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This report is the collective work of all the members of the Task Force.
Authors of the first draft of each chapter are given below.

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Foreword

Cardiovascular disease, including coronary heart disease, strokes and diseases of other arteries, is a major cause of early death and disability. For many years the major markers of disease risk have been well recognised: these include high blood cholesterol levels and smoking. But it has also been recognised that these markers do not account for all cardiovascular risk. Furthermore, treatments that are highly effective in altering these markers, for instance the ‘statin’ drugs used to lower cholesterol, do not remove risk entirely: typically they reduce it by about 30% or less. These observations have prompted a search for other, perhaps more subtle, indicators of risk of cardiovascular disease. Over the past few years, a number of such risk markers have emerged. These include subtle alterations of types of fat in the bloodstream other than cholesterol, factors associated with inflammation and with clotting, lowered resistance to oxidative stress and impaired functioning of blood vessels. In addition, it has been recognised that experiences in early life, even before birth, may influence later disease risk. Although these so-called ‘emerging’ or ‘novel’ risk markers are now becoming clear, we still know little about how they may be altered to reduce risk of cardiovascular disease. In particular, we know little about how they may be influenced by the diet, although the rapid changes in risk of cardiovascular disease that occurred throughout the twentieth century suggest that features of our lifestyle such as diet may play a fundamental role.

This Task Force was asked to consider the emerging risk factors for cardiovascular disease, and their relationship to diet. The panel of experts that constituted the Task Force represented a range of different disciplines. Each chapter in the report was written initially by one or two of the members of the Task Force, but then all members commented and may have contributed to each chapter. We divided the chapters to cover each of the major themes: novel lipid factors, vascular function, clotting factors, inflammatory factors, oxidative stress, homocysteine and ‘early origins of adult disease’. But there were certain consistent themes emerging in all chapters, such as obesity, insulin resistance and genetic predisposition (especially the way that diet may interact with genes) and we decided to make these the subject of a separate chapter (Chapter 2). We also considered one novel area, that of factors related to adipose tissue, in which links with cardiovascular disease are at present not well established, but which we feel may become increasingly important over the next few years. In each chapter we have tried to assess the strength of the evidence for links with cardiovascular disease, and the state of knowledge about the relationship to diet. The Report includes, as is usual now for British Nutrition Foundation reports, a Question and Answer section and a Public Health chapter, in which we hope everyone will be able to find ‘take-home messages’ emerging from our work.

I would like to thank all the members of the Task Force who worked hard and willingly on this project, and also others who corresponded with us. I extend special thanks to the British Nutrition Foundation staff who participated, both as authors and by providing administrative support.

Professor Keith Frayn
Terms of Reference

The Task Force was invited by the Council of the British Nutrition Foundation to:

(1) Review the present state of knowledge of the link between emerging aspects of diet (and related factors) and cardiovascular disease.
(2) Prepare a report and, should it see fit, draw conclusions, make recommendations and identify areas for future research.
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1 The Aetiology and Epidemiology of Cardiovascular Disease

1.1 Definitions
This report is concerned with the causes or factors that relate to the risk of developing cardiovascular disease and how these may be influenced by diet. Cardiovascular disease includes arterial disease affecting the blood supply to the heart or to the brain, or to the peripheral regions of the body. Cardiovascular diseases account for over half of all deaths in middle age and one-third of all deaths in old age in most developed countries.

1.1.1 Coronary heart disease (CHD)
Coronary heart disease (CHD) is a condition in which the walls of the arteries supplying blood to the heart muscle (coronary arteries) become thickened. This thickening, caused by the development of lesions in the arterial wall, is called atherosclerosis; the lesions are called plaques. It can restrict the supply of blood to the heart muscle (the myocardium) and may manifest to the patient as chest pain on exertion (angina) or breathlessness on exertion. More seriously, if the cap covering the plaque ruptures, exposing the contents to the circulation, the blood may clot and obstruct the flow completely, resulting in a myocardial infarction or heart attack. CHD is also known as ischaemic heart disease.

The term acute coronary syndromes is used to denote a hospitalisation for unstable angina, or thrombolysis for suspected myocardial infarction or an emergency revascularisation procedure for relief of ischaemic chest pain at rest.

There are several causes of sudden death, but most are related to CHD or cerebrovascular disease (see Section 1.1.2). Sudden cardiac death may be due to myocardial infarction or to cardiac arrhythmia. Cardiac arrhythmias are situations where the heart rate becomes irregular, and/or too rapid or too slow. Arrhythmias may be provoked by intercurrent stress or illness but are more common, and more frequently fatal, in hearts previously damaged by ischaemic heart disease or any other cause of cardiac dysfunction, such as hypertension or excess alcohol consumption. The main risk factors for arrhythmias and sudden cardiac death are thus very similar to those for CHD.

1.1.2 Cerebrovascular disease
Cerebrovascular disease involves interruption of the blood supply to part of the brain and may result in a stroke or a transient ischaemic attack. There are two types of stroke. The most common type of stroke in Western countries is ischaemic stroke, in which there is a blockage in the blood supply to the brain. The loss of blood supply to part of the brain may lead to irreversible damage to brain tissue. The blockage most commonly arises from the process of thromboembolism, in which a blood clot formed somewhere else (e.g. in the heart or in the carotid artery) becomes dislodged and then occludes an artery within the brain (cerebral arteries). Narrowing of the intracerebral arteries with atherosclerotic plaque may increase the risk, and may also lead to local formation of a blood clot. The aetiology is similar to that of CHD. In the other main type there is rupture of a blood vessel supplying the brain, with release of blood into the brain (haemorrhagic stroke). High blood pressure (hypertension) is a major risk factor for haemorrhagic stroke, but otherwise the aetiology is different and will not be considered in detail in this report.
1.1.3 Peripheral vascular disease (PVD)

Peripheral vascular disease (PVD) involves athero-
sclerotic plaques narrowing the arteries supplying
other regions apart from the myocardium and brain.
A common form involves narrowing of the arteries
supplying blood to the legs. The result may be pain
on exercise (claudication). In more severe cases,
impaired blood supply leads to death of leg tissues,
which require amputation.

**Terminology used in the report**

**Saturates, polyunsaturates, monounsaturates**

As saturated fatty acids are referred to as
saturates on food labels, this term will be used
throughout this report. Similarly, polyunsatur-
ates and monounsaturates will be used to denote
polyunsaturated and monounsaturated fatty
acids, respectively. The abbreviations SFA,
MUFA and PUFA are also in common usage
and have been used in some tables to denote
saturated, monounsaturated and polyunsatur-
ated fatty acids, respectively.

Although fatty acids are generally grouped
according to the degree of unsaturation (num-
ber of double bonds), it should be noted that
their chemical and biochemical properties are
also dependent upon chain length. For example,
not all saturates raise blood cholesterol levels,
and they do so to different extents. Saturates
with chain lengths of 18:0 (stearic) and above
and 10:0 (capric) and below have essentially no
effect on blood cholesterol. Only 12:0 (lauric),
14:0 (myristic) and 16:0 (palmitic) raise serum
cholesterol with the order of effectiveness being
14:0 > 12:0 > 16:0.

**n-6 and n-3 fatty acids**

Polyunsaturates of the omega-6 and omega-3
families are referred to as n-6 and n-3 polyunsat-
urates, respectively.

**Triglycerides**

The traditionally used term triglycerides will be
used throughout this report, but an alternative
term is triacylglycerols (often abbreviated to
TAG).

1.2 Pathogenesis

Cardiovascular diseases, whether affecting the
coronary, cerebral or peripheral arteries, share a
common pathophysiology involving atherosclerosis
and thrombosis (or clotting). The causes of cardio-
vascular disease and why it affects some individuals
and not others, or why it more severely affects one
region rather than another, are discussed later in this
chapter and elsewhere in this report.

1.2.1 Atherosclerosis

The term atherosclerosis comes from the Greek athere,
meaning porridge or gruel and referring to the soft
consistency of the core of the plaque (mainly lipid),
and sclerosis, meaning hardening. The lipid of the
atherosclerotic plaque is mainly cholesterol from
low-density lipoprotein (LDL) particles that have
left the circulation. Current understanding is that the
LDL particles must be chemically modified in some
way before they are taken up by the so-called scav-
enger receptors of macrophages (white blood cells that
have become resident in the arterial wall, see below).
This chemical modification may involve lipid
peroxidation (see Chapter 5), which leads in turn to
peroxidation of the large protein known as apoli-
ipoprotein-B100 that is associated with each LDL
particle. Whilst uptake of cholesterol by cells is nor-
mally tightly controlled so that cellular cholesterol
levels do not become excessive, lipid uptake by the
scavenger receptor pathway is not subject to such regu-
lation. Therefore, the macrophages may engulf large
amounts of lipid, giving them a foamy appearance
under the microscope. These are so-called foam cells,
and are characteristic of the atherosclerotic plaque.

Accumulation of these foam cells in the arterial
wall leads to the first visible stage in atherosclerosis,
formation of a yellowish, minimally raised spot (the
spots later merging into streaks) in the arterial wall.
These are known as fatty streaks. The process at this
stage must be largely reversible since more than 40%
of infants coming to post-mortem examination
during the first year of life have fatty streaks in their
aortas (Woolf, 1990).

These macrophages send chemical signals that
trigger further events associated with atherosclerosis.
Blood monocytes and T-lymphocytes (other types
of white blood cells) adhere to an area of the cellular
lining of an artery, the endothelium. The monocytes
migrate into the subendothelial space where they differentiate into further macrophages and engulf further lipid. Development of the atherosclerotic plaque involves proliferation of smooth muscle cells of the arterial wall and the elaboration of a connective tissue matrix, forming a fibromuscular cap to the lesion (Fig. 1.1). These processes may be seen as reparative, and this has led to the description of these events as the ‘response to injury’ hypothesis of atherosclerosis.

Within the lesion there may be breakdown of dead macrophages and release of their contents, with formation of a semi-liquid pool of extracellular lipid. At the same time, calcification of the arterial wall leads to hardening (lack of elasticity). The lid of the lesion may remain firm, in which case the lesion may protrude into the arterial lumen, obstructing flow but not causing acute damage. Some plaque caps, however, become unstable and are damaged, exposing the contents of the plaque. This results in the normal response to vessel wall damage – thrombus formation (blood clotting).

1.2.2 Blood clotting

The process of blood clotting will be described fully in Chapter 6, but briefly it begins when the endothelial lining of a blood vessel is damaged, exposing cells and surfaces that are normally covered by the endothelium (Fig. 1.2). This may happen at the site of an atherosclerotic plaque, especially following rupture of the plaque cap. Proteins thus exposed activate the pathway. Formation of a clot depends upon a cascade of proteolytic reactions, with enzymes initially in an inactive, precursor (or ‘zymogen’) form becoming activated sequentially. Because of the cascade nature of this process, there is amplification, each enzyme catalysing the production of many of its product enzymes. In the course of this activation process, blood platelets are drawn to the site of injury where they aggregate and form a primary plug. Upon this is built a mesh of fibrils of the protein fibrin, formed by cleavage of the circulating precursor protein fibrinogen. If the coagulation process is brought about by bleeding outside the blood vessel, then the product is known as a clot. If it is brought about by damage to the endothelium, as for instance at the site of an atherosclerotic plaque, then the product is known as a thrombus. Part of the thrombus may become loose and then be carried to other sites where it can lodge and

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**Fig. 1.1** Outline of the development of the atherosclerotic plaque. Low-density lipoprotein (LDL) particles from the circulation enter the arterial intima. After oxidation (oxLDL) they may be engulfed by macrophages. The resultant lipid-laden macrophages are known as foam cells. Through a process of ‘response to injury’ with proliferation and migration of smooth muscle cells and collagen, the arterial wall becomes thickened and hardened. The processes involved are described in more detail in the accompanying text and developed in later chapters (see Figs 5.7, 7.2).
obstruct flow, the process of thromboembolism (e.g. in ischaemic stroke).

Not surprisingly, there is also a pathway for the dissolution of clots or thrombi, the fibrinolytic pathway (also known as fibrinolysis); the process of coagulation is a balance between the activities of the coagulation and fibrinolytic pathways. As will be described later, components of both these pathways may be risk markers for cardiovascular disease.

1.2.3 Raised blood pressure

Blood is pumped around the body by the left ventricle of the heart. The pressure resulting from this process is opposed by the resistance of the vessels through which the blood flows, and the balance of these two opposing forces is known as blood pressure. Blood pressure is conventionally recorded as systolic (highest) over diastolic (lowest) pressure and needs to be sufficiently high to ensure adequate blood flow to the brain and other tissues, but not so high that it creates extra work for the heart and risks tissue damage. Elevated blood pressure is known as hypertension.

Until recently, the importance of blood pressure for risk of CHD or stroke was seriously underestimated in epidemiological studies due to regression dilution bias (random error that occurs as a consequence of using a single blood pressure measurement rather than the ‘usual’ or average value over a period of time) (Clarke et al., 1999, 2002). A recent meta-analysis (a statistical technique that combines the results of previous studies) was carried out to take account of regression dilution bias. It involved 56,000 vascular deaths of people between the ages of 40 and 89 years among one million adults without known vascular disease at baseline, and showed that each 20 mmHg difference in usual systolic blood pressure (which is approximately equivalent to a 10 mmHg difference in usual diastolic blood pressure) was associated with a two-fold difference in death rates from CHD, stroke and other vascular deaths (Prospective Studies Collaboration, 2002). All of these proportional differences in vascular mortality were half as extreme at 80–89 years as at 40–49 years, but the annual absolute differences in risk (i.e. the differences in the number of actual events) are greater in old age.

Randomised trials (which have usually lasted only a few years) have confirmed the findings of epidemiological studies and shown that modest reductions in blood pressure are associated with a reduction in risk of stroke and CHD mortality and morbidity (Bosch et al., 2002; PROGRESS Collaborative Group, 2003).

1.2.4 Relationship of risk factors to the pathological processes

The nature of the processes described above provides a framework for understanding how certain factors
may predispose to atherosclerosis. The role of plasma lipids explains why elevated serum total cholesterol level has long been recognised as a predisposing factor to atherosclerosis. However, evidence has accumulated over the past few decades that has highlighted the importance of other processes, including an impairment of endothelial function, the tendency to oxidation in the subendothelial space, the inflammatory processes involved in formation of a plaque, and blood clotting. In this report, the evidence for emerging risk factors for cardiovascular disease relating to these processes is considered in some detail.

1.3 Epidemiology of cardiovascular disease

1.3.1 The burden of cardiovascular disease globally and in the UK

Cardiovascular disease is the leading cause of death worldwide, accounting for around 18 million deaths each year (33% of the 55 million total) (World Health Organization, 2001). Mortality rates vary considerably between countries, being lower in Japan and the Mediterranean countries such as France, Spain, Portugal and Italy, and highest in Eastern European countries such as Latvia, the Russian Federation and Lithuania (Fig. 1.3) (World Health Organization, 2001). Around 50% of these deaths are from CHD and a further 25% from stroke.

Cardiovascular disease mortality rates in the UK are currently amongst the highest in the world, accounting for 36% of premature deaths in men (i.e. those aged less than 75 years) and 27% amongst women (Fig. 1.4). CHD is the most common cause of death in the UK, with one in four men and one in six women dying from the disease (British Heart Foundation, 2003a).

Cardiovascular disease is also a major cause of ill health and disability. It has been estimated that 1.2 million people in the UK have survived a heart attack and 2 million have angina (British Heart Foundation, 2003a). CHD alone is thought to cost the UK economy around £7 billion per year, more than any other single disease for which a comparable analysis has been carried out (British Heart Foundation, 2003a).

1.3.2 Temporal trends

Increased death rates from cardiovascular disease have been noted recently in the former socialist economies in Eastern and Central Europe and in developing countries undergoing the ‘epidemiological transition’, where control of infectious, parasitic and nutritional diseases allows most of the population to reach the ages at which cardiovascular disease manifests itself. Accompanying changes in diet and lifestyle are also leading to a growing epidemic of overweight/obesity and type 2 diabetes in these
countries, which are major risk factors for cardiovascular disease (see Chapter 2). In fact, by the mid 1990s, cardiovascular disease had become the leading cause of death in developing as well as developed countries (Pearson, 1999).

In contrast, CHD mortality rates have been declining since the 1960s or 1970s in most European countries, North America and Australia/New Zealand (Sarti et al., 2000; Tunstall-Pedoe et al., 2000). The UK has also experienced declines, although the reduction is less than that achieved in other developed countries with initially higher death rates. For example, the death rate from CHD for men aged 35–74 years fell by 39% between 1988 and 1998 in the UK, whilst a fall of 49% was achieved in Denmark and 45% in Australia and Norway over the same period (Fig. 1.5) (British Heart Foundation, 2003a). Amongst men in the UK, CHD death rates have declined faster in the younger age groups. For example, between 1989 and 1999 there was a 43% fall amongst men aged 45–54 years compared to 34% in those aged 65–74 years (British Heart Foundation, 2003a).

While death rates from haemorrhagic stroke have been falling in the UK since the 1940s, secular trends in ischaemic stroke have paralleled those of CHD mortality (i.e. fallen from a peak in the 1970s) (Lawlor et al., 2001). Overall stroke mortality fell by 21% from 1990 to 2000 (Fig. 1.6), but this decline has slowed in recent years, particularly in the younger age groups. As with CHD, death rates from stroke have not declined as fast in the UK as in some other developed countries (e.g. USA, Denmark).

The WHO MONICA project (monitoring trends and determinants of cardiovascular disease) indicated that, although trends vary by population, a fall in the rate of new major CHD events is the primary contributor to declining CHD mortality (accounting for about two-thirds of the decline), with improvements in survival having a lesser, but nevertheless substantial, role (Tunstall-Pedoe et al., 1999). However, other studies within Western Europe and the USA have suggested that improved survival may play a greater role in the declines in CHD mortality than falling incident myocardial infarction rates or changes in population cardiovascular risk factors (Capewell et al., 1999; Ferrario et al., 2001; Rosamond et al., 2001). Moreover, recent studies supported by data from population-based stroke registers have not shown any significant decline in the incidence of stroke in Western European countries (Bamford et al., 1990; Harmsen et al., 1992).

Morbidity data are less reliable than mortality data so trends are harder to discern, but national
surveys (e.g. the Health Survey for England and the General Household Survey) suggest that, while mortality from cardiovascular disease is falling rapidly in the UK, morbidity is not falling and, in older age groups, has risen by around a quarter since the late 1980s (Department of Health, 1999a; Office for National Statistics, 2001; British Heart Foundation, 2003a) (Fig. 1.7). The British Regional Heart Study,
Cardiovascular Disease

Fig. 1.7  Rate of reporting longstanding cardiovascular disease for adults by age group, Great Britain, 1988–2000. Reproduced with permission from British Heart Foundation (2003a).

Fig. 1.8  Changes in prevalence rates in diseases of the circulatory system, men and women, between 1981–82 and 1991–92, England and Wales. Reproduced with permission from British Heart Foundation (2003a).

a large longitudinal cohort study of British men aged 50–64 years, showed a substantial fall in CHD mortality paralleled by a fall in the prevalence of angina between 1978 and 1996 but no evidence of any reduction in the prevalence of diagnosed CHD (Lampe et al., 2001). Unfortunately, this study did not investigate trends in older age groups. Statistics from general practice have suggested a rise in the prevalence of stroke in England and Wales between 1981/82 and 1991/92 (Royal College of General Practitioners, 1995) (Fig. 1.8), although temporal trends from other sources are less clear (Office of Population Censuses and Surveys, 2000). There is little evidence of significant improvements in many cardiovascular disease risk factors acting at the primary prevention level (preventing onset of disease) amongst the adult UK population (see Section 1.4.7).

1.3.3 Variation in cardiovascular disease in the UK

Cardiovascular disease (predominantly CHD) is significantly higher amongst males than females, although the difference in risk varies widely between countries. In the UK, a man is nearly three times more likely to die from premature CHD than a woman (British Heart Foundation, 2003a). Although men and women share most of the major risk factors for heart disease (see Section 1.4), it has been speculated that before the menopause endogenous oestrogens may confer some protection against CHD
in women. Although hormonal factors may be important contributors to lower CHD rates amongst women, secular and geographical trends indicate that environmental factors (i.e., diet and lifestyle) are also likely to play a part (Lawlor et al., 2001). Rates of CHD amongst women increase around the fifth to sixth decade of life, indicating that any protective effect is lost after this time. This may be linked to changes in fat distribution as longitudinal data support an increase in central body fatness occurring after the menopause (Barrett-Connor, 1997).

There are also marked regional, social and ethnic differences in risk of cardiovascular disease within the UK, and these differences appear to be increasing. Mortality and morbidity from cardiovascular disease is highest in Scotland and the north of England and lowest in the south of England (British Heart Foundation, 2003a). For example, the premature death rate from CHD for men living in Scotland is over 50% higher than in East Anglia and over 80% higher for women. Within those areas of high cardiovascular disease mortality, the highest death rates are concentrated in certain urban areas such as inner city areas of Glasgow, Manchester, Liverpool, Leeds, Birmingham, Cardiff and Belfast. CHD and cerebrovascular disease patterns within the UK and other countries have consistently shown that cardiovascular disease is more common among those in less privileged socioeconomic groups. For example, amongst male manual workers the premature CHD mortality rate is 58% higher than that for trained professionals. There is also evidence that the socioeconomic gradient in cardiovascular disease mortality appears to be widening. In the 1970s, the difference in CHD risk between men in social classes V and I of dying of a heart attack was two-fold, but by 1990s this had risen to three-fold (Fig. 1.9) (Department of Health, 2000a). Whilst some of the social class differences in cardiovascular disease mortality and morbidity can be attributed to a higher proportion of smokers amongst men and women in lower socioeconomic groups, there is also evidence of variation in diet-related risk factors (see Section 1.4.4 for a description of these factors). Whilst the social or regional variations cannot be explained by differences in the consumption of fat or saturates, or in blood cholesterol levels, there has been a social class gradient for obesity and blood pressure, as well as for fruit and vegetable intake, throughout the past decade. There is also evidence of geographical differences in fruit and vegetable consumption, with the north of England and Scotland having lower intakes (DEFRA, 2001). Other proposed explanations for the social class differences include early life factors (see Chapter 10), psychological factors and access to health care (Marmot et al., 1991).

Significant differences in premature cardiovascular disease incidence and prevalence also exist for some ethnic populations living in the UK compared with the indigenous population. For example, South Asian men living in the UK (Indians, Bangladeshis, Pakistanis, Sri Lankans) have higher premature death rates from CHD and stroke than the national average (Fig. 1.10) (Department of Health, 2001). The difference in the death rates between South Asian men and the rest of the population is also increasing because the death rate from cardiovascular disease is not falling as fast in South Asians as it is in the rest of the population (Wild & McKeigue, 1997). Studies have suggested that these high rates of CHD, as well as concomitant high rates of type 2 diabetes, are most easily explained by the existence of an insulin resistance syndrome, prevalent in South Asian populations and associated with a pronounced tendency to central obesity in this group (see Chapter 2, Section 2.4.2) (Wild & McKeigue, 1997). In contrast, premature death rates from CHD for Caribbeans and West Africans living in the UK are much lower than average—around half the rate found in the general population for men and two-thirds of the rate found in women. However, individuals of African-Caribbean descent have an increased risk
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1.4 Risk factors for cardiovascular disease

1.4.1 Definition of risk factors

The term risk factor describes those characteristics found to be related to the subsequent occurrence of cardiovascular disease. This term includes modifiable lifestyle, biochemical and physiological characteristics, as well as unmodifiable personal characteristics such as age, sex and family history of cardiovascular disease.

In any given study, it is necessary to consider whether the relationship between a risk factor and cardiovascular disease is likely to be causal (i.e. whether modification of this factor will lead to a change in cardiovascular disease risk). Such a judgement must be made in the context of all the available evidence and as such must be re-evaluated with new findings. Criteria that aid in the judgement of causality include the strength of the association, biological credibility of the hypothesis, consistency of the findings, as well as other information concerning the temporal sequence and the presence of a dose–response relationship (Bradford Hill, 1965). Inferences on causality can also be derived from genetic associations if the gene associated with a particular characteristic is associated with disease (referred to as Mendelian randomisation).

Risk factors may be related to one another. Family history may involve elevated LDL-cholesterol, for instance, and male gender is associated with lower high-density lipoprotein (HDL) cholesterol. If a risk factor is not related to other known risk factors it is said to be independent. In this report, we will attempt to distinguish which risk factors have independent status.

The term risk markers is sometimes preferred to risk factors, to avoid the implication that cause and effect are known. For instance, church attendance is associated with lower risk of cardiovascular disease (Kawachi et al., 1996), but most would argue that a causal relationship is less likely than the fact that this reflects some other aspect of lifestyle that has a bearing on cardiovascular disease. In this report we will generally use the term risk factor, but recognising that cause and effect may not have been demonstrated unequivocally.

1.4.2 Approaches used to investigate the relationship between risk factors and disease

The observational epidemiological evidence in support of particular risk factors for cardiovascular disease varies according to the study design. Cross-sectional studies (where the measurement of risk

Fig. 1.10 Standardised mortality ratios (SMRs) for CHD and stroke in different ethnic groups in the UK. The average UK value is represented as an SMR of one. Reproduced from Department of Health (2001) with permission of The Stationery Office. Crown copyright.
factors and occurrence of disease are recorded at one point in time) compare the distribution of risk factors and of the presence, or severity, of disease in individuals, and examine relationships between these. Cross-sectional studies may give spurious results if two risk factors (A and B) are highly correlated and B is related to disease but A is not. Failure to take account of the relationship between A and B may inappropriately lead to the conclusion that A is related to disease. This is known as confounding. To illustrate this, a cross-sectional study may report a higher mortality among people taking cholesterol-lowering drugs. This may imply that the drugs cause death from CHD, whereas the true explanation is that use of such medication and prior elevated levels of cholesterol may relate to the fact that these people are at higher risk of death from CHD.

In a case-control study, people with the disease (in this case CHD) are compared with people without it and matched for relevant factors such as age and sex. A limitation of the case-control study is the inability to distinguish whether the disease may cause some biochemical abnormality rather than vice versa (referred to as reverse causality). Case-control studies cannot demonstrate cause and effect, although this may be inferred from a knowledge of the pathological processes involved (thus, it seems more likely that a high serum cholesterol concentration causes CHD than the reverse).

In prospective (or cohort) studies, healthy people are investigated and followed up for several years to record the onset of disease. At the end of a particular follow-up period, the characteristics of people who developed (or died from) the disease are compared with people who did not. However, while prospective studies can demonstrate an association or relationship between disease and some factor, whether or not this is causal is often uncertain. It is possible that the association may be mediated through some other factor to which both are related (i.e., confounding).

The most convincing evidence in support of causality for a particular risk factor comes from an intervention study in which that factor is modified and the effects on subsequent disease are studied. Thus, intervention trials with cholesterol-lowering drugs consistently show reductions in mortality from CHD and have led to acceptance of the 'lipid hypothesis' (that a high blood lipid level is causally related to development of CHD). In this report we shall distinguish between levels of evidence for each of the risk factors discussed. Clearly, for the emerging risk factors, the evidence for causality is still incomplete.

1.4.3 Interpretation of the association

The magnitude of the association between a risk factor and a disease is often expressed as a relative risk (RR) or risk ratio. This indicates the likelihood of developing a disease in those exposed to a risk factor (or treatment) relative to those who are not exposed, and is defined as the ratio of the incidence of the disease in the exposed group divided by the corresponding incidence of the disease in the non-exposed group. A relative risk of 1.0 indicates that the rates of disease are the same in the exposed and unexposed groups. A value greater than 1.0 indicates a positive association or an increased risk among those exposed to a risk factor. A value below 1.0 indicates a reduction in risk among those exposed to a risk factor.

Epidemiological studies also commonly use the term attributable risk (AR). AR measures the excess risk accounted for by exposure to a particular factor and is defined as the disease rate in the exposed group minus that in the unexposed group. If the attributable risk of an exposure is greater than zero this indicates an increase in the risk of disease; if it is negative (e.g. if the exposure is a treatment) this demonstrates a beneficial effect. AR is the measure of association that is most relevant when making decisions for individuals, because it relates to their risk of developing a disease. If a condition is common, such as CHD, the importance of reducing an individual’s risk is much greater than if the condition is rare.

The population attributable risk (also known as attributable fraction) measures the reduction in disease in the whole population that might be achieved by eliminating a risk factor. It is calculated by multiplying the attributable risk by the prevalence of exposure to a risk factor in a population. This may be expressed as a percentage and is the most useful measure for public health purposes. A particular risk factor may substantially increase the risk of cardiovascular disease (i.e. have a high RR), but if exposure to this risk factor is relatively rare its influence on the rates of cardiovascular disease in a particular population may be limited. The converse is also true; if a risk factor is common it may exert a substantial influence on the occurrence of a disease in a population even if it exerts a comparatively small increase in
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risk for an individual. For example, people who are physically active have a lower risk of CHD. In the UK, 63% of men and 75% of women do not meet the current guidelines suggested by the government (i.e. 30 minutes of at least moderate intensity activity on five days of the week or more) and, as a consequence, a large number of deaths from CHD have been attributed to physical inactivity. For example, a recent estimate in a report from the National Heart Forum estimated that around 36% of CHD deaths in men and 38% in women might be attributed to their lack of physical activity (National Heart Forum, 2002) (see Chapter 2, Section 2.8 and Chapter 12). More recent research from the World Health Organization suggests that these estimates may be too high, but confirms the importance of encouraging physical activity to reduce the burden of CHD in developed countries (World Health Organization, 2002) (see Chapter 12).

1.4.4 Conventional risk factors for CHD

The aetiology of CHD began to be unravelled seriously in the 1950s. The American nutritionist Ancel Keys was interested in the low rates of CHD that he observed in countries bordering the Mediterranean. He established the Seven Countries Study to compare CHD rates and diet in different countries. An early finding from this study was that there was a strong relationship, when comparing one country with another, between the incidence of CHD and the dietary intake of saturates compared with polyunsaturates (a high ratio of dietary saturates to polyunsaturates was associated with a high rate of CHD). It was then found that the average level of serum cholesterol, comparing one country with another, correlated positively both with the incidence of CHD (Fig. 1.11) and with the average ratio of saturates to polyunsaturates ingested. This relationship was later shown to exist even within a country. Thus, an elevated serum cholesterol concentration became the first well-documented risk factor for development of CHD.

Until perhaps 10 years ago, there were a small number of factors that were recognised to mark a predisposition to CHD. These are the so-called classical, or conventional, risk factors (Table 1.1). As well as the ‘unmodifiable’ risk factors (e.g. age, sex, genetic predisposition), and the regional, social and ethnic differences described in Section 1.3.3, these include smoking, raised blood cholesterol, raised blood pressure, physical inactivity and obesity. People with type 2 diabetes also have a two- to four-fold greater risk of death from cardiovascular disease than non-diabetic individuals (see Chapter 2, Section 2.5). These ‘classical’ risk factors have been reviewed in a previous British Nutrition Foundation report (British Nutrition Foundation, 1997). Some of the newer insights into the relationship between diet and these risk factors will be discussed in Chapter 11. However, over the past decade a number of new factors have been recognised and these novel, or emerging, risk factors are the subject of this report. Inevitably, however, the definition of what is conventional and what is novel, or emerging, is somewhat subjective. The risk factors that are considered in detail in this report are listed in Table 1.2.

Fig. 1.11 CHD incidence and serum cholesterol levels in selected countries (data collected in the 1960s). The x-axis shows average serum cholesterol concentration in each country. Since most of the serum cholesterol is in the low-density lipoprotein (LDL) fraction, total serum cholesterol is strongly related to LDL-cholesterol, the fraction thought to predispose to CHD. Adapted from Simons (1985) with permission from Excerpta Medica.
Table 1.1 Conventional risk factors for cardiovascular disease.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Direction of association</th>
<th>Modifiable by dietary factors?</th>
<th>Relevance to cardiovascular disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Increases with age</td>
<td>No</td>
<td>Increased prevalence as population ages</td>
</tr>
<tr>
<td>Gender</td>
<td>Males at higher risk</td>
<td>No</td>
<td>Risk in men and women is equalised after the menopause, possibly due to protection by oestrogens in younger women or to increases in male pattern (central) obesity amongst post-menopausal women</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>Lower socioeconomic status increases risk</td>
<td>Some dietary changes may help to reduce the higher risk in lower socioeconomic groups</td>
<td>Highly related to other factors (e.g. diet, smoking, physical inactivity)</td>
</tr>
<tr>
<td>Ethnic group</td>
<td>People from the Indian subcontinent are at particularly high risk of CHD</td>
<td>No</td>
<td>May reflect abdominal obesity and insulin resistance (see Chapter 2, Section 2.4.2)</td>
</tr>
<tr>
<td></td>
<td>People of African-Caribbean descent in the UK are at greater risk of stroke</td>
<td></td>
<td>Possibly reflects genetic predisposition to hypertension. Rates are falling as a result of better detection and treatment of hypertension</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoking increases risk</td>
<td>No</td>
<td>Increases oxidative stress (see Chapter 5) and impairs endothelial function (see Chapter 4)</td>
</tr>
<tr>
<td>Serum total cholesterol concentration</td>
<td>Higher blood cholesterol level increases risk</td>
<td>Yes</td>
<td>Uptake of cholesterol by macrophages is the origin of the core of the atherosclerotic plaque</td>
</tr>
<tr>
<td>Serum LDL-cholesterol concentration</td>
<td>Higher LDL-cholesterol level increases risk</td>
<td>Yes</td>
<td>LDL particles carry cholesterol that may be deposited in arterial walls</td>
</tr>
<tr>
<td>Serum HDL-cholesterol concentration</td>
<td>Lower HDL-cholesterol level increases risk, particularly amongst women</td>
<td>Yes</td>
<td>HDL may transport excess cholesterol to the liver for excretion</td>
</tr>
<tr>
<td>Serum triglyceride concentration</td>
<td>Higher triglyceride level increases risk</td>
<td>Yes</td>
<td>Strongly inversely related to HDL-cholesterol although serum triglyceride is also an independent risk factor, perhaps reflecting more subtle alterations in lipid metabolism</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>Higher blood pressure increases risk</td>
<td>Yes</td>
<td>Hypertension increases the risk of haemorrhagic/ ischaemic stroke, induces endothelial dysfunction, exacerbates the atherosclerotic process and contributes to the instability of the atherosclerotic plaque</td>
</tr>
<tr>
<td>Diabetes</td>
<td>Diabetes increases risk</td>
<td>Somewhat</td>
<td>See Chapter 2, Section 2.5</td>
</tr>
<tr>
<td>Physical inactivity</td>
<td>Being inactive increases risk</td>
<td>No</td>
<td>See Chapter 2, Section 2.8</td>
</tr>
<tr>
<td>Obesity</td>
<td>Overweight and obesity increase risk partly via other CHD risk factors (e.g. blood pressure, diabetes, blood cholesterol)</td>
<td>Yes</td>
<td>See Chapter 2, Section 2.4</td>
</tr>
</tbody>
</table>

HDL: high-density lipoprotein; LDL: low-density lipoprotein.
Conventional risk factors for cerebrovascular disease

The most important modifiable risk factor for cerebrovascular disease is hypertension (see Section 1.2.3); higher systolic and diastolic blood pressure has been associated with an increased incidence of ischaemic and hemorrhagic stroke (MacMahon, 1996). Historically, salt (sodium) is viewed as the most important dietary determinant of blood pressure, in part influencing the rise in blood pressure with age. Sodium is the principal cation in the extracellular fluid and plays a key role in maintaining water balance in the body. There is an upper limit to the rate at which excretion of excess sodium can occur, causing an increase in body sodium content and water retention. If this situation persists, one important manifestation may be the development of raised blood pressure. In 1994, the Committee on Medical Aspects of Food Policy (COMA), recommended a reduction in average salt intake (to 6 g/day for the adult population) (Department of Health, 1994) (see Sections 1.5.1 and Chapter 13, Section 13.4.3). More recently, the UK government’s Scientific Advisory Committee on Nutrition (SACN) reviewed the available studies and concluded that the strength of the evidence for an association between salt intake and elevated blood pressure has increased and supported the recommendation for a reduction in average intake (Scientific Advisory Committee on Nutrition, 2003). The strongest evidence comes from the Dietary Approaches to Stop Hypertension (DASH) Sodium Trial, in which stepwise reductions in blood pressure were demonstrated in response to a lowering of dietary salt levels (Sacks et al., 2001). In this study, the greatest reductions were observed when a decrease in dietary salt was combined with a diet rich in fruit and vegetables and low fat dairy products, indicating that a whole diet approach is likely to be the most effective population-based method of lowering blood pressure (see Chapter 11, Section 11.4).

Observational studies also support an association of other lifestyle-related risk factors with increasing risk of stroke (e.g. lack of exercise, alcohol consumption, diabetes, obesity, smoking). The role of fat intake as a risk factor for stroke remains uncertain,
although the use of cholesterol-lowering drugs reduces stroke risk (Gariballa, 2000). Recent research has also investigated links with other risk factors, such as hyperhomocysteinaemia, antioxidant nutrients (e.g. vitamins C and E, carotenoids, selenium), fibrinogen and clotting factors, which will be discussed further in this report (see Chapters 5, 6, 8, 11).

1.4.6 Smoking and PVD

The risk factors that contribute to PVD are similar to those associated with CHD and cerebrovascular disease (e.g. diabetes, hypercholesterolaemia, high blood pressure, physical inactivity, low levels of HDL cholesterol, a high body mass index, hyperhomocysteinaemia). However, data from the Framingham Study (Murabito et al., 2002) and other population studies indicate that cigarette smoking has a particularly strong association with PVD and is one of the most important risk factors for the condition. A possible explanation for this relation is that smoking induces a wide variety of physiological responses, some of which appear likely to be involved in development of atherosclerosis or increased probability of thrombosis. These responses include modified lipid levels, decreased fibrinolysis, increased fibrinogen levels and changes in endothelial and platelet functions, which are themselves either known risk factors for, or early features of, atherosclerosis. The rapid amelioration of the risk of cardiovascular disease after cessation of smoking suggests that these processes are readily reversible.

1.4.7 Trends in the classic cardiovascular risk factors

The WHO MONICA project studied trends in the classic risk factors in several populations throughout Europe over a 10-year period (within a total study period of 1979 to 1996). This survey demonstrated declining trends in the prevalence of smoking in men in most of the study populations but an increase in the prevalence of female smokers in several countries. Systolic blood pressure increased during this period in most centres in both sexes, while cholesterol generally showed a small downward trend. The most disturbing feature of the results was the rise in body mass index, particularly amongst men, which occurred in three-quarters of the populations studied (Evans et al., 2001).

Similar findings have also been reported by surveys within the UK. There has been a long-term decline in smoking prevalence in men and women in Britain since the 1970s, with a greater decline among men than women (Bennett et al., 1996). However, data from the Health Survey for England show little change in average cholesterol levels since reliable measurements of this risk factor have been made (i.e. since 1991) (Department of Health, 1999a), and recent estimates amongst adults in Great Britain show nearly half to have blood cholesterol levels of 5.2 mmol/l and above (Food Standards Agency & Department of Health, 2004) (Fig. 1.12). Whilst there appears to have been a slight decrease in average blood pressure independent of treatment since
1994, the proportion of those suffering from hypertension has also remained fairly stable (Department of Health, 1999a). In England, 41% of men and 33% of women have hypertension (defined as a systolic blood pressure of 140 mmHg or over, or a diastolic blood pressure of 90 mmHg or over) or are being treated for the condition (Department of Health, 1999a). Of most concern, however, is the continued and rapid rise in the prevalence of overweight and obesity and the associated increase in the number of people suffering from type 2 diabetes (see Chapter 2). The prevalence of obesity in England has more than trebled since 1980, with 21% of women and 17% of men being clinically obese (National Audit Office, 2001). The prevalence of obesity is also rising amongst children and has led to the first reports of type 2 diabetes in white adolescents in the UK (Drake et al., 2002). Primary prevention strategies to bring about a continued decline in cardiovascular disease incidence therefore remain a major challenge (Lampe et al., 2001) (see Chapter 13).

1.4.8 The emergence of new risk factors

Although a high serum cholesterol level undoubtedly increases the risk of CHD in an individual, it is nevertheless true that in surveys of people with documented CHD (e.g. those presenting with myocardial infarction), the distribution of serum cholesterol levels does not clearly distinguish those with from those without the disease (Fig. 1.13). This reflects partly the concept of attributable risk discussed previously (see Section 1.4.1): the number of people in the population with particularly high serum cholesterol levels is not great. But it also shows that other factors must underlie the risk of CHD in many people. In fact, although the importance of the major cardiovascular risk factors (elevated blood cholesterol, cigarette smoking and high blood pressure) have been strongly substantiated and it is likely that they account for most cases of heart disease (Magnus & Beaglehole, 2001), it is also likely that other novel risk factors may account for a substantial proportion of cardiovascular disease cases. This has initiated a search for alternative risk factors. In addition, new research on the mechanisms of atherosclerosis has suggested the presence of novel risk factors, such as oxidative stress, in the aetiology of CHD. Understanding of the role of lipid peroxidation in atherosclerosis has led to a search for indicators of oxidative stress that may predict development of CHD (see Chapter 5, Section 5.12). Observations from basic science and epidemiological evidence have prompted interest in the role of antioxidants. However, nutrients with antioxidant properties may also affect cardiovascular risk by other (not directly antioxidant-type) mechanisms of action, such as effects on the immune system, markers of endothelial damage and effects on gene expression and cell signalling. This situation needs to be taken into account in the design of studies (Buttriss et al., 2002a).

1.5 Role of diet

Because of the large variation in risk of mortality from CHD and stroke, it is likely that behavioural risk factors play an important role in the aetiology of these diseases. It is now well recognised that diet, as well as other lifestyle factors (e.g. alcohol consumption, smoking, physical inactivity), is strongly related to several of the main risk factors for cardiovascular disease.

1.5.1 Dietary recommendations to reduce cardiovascular disease

Epidemiological studies have shown a log-linear relationship of increasing risk of CHD with increasing...
levels of total cholesterol, with no threshold value below which a lower level is not associated with lower risk of CHD. Moreover, controlled clinical trials have shown the importance of pharmacological lowering of serum cholesterol levels in reducing cardiovascular mortality (and morbidity) in individuals at high-risk of CHD (Heart Protection Study Collaborative Group, 2002a). Lowering the population mean level of LDL-cholesterol is probably the most important public health strategy to prevent CHD mortality. Whilst most dietary measures to achieve this are less effective than a statin drug, they are likely to have substantial benefit for cardiovascular disease risk as they may impinge simultaneously on several of the risk markers described in this report. There is therefore considerable potential for dietary modification in the primary and secondary prevention of CHD.

As a result of the interest in blood cholesterol levels, dietary recommendations for cardiovascular disease prevention have concentrated primarily on reducing fat intake. However, the focus is no longer simply a need to reduce total fat and saturates intake. Recent research has emphasised the importance of monounsaturates in helping to keep levels of blood cholesterol (and other blood lipids) down, and it is important to note that whilst monounsaturates reduce LDL-cholesterol, they do not reduce protective HDL-cholesterol to the same extent as \( n-6 \) polyunsaturates (see Chapter 11, Section 11.11.1). Research also now indicates that the balance of \( n-3 \) and \( n-6 \) polyunsaturates is important. The \( n-3 \) polyunsaturates, of which oil-rich fish is an important source of the longest chain length members of this group (eicosapentaenoic acid and docosahexaenoic acid), have little effect on blood cholesterol but reduce blood triglyceride levels. There is also evidence for other beneficial effects, for example with regard to blood clotting (see Chapter 6). Former concerns about the \( trans \) fatty acid content of the diet have now largely disappeared in Britain for the majority of people eating a varied diet, since manufacturers have taken steps to reduce the \( trans \) content of margarines and spreads to a minimum.

In 1994, COMA published dietary (food and nutrient) recommendations to prevent cardiovascular disease (Department of Health, 1994) (Table 1.3). These included reducing the average contribution of total fat to dietary energy (i.e. from food and alcohol) in the population to about 35% and reducing the average contribution of saturates to dietary energy to no more than 10%. COMA recommended that average intakes of \( trans \) fatty acids should not increase but made no specific recommendations for monounsaturates. The report recommended that average intakes of \( n-6 \) polyunsaturates need not increase above current levels, and intakes of long-chain \( n-3 \) polyunsaturates (eicosapentaenoic acid and docosahexaenoic acid) should double from 0.1 g/day to 0.2 g/day. Information about dietary sources of these fatty acids can be found in Chapter 13,

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Nutrient recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>Reduce population average to 35% of dietary energy</td>
</tr>
<tr>
<td>Saturates</td>
<td>Reduce to no more than 10% of dietary energy</td>
</tr>
<tr>
<td>( n-6 ) polyunsaturates</td>
<td>No further increase in average intake of ( n-6 ) polyunsaturates</td>
</tr>
<tr>
<td>( n-3 ) polyunsaturates</td>
<td>Increase population average consumption of long-chain ( n-3 ) polyunsaturates from 0.1 g/day to 0.2 g/day (1.5 g/week)</td>
</tr>
<tr>
<td>( Trans ) fatty acids</td>
<td>( Trans ) fatty acids should not provide more than 2% of dietary energy</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>No increase in average dietary cholesterol intake (250–300 mg/day)</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Increase the average proportion of dietary energy derived from carbohydrate to approximately 50%</td>
</tr>
<tr>
<td>Salt (sodium)</td>
<td>Reduce salt intake from 9 g/day to 6 g/day</td>
</tr>
<tr>
<td>Potassium</td>
<td>Increase potassium intake to around 3.5 g/day</td>
</tr>
</tbody>
</table>

Table 13.5. The population was also advised to increase the proportion of dietary energy derived from carbohydrate to approximately 50% and to reduce salt intake by at least one-third from its current level of 9 g/day to 6 g/day (Department of Health, 1994). The practical food-based advice arising from the COMA recommendations for cardiovascular disease prevention is therefore to maintain a healthy body-weight, eat five or more portions of fruit and vegetables each day, reduce intake of fat (particularly saturates), reduce salt intake and eat at least two portions of fish, of which one should be oily fish, each week (see Chapter 11, Section 11.8.3 for an update).

More recent dietary recommendations have been published in the US (American Heart Association, 2000) and also in The Netherlands (Fernandes, 2002) (see Chapter 13, Section 13.3). These have incorporated some of the newer insights into the role of diet in cardiovascular disease risk; for example, they have included specific recommendations to increase the ratio of monounsaturates to other fatty acids (for further discussion of these recommendations see Chapter 13, Section 13.5).

1.5.2 The role of low fat diets in cardiovascular disease prevention

As discussed in Section 1.5.1, most dietary recommendations for cardiovascular disease prevention have included a reduction in total fat intake in order to reduce serum cholesterol concentration. Low fat diets have also been shown, in meta-analyses, to confer benefit in weight reduction programmes (Astrup et al., 2000). This is generally thought to reflect the strong correlation between fat content of the diet and its energy density (kJ/g). However, there has recently been controversy over the benefits of low fat diets. This centres upon the long-standing observation that dietary carbohydrate tends to raise serum triglyceride and depress serum HDL-cholesterol concentrations (when substituted for dietary fat) (see Chapter 3, Section 3.2). Therefore, it is argued, the lowering of serum cholesterol concentration seen on a low fat diet reflects, at least in part, reduction in the beneficial HDL component, and a reduction in HDL-cholesterol together with an elevation of triglycerides could be seen as risk-promoting. It has been argued that it is preferable to concentrate upon changing the nature of dietary fat, for instance replacement of saturates with monounsaturates, rather than reducing fat intake (see Chapter 11, Section 11.11.1). These claims have been highly controversial. The potentially adverse effect of dietary fat restriction may be relatively transient. In the Carbohydrate Ratio Management in European National diets (CARMEN) study, a six-month low fat diet intervention (high in simple or complex carbohydrate) had no adverse effect on serum lipid concentrations and resulted in a modest, but significant, reduction in body weight (Saris et al., 2000). The effect of a low fat diet is particularly marked upon postprandial triglyceride concentrations (discussed further in Chapter 3), at least in the short-term (Koutsari et al., 2001). This may provide the link with lowering of HDL-cholesterol concentrations. However, these observations have been made in laboratory studies in which subjects on either type of diet consume identical test meals. In reality, someone consuming a low fat diet will have a correspondingly lower fat load to dispose of at each meal. Further research is needed in this area to attempt to reconcile the differing interpretations of the effects of low fat diets.

1.5.3 Paradoxes in CHD epidemiology

In most countries, high intake of saturates is positively related to high mortality from CHD. However, the situation in France is paradoxical in that there is high intake of saturates but low incidence and mortality from CHD. In fact, the prevalence of CHD in France is noticeably lower than in most European or English-speaking countries (Fig. 1.3) (premature CHD mortality rates among men are 2.5 times higher in the USA and three times higher in the UK than in France) (British Heart Foundation, 2003a). This peculiarity does not seem to be ascribable to the level of the ‘classical’ coronary risk factors in France (serum cholesterol levels, blood pressure and prevalence of smoking are no lower there than elsewhere) and has been termed the ‘French paradox’ (Renaud et al., 1992). The relative immunity of the French to CHD has been attributed in part to their custom of drinking wine with meals (Criqui & Ringel, 1994). Epidemiological studies indicate that consumption of alcohol at the level of intake in France (20–30 g ethanol per day; 2–4 drinks) can reduce risk of CHD by around 40% (see Chapter 11, Section 11.9.8). Much of this effect has been attributed to the ethanol content per se, but the consumption of wine, especially red wine, may be more favourable than other
alcoholic beverages because of its high content of polyphenols, some of which are absorbed through the gut and have potentially promising antioxidant effects in vitro (British Nutrition Foundation, 2003a). The difference in CHD risk may also reflect a higher fruit and vegetable intake in France (Parodi, 1997). Additionally, it has also been been suggested that the various measures implemented in the early 1900s to improve the nutrition and health of French children may have contributed to the low rates of CHD in later life (Barker, 1999; see Chapter 10).

There are also a number of trends within the UK relating to the prevalence of cardiovascular disease and its risk factors that are worthy of some discussion. Firstly, regional and socioeconomic differences in cardiovascular disease mortality in the UK (see Section 1.3.3) appear to be unrelated to total blood cholesterol level, the dominant diet-related risk factor for cardiovascular disease, or to dietary fat intake. Total blood cholesterol levels show little social class variation in either sex, and there is little difference in the fat or saturates intake between classes. Similarly, when regional differences in consumption of total fat and saturates are examined, no clear pattern emerges and differences between regions are very small (DEFRA, 2001; Fig. 1.14a). There is, however, evidence of other dietary differences. For example, consumption of fresh fruit and vegetables is higher in households with higher incomes and there is a strong north–south gradient for both fruit and vegetable consumption, with people in Scotland, Northern Ireland and the north of England eating considerably less than people in the south of England (Fig. 1.14b). Salt intake also varies by region. These dietary factors may be influencing cardiovascular risk without affecting blood cholesterol levels (see Chapter 13, Section 13.4).

The decline in cardiovascular disease experienced in the UK has also occurred without any corresponding fall in the reported proportion of dietary energy obtained from fat. According to the National Food Survey, total fat intake has fallen by over 30 g/day since the mid-1970s and intake of saturates has dropped by more than 20 g/day over the same period, but the simultaneous decline in the energy content of the diet has meant that there has been relatively little change in the proportion of either to total energy intake (total fat has fallen from around 42% to 38% since the 1970s and saturates from 20% to 15% during the same period) (Fig. 1.15) (DEFRA, 2001).

![Fig. 1.14](image_url) Regional differences in diet in the UK. Reproduced from DEFRA (2001) with permission of The Stationery Office. Crown copyright.

Despite the apparent fall in energy intake in the UK, rates of obesity have continued to rise (see Chapter 2, Section 2.4.3). National Food Survey data suggest a substantial fall in average energy intake of around 130 kilojoules (kJ) since 1975 (DEFRA, 2001), while obesity rates have more than trebled during this period (British Nutrition Foundation, 1999a; National Audit Office, 2001). As obesity can only occur when energy intake is not matched by energy output, this must reflect a decline in average levels of physical activity. Although corresponding data showing trends in physical activity are not available, it is generally believed that physical
activity levels have declined in the UK over the past 20 years. In fact, the UK currently has high rates of physical inactivity compared to other countries in Europe (Fig. 1.16) (British Heart Foundation, 2003a). Initiatives that combat a sedentary lifestyle and provide opportunities to increase physical activity are therefore an essential adjunct to promoting a healthy diet if reductions in the major diet-related cardiovascular disease risk factors are to be achieved. Increasing physical activity in children is also an attractive and non-restrictive approach to obesity prevention (see Chapter 12).

1.6 Structure of the report

The Task Force was asked to review the present state of knowledge of the link between emerging aspects of cardiovascular disease and diet (and related factors). Chapter 2 deals with common themes (e.g. insulin resistance, obesity) that are linked to several risk factors and are therefore consistently referred to throughout the report. Chapters 3 to 10 present the evidence for the role of the major emerging risk factors, providing information about methods of assessment (including the robustness of these
1.7 Key points

- Cardiovascular disease refers to disease of the arteries supplying the muscle of the heart (coronary heart disease), the brain (cerebrovascular disease) and the extremities, especially the legs (peripheral vascular disease). It involves the processes of atherosclerosis (lesions in the arterial wall) and thrombosis (blood clotting), as well as changes to the function of the arterial lining.

- Cardiovascular disease is the leading cause of death worldwide, accounting for around 18 million deaths each year. Around 50% of these deaths are from CHD and a further 25% from stroke.

- The UK has one of the highest mortality rates from cardiovascular disease in the world, with more than one in three people dying from this condition. The disease is also a major cause of illness and disability, including angina and heart attacks.

- Death rates from CHD have been falling in the UK since the early 1970s, while death rates from stroke have declined throughout the latter part of the twentieth century. Both lifestyle modifications and medical therapies have played an important role in this decline.

- Whilst CHD mortality has been falling, surveys suggest that morbidity from CHD is not falling and, in older age groups, may even be rising. This reflects both ageing of the population and the survival of those who would previously have died.

- There are major social, regional and ethnic inequalities in cardiovascular disease morbidity and mortality in the UK, which appear to be widening.

- Conventional risk factors for cardiovascular disease include smoking, raised cholesterol levels, raised blood pressure, physical inactivity, obesity and diabetes. These ‘classical’ risk factors were reviewed in a previous British Nutrition Foundation report. However, these risk factors cannot fully explain the regional, gender, socioeconomic and ethnic differences in cardiovascular disease, and emerging evidence suggests that other novel risk factors may play an important role.

- This Task Force considers some of these novel or emerging risk factors for cardiovascular disease and reviews the potential role of diet in their modification.

1.8 Key references


2.1 Aim of this chapter

The aim of the chapter is to introduce concepts that appear to be of relevance to several of the emerging risk factors detailed in later chapters. Four of these factors,

- insulin resistance
- obesity
- glucose intolerance/diabetes mellitus, and
- hypertension

are strongly inter-related and cluster together in epidemiological studies. Other studies reveal compelling mechanisms that underly these clusters suggesting these are real, biological, links.

This chapter also introduces two other factors that lead to the above inter-related factors and also to other emerging risk factors detailed in the later chapters; these are:

- physical inactivity
- genes.

All six factors will be reprised in later chapters so the topics will be specifically introduced here.

Whilst this chapter focuses primarily on the relationship between these risk factors and cardiovascular disease, a discussion of the impact of diet on their modification can be found in Chapters 11, 12 and 13.

2.2 Overlap of insulin resistance, obesity and physical inactivity

There is a significant tendency for several risk markers to co-segregate across different societies and in different individuals within a single society. The problems that this can cause when one is searching for risk factors and trying to determine their importance has been discussed (Chapter 1, Section 1.4.1). One of the most difficult of these co-segregations to ‘untangle’ is the overlap between insulin resistance, obesity and physical inactivity. The associations between these factors are very strong (correlation coefficients usually exceed 0.7 between individuals in population studies), but we shall initially discuss these factors independently.

2.3 Insulin resistance

2.3.1 Concept/definition of insulin resistance

The responses to insulin vary considerably between different individuals. When the response is muted, this is termed insulin resistance. Insulin resistance has been simply defined as the situation when the response to insulin is less than normal. However, insulin is a pleiotropic hormone with a range of actions occurring in multiple tissues (e.g. glucoregulation, anti-lipolysis, protein synthesis; Table 2.1, Fig. 2.1; Stumvoll & Jacob, 1999). Each action of insulin is determined by both the circulating insulin concentration and the shape of the individual insulin dose–response curve. There are thus multiple possible insulin resistances, for example insulin resistance for glucose uptake in skeletal muscle, insulin resistance for glucose release in the liver, insulin resistance for lipolysis in adipose tissue, insulin resistance for amino acid metabolism in skeletal muscle etc., or any combination. Thus, theoretically, there may be
Metabolic Syndrome

2.3.2 The insulin resistance syndrome

Fig. 2.2 shows the 24 hour profiles of insulin and glucose in two groups of subjects, a lean control group and an obese, hypertensive group. As can be seen, the latter are producing far more insulin to regulate their blood glucose. The fact that they need more insulin to achieve the same levels of glycaemia means that they are insulin resistant.

It has been recognised for several decades that individuals with insulin resistance often have other characteristics, most notably high plasma triglyceride concentrations, low high-density lipoprotein (HDL) cholesterol, hypertension and hyperinsulinaemia (Reaven, 1988). Hypertension is a long established risk factor for cardiovascular disease.
Cardiovascular Disease

A dyslipidaemia characterised by low HDL/hypertriglyceridaemia (see also Section 2.4.7) is generally accepted as a cardiovascular risk factor (discussed more fully in Chapter 3). There remains debate about whether it is independent of co-segregating factors, and whether low HDL-cholesterol and hypertriglyceridaemia are one or two independent

**Fig. 2.1** Insulin actions in peripheral tissues.

![Insulin actions in peripheral tissues](image)

**Table 2.2** Tests for measuring insulin resistance.

<table>
<thead>
<tr>
<th>Test</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma insulin concentrations</td>
<td>Measurement of insulin concentrations under standard physiological conditions (e.g. fasting state) correlates broadly with insulin resistance</td>
</tr>
<tr>
<td>HOMA (HomeOstatic Model Assessment)</td>
<td>An index based upon fasting glucose and insulin concentrations</td>
</tr>
<tr>
<td>Minimal modelling</td>
<td>A model-based method estimating insulin resistance from results of frequently-sampled intravenous glucose tolerance test (fsIVGTT)</td>
</tr>
<tr>
<td>Insulin tolerance test</td>
<td>High dose insulin is given intravenously and the decline of glucose concentration measured over about 20 minutes</td>
</tr>
<tr>
<td>Steady state plasma glucose (SSPG) and variants</td>
<td>A sophisticated method but currently little used</td>
</tr>
<tr>
<td>Clamp methods</td>
<td>This test involves infusing a known amount of insulin. This will necessarily cause the blood glucose to decline. Glucose is then infused to ‘clamp’ or maintain the blood glucose. The amount of glucose that must be infused is a direct measurement of the response to insulin</td>
</tr>
<tr>
<td>(a) hyperinsulinaemic, euglycaemic clamp</td>
<td>The hyperinsulinaemic euglycaemic variant is probably the gold standard method</td>
</tr>
<tr>
<td>(b) hyperglycaemic clamp</td>
<td>The hyperglycaemic variant also allows estimate of insulin secretion</td>
</tr>
</tbody>
</table>

Metabolic Syndrome

Table 2.3  Diagnostic criteria for insulin resistance/metabolic syndrome.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance</td>
<td>Top 25% of population distribution</td>
<td>Top 25% of population distribution</td>
<td>Not considered</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>Not considered</td>
<td>Top 25% of population distribution</td>
<td>Not considered</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>Impaired fasting glucose, or impaired glucose tolerance or diabetes (as defined in Table 2.13)</td>
<td>≥6.1, but not diabetic</td>
<td>≥6.1</td>
</tr>
<tr>
<td>Hypertension (mmHg)</td>
<td>≥160/≥90</td>
<td>≥140/≥90 or on treatment</td>
<td>≥130/≥85</td>
</tr>
<tr>
<td>Central obesity</td>
<td>Waist:hip ratio &gt;0.9 (men)</td>
<td>&gt;0.85 (women) and/or BMI &gt; 30 kg/m²</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>Not considered</td>
<td>≥94 (men)</td>
<td>≥102 (men)</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>&lt;1.0 mmol/l or treated for dyslipidaemia</td>
<td>&lt;1.0 mmol/l or treated for dyslipidaemia</td>
<td>&lt;1.07 mmol/l (men)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&gt;2.0 mmol/l or treated for dyslipidaemia</td>
<td>&gt;2.0 mmol/l or treated for dyslipidaemia</td>
<td>≥1.69 mmolar</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>Present</td>
<td>Not considered</td>
<td>Not considered</td>
</tr>
<tr>
<td>Criteria</td>
<td>One of first two, plus two other features</td>
<td>One of first two, plus two other features</td>
<td>Three of above</td>
</tr>
</tbody>
</table>

BMI: body mass index; EGIR: European Group for the study of Insulin Resistance; HDL: high-density lipoprotein; NCEP ATPIII: 3rd Recommendations of the Adult Treatment Panel of the National Cholesterol Education Program. Values in NCEP definition are approximations of values in mg%. Sources: Alberti & Zimmet (1998); Balkau & Charles (1999); Balkau et al. (2002); Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults (2001).

Risk factors. Even hyperinsulinaemia has been plausibly suggested to be an independent cardiovascular risk factor. This complex of co-segregating risk factors has been labelled as the insulin resistance syndrome (Reaven, 1988). Various definition criteria for the syndrome(s) are shown in Table 2.3. Of these definitions, it should be noted that the World Health Organization (WHO) and the European Group for the study of Insulin Resistance (EGIR) definitions include an attempt to measure insulin resistance, and therefore may define an ‘insulin resistance syndrome’. However, this is difficult to measure in routine practice. In contrast, the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) claims to define a ‘metabolic syndrome’ rather than an ‘insulin resistance syndrome’, but this is based upon parameters routinely available in clinical practice. For this reason alone, the NCEP criteria are being more widely adopted. The syndrome identified by the NCEP ATPIII can be shown to predict those with a 24.5-fold increased risk of subsequent development of type 2 diabetes mellitus (Sattar et al., 2003).

The prevalence of the insulin resistance syndrome varies depending upon which definition is adopted: the NCEP definition includes patients with established diabetes; the EGIR definition specifically excludes these people. Although type 2 diabetes may occur in people who do not have the insulin resistance syndrome or did not have the syndrome previously, this is unusual, so that in general these syndromes are a prodrome to type 2 diabetes (Sattar et al., 2003).

The putative components of the insulin resistance syndrome are indicated in Table 2.4, which divides
the components into those which are established members of the syndrome and those which have been proposed more recently and are not universally accepted to be part of the syndrome. Reaven (1988) and others would argue that insulin resistance is the prime defect underlying this syndrome; hence the name ‘insulin resistance syndrome’. Others who had noted the association were not so sure that insulin resistance was the primary defect and gave the syndrome more non-committal names (Table 2.5). Reaven’s hypothesis is that the failure of insulin’s hypoglycaemic action causes glucose intolerance (which may be severe enough to be type 2 diabetes mellitus). Reaven’s explanation for the whole syndrome is based upon the fact that insulin is a pleiotropic hormone, and that simultaneous problems with several of insulin’s actions generate a syndrome of co-segregating problems. In his view, some actions of insulin (i.e. its hypoglycaemic action, its anti-lipolytic action, its effect on liver very-low-density lipoprotein (VLDL) secretion) are reduced because of insulin resistance whilst simultaneously other actions of insulin (notably those causing hypertension) are increased because of the hyperinsulinaemia.

It has been estimated that having the metabolic syndrome (as defined by either WHO or NCEP, see Table 2.3) increases cardiovascular disease deaths approximately three-fold and total mortality approximately two-fold, even after correction for
Metabolic Syndrome

Table 2.5 Names of syndromes similar to insulin resistance syndrome.

<table>
<thead>
<tr>
<th>Name</th>
<th>Components</th>
<th>Reference (useful reviews)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleurimetabolic syndrome</td>
<td>Obesity, hypertension, hyperlipidaemia and glucose intolerance</td>
<td>Crepaldi (1993)</td>
</tr>
<tr>
<td>Trisyndrome métabolique</td>
<td>Hyperglycaemia, hyperlipidaemia and hyperuricaemia</td>
<td>Camus (1966)</td>
</tr>
<tr>
<td>Deadly quartet</td>
<td>Hypertension, glucose intolerance, hypertriglyceridaemia, upper body obesity</td>
<td>Kaplan (1989)</td>
</tr>
<tr>
<td>Syndrome X, insulin resistance syndrome,</td>
<td>Hyperinsulinaemia, glucose intolerance, hypertension, low HDL,</td>
<td>Reaven (1988)</td>
</tr>
<tr>
<td>Reaven’s syndrome</td>
<td>hypertriglyceridaemia</td>
<td></td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>Fasting hyperglycaemia, hypertriglyceridaemia, low HDL, hypertension,</td>
<td>Expert Panel on Detection Evaluation and</td>
</tr>
<tr>
<td></td>
<td>increased waist girth</td>
<td>Treatment of High Blood Cholesterol in</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adults (2001)</td>
</tr>
</tbody>
</table>

HDL: high-density lipoprotein.

conventional risk factors (Lakka et al., 2002), and that 18% of the total variance of coronary heart disease (CHD)/cardiovascular disease variance is attributable to insulin resistance.

2.3.3 Mechanism(s) of insulin resistance

As indicated in Section 2.3.1, there may be different insulin resistances depending upon which actions of insulin are involved. Similarly, there are probably many different mechanisms of insulin resistance. There are scores of different animal models of insulin resistance, including dozens due to single gene defects or even tissue-specific knockout animals in which a single gene has been removed from a single tissue. Some of the models based upon genetic defects are shown in Table 2.6, whilst Table 2.7 indicates models where the defect is not primarily of genetic origin. Although Tables 2.6 and 2.7 indicate the wide range of factors that can cause insulin resistance in animals, across human populations, the majority (>75%) of the variation can be explained by obesity. Section 2.4.7 discusses the likely mechanisms for the common phenotype of insulin resistance caused by human obesity.

Normal insulin signalling has been extensively studied, and large parts of the elaborate intracellular second messenger system responsible for insulin’s pleiotropic effects are well understood. Some of the major second messenger systems are mentioned in Table 2.7. These insulin second messenger systems are generally similar in all insulin-responsive cells, although some modest differences are found between different tissues. In general, the insulin second messenger systems show redundancy and have multiple downstream effects on both the cytoplasm and nucleus of the cell. The cytoplasmic effects are usually rapid, occurring within minutes or hours, whilst the nuclear actions involve the synthesis of new proteins and may take days to occur.

Of the various mechanisms implicated in insulin resistance, one group of mechanisms apparently involves insulin antagonists of some kind. A wide range of substances, from neurotransmitters such as catecholamines, to hormones such as cortisol and growth hormone, to cytokines such as tumour necrosis factor, can antagonise many of insulin’s actions. When insulin antagonists induce insulin resistance, multiple intracellular insulin responsive enzymes can be affected.

Another group of insulin resistance mechanisms have the common theme of cellular satiety, such that when cells are loaded with excess fuel, either carbohydrate (perhaps by recent over-eating) or lipid (as occurs in many cells in obese individuals), these tissues become insulin resistant. Within cells, there appear to be critical metabolic processes (e.g. accumulation of uridine diphosphate (UDP), glucosamine) that, when saturated, cause multiple changes in cellular enzyme activities that switch off insulin responses.

In contrast, other work, often based on knockout or over-expressing genetically modified mice demonstrates that a single cellular enzyme change can
Cardiovascular Disease

Table 2.6 Candidate mechanisms for the causes of insulin resistance in the insulin resistance syndrome: some genetic candidates from animal studies.

<table>
<thead>
<tr>
<th>Genes related to insulin signalling</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin receptor</td>
<td>Knockout causes diabetes</td>
<td>Kadowaki (2000)</td>
</tr>
<tr>
<td>Insulin receptor substrate-1</td>
<td>IRS-1 knockout causes insulin resistance in muscle and adipose tissue</td>
<td>Clausen et al. (1995); Kadowaki (2000)</td>
</tr>
<tr>
<td>Insulin receptor substrate-2</td>
<td>IRS-2 knockout causes insulin resistance in liver</td>
<td>Clausen et al. (1995); Kadowaki (2000)</td>
</tr>
<tr>
<td>Phosphoinositid 3-kinase, MAP kinase</td>
<td>Each of these knockouts has features of insulin resistance</td>
<td>Mauvais-Jarvis et al. (2002)</td>
</tr>
<tr>
<td>Protein kinase B, protein kinase C, GLUT-4</td>
<td>Knockout mice are hyperinsulinaemic but not diabetic</td>
<td>Eriksson et al. (1992); Kadowaki (2000)</td>
</tr>
</tbody>
</table>

Other genes

| Lipoprotein lipase | Causes some elements of insulin resistance syndrome | Ahn et al. (1993) |
| Leptin and leptin-receptor | Causes obesity and insulin resistance | Silva et al. (1999) |
| FAT/CD36 and other fatty acid binding proteins | Suggested a gene underlying insulin resistance syndrome, but evidence now against this in humans | Aitman et al. (1999) |
| ACE gene | Contributes to insulin resistance | |
| PPARγ | PPARγ heterozygote knockouts remain insulin sensitive on a high fat diet | Katsuya et al. (1995) |
| Neuronal nitric oxide synthase | Knockout mice are insulin resistant at peripheral tissues | Shankar et al. (2000) |
| Endothelial nitric oxide synthase | Knockout mice are insulin resistant at the level of liver and peripheral tissues | Shankar et al. (2000) |

ACE: angiotensin converting enzyme; FAT/CD36: CD36 fatty acid translocase; GLUT: glucose transporter; GSK3: glycogen synthase kinase 3; I kappa B kinase: inhibitor I kappa B kinase; IRS: insulin receptor substrate; MAP: mitogen-activated protein; PPAR: peroxisome proliferator-activated receptor.

induce insulin resistance. Indeed, some very elegant work demonstrates that whole body insulin resistance can be induced by knocking out a single insulin-responsive element in a single tissue (Kadowaki, 2000).

It is thus apparent that there are multiple genetic and acquired factors that can induce insulin resistance and/or some elements of the insulin resistance syndrome. It appears highly likely that not all people with insulin resistance are the same, so there are probably several different forms of insulin resistance in human populations.

The above section emphasises the heterogeneity of the causes of insulin resistance in various (animal) research models. However, in man, as already stated, obesity and low physical fitness account for the large majority of the variance in insulin resistance seen within populations; this common phenotype of insulin resistance due to obesity is linked to vascular disease and other risk factors as indicated above.

2.4 Obesity

Obesity is the topic of a previous report from a British Nutrition Foundation Task Force (British Nutrition Foundation, 1999a). Obesity has an impact upon the behaviour of adipose tissue which is discussed more fully in Chapter 9. This introductory chapter will confine itself to definitions and an overview of obesity in relation to cardiovascular disease. There is no doubt that obesity is a risk factor for cardiovascular disease and increased mortality rates (British Nutrition Foundation, 1999a; Fig. 2.3). Several studies suggest that it is an independent risk
Metabolic Syndrome

Table 2.7 Non-genetic candidate mechanisms for the causes of insulin resistance in the insulin resistance syndrome.

(These mechanisms are not necessarily independent; for example, central obesity and hepatic triglyceride deposition may be commonly caused by high fat diets.)

<table>
<thead>
<tr>
<th>Candidate mechanism</th>
<th>Comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low birthweight</td>
<td>Some evidence for association with insulin resistance syndrome. Major determinants of low birthweight are currently only partially understood, some strong environmental effects</td>
<td>See Chapter 10</td>
</tr>
<tr>
<td>Randle’s glucose:fatty acid cycle</td>
<td>Increases in lipid fuel availability reduces carbohydrate metabolism</td>
<td>Randle et al. (1963)</td>
</tr>
<tr>
<td>Central obesity</td>
<td>Good evidence for association with insulin resistance syndrome, may have some genetic cause, strong environmental effects. Some argue this should be a defining part of the syndrome</td>
<td>Alberti &amp; Zimet (1998); Balkau &amp; Charles (1999)</td>
</tr>
<tr>
<td>Increased triglyceride deposition</td>
<td></td>
<td>Pan et al. (1997)</td>
</tr>
<tr>
<td>(a) Within specific adipose depots</td>
<td>This relates to regional adiposity</td>
<td></td>
</tr>
<tr>
<td>(b) Within hepatocytes</td>
<td>This is also known as NASH, non-alcoholic steatohepatitis</td>
<td></td>
</tr>
<tr>
<td>(c) Within skeletal muscle</td>
<td>Such triglyceride deposition may be either within myocytes or between myocytes Each are related to insulin resistance. Genetic and environmental effects are unclear</td>
<td></td>
</tr>
<tr>
<td>Diet</td>
<td>High fat diets may underlie whole syndrome Diet deficient in certain fatty acids or micronutrients can cause insulin resistance</td>
<td>Purnell &amp; Brunzell (1997); Mayer-Davis et al. (1997)</td>
</tr>
<tr>
<td>Low levels of physical fitness</td>
<td>Good evidence for association with insulin resistance syndrome, may have some genetic cause, very strong environmental effects</td>
<td>Mayer-Davis et al. (1998); Nyholm et al. (1996)</td>
</tr>
<tr>
<td>Histological changes in skeletal muscle</td>
<td>Each associated with insulin resistance syndrome, the relative role of genes and environmental effects is uncertain</td>
<td>Lillioja et al. (1987)</td>
</tr>
<tr>
<td>(a) Increased muscle triglyceride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Decreased capillary density</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(c) Fibre type (more type 2b, fast twitch)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endothelial dysfunction</td>
<td>May reduce insulin transport to its site of action on target cell plasma membrane, but doubtful as to whether this is major cause of insulin resistance</td>
<td>Castillo et al. (1994); Unger &amp; Tremblay (1985)</td>
</tr>
<tr>
<td>Cytokines</td>
<td>Can cause insulin resistance, but physiological relevance is currently uncertain</td>
<td>Hotamisligil (1999)</td>
</tr>
<tr>
<td>Other insulin antagonists</td>
<td>Increased adrenal and sympathetic nervous system tone have long been suggested as being part of the syndrome</td>
<td>Björntorp &amp; Rosmond (2000)</td>
</tr>
<tr>
<td>Polycystic ovary syndrome (PCOS)</td>
<td>Good evidence for association with insulin resistance syndrome in some women. PCOS itself has strong genetic factors</td>
<td></td>
</tr>
</tbody>
</table>

factor, but arguably no study has examined whether it is a risk factor independent of all the other putative components of the insulin resistance syndrome and independent of level of physical activity (see Section 2.7).

2.4.1 Definitions and measurement

The WHO has adopted definitions of adult obesity based upon the body mass index (BMI; weight in kilograms divided by the square of the height in
metres) (Table 2.8; World Health Organization, 1998). Although generally acceptable for epidemiology, this method of defining obesity cannot account for differences in muscle mass between subjects, and it is well known that heavily muscled individuals may fulfill WHO criteria to be labelled obese even though their total and percentage body fat content is low (e.g. most champion heavyweight boxers). This is a limitation with the WHO definition because the ‘co-morbidities of obesity’ are probably more related to the amount of fat tissue that an individual carries than to their total weight. Thus the co-morbidities of obesity are perhaps more accurately described as the co-morbidities of adiposity. With the exception of comparisons between sexes (where female percentage body fat is generally higher than that in males, whilst cardiovascular risk is higher in men) the risk is greater in those with greater percentage body fat. Assessing percentage body fat is thus generally a better indicator of risk than BMI.

### 2.4.2 Body fat distribution, ethnic and gender issues

In obesity, the increased amount of fat is found in adipose depots (which are mostly subcutaneous), as well as in smaller depots within the abdominal cavity and around organs such as heart and kidneys. Excess lipid is also deposited between cells in non-adipose tissues such as skeletal muscle. Furthermore, intracellular lipid increases within non-adipose cells such as skeletal muscle, heart and liver, and can have major impacts upon the metabolic function of the affected tissue. It is not known whether fat accumulation in any specific tissue is more strongly predictive of morbidity and mortality than adiposity in general. In most subjects, accumulation of intracellular lipid broadly parallels total adiposity. Although there are no prospective data, in cross-sectional studies fat deposition within the liver (sometimes called fatty liver or non-alcoholic steatohepatitis or NASH) is associated especially strongly with insulin resistance syndrome metabolic abnormalities.

### Table 2.8 WHO definition of adult obesity by body mass index (BMI) in caucasians.

<table>
<thead>
<tr>
<th>Category</th>
<th>BMI (kg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
</tr>
<tr>
<td>‘Healthy’, ‘normal’ or ‘acceptable’</td>
<td>18.5–24.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>25.0–29.9</td>
</tr>
<tr>
<td>Obese</td>
<td>30.0–39.9</td>
</tr>
<tr>
<td>Morbid obesity</td>
<td>≥40.0</td>
</tr>
</tbody>
</table>

In Asians a BMI of 27 kg/m² is equivalent to a value of 30 kg/m² in other groups. Source: World Health Organization (1998), reproduced with permission.
Another common problem with the BMI definition of obesity is that different racial groups have differing muscularity. For example, South Asians typically have a higher percentage body fat content for a given BMI than Europid or African-Caribbean individuals. In South Asians the propensity to develop the typical co-morbidities of obesity is again more closely related to adiposity than to BMI. In recognition of this problem, the WHO has suggested that the cut-offs of BMI used to define obesity are reduced in South Asian groups (Weisell, 2002).

To further complicate the relationship between obesity/adiposity and ill-health, some studies show that different distributions of fat (in adipose tissue depots and elsewhere) are particularly related to co-morbidities. In particular, there is evidence that ‘android’ (also known as ‘central’, ‘visceral’, ‘upper-body’) obesity is more detrimental than ‘gynoid’ (also known as ‘peripheral’, ‘limb’, ‘lower-body’) obesity. The exact definitions of these variants of obesity depend upon the method used to describe the fat distribution (e.g. tape measure, skin calipers, computed tomography (CT), magnetic resonance imaging (MRI) scanner). For most large studies, only simple measurements such as the circumferences of waist (Table 2.9), hip or thigh can be taken in practice. Although some suggest the waist circumference alone is the best predictor of ill-health, the waist/hip ratio is perhaps the most established of this sort of index of regional adiposity. In some studies, waist/hip ratio or waist measurement is a better marker of risk than BMI, although the reason for this is not clear (see Section 2.4.4) (Rexrode et al., 1998).

Table 2.9 Definitions of risk related to waist size.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Alerting zone’</td>
<td>&gt;94 cm</td>
<td>&gt;80 cm</td>
</tr>
<tr>
<td>‘Action level’</td>
<td>&gt;102 cm</td>
<td>&gt;88 cm</td>
</tr>
</tbody>
</table>

Source: Amended from Lean et al. (1995) with permission from the BMJ Publishing Group.

Patients with polycystic ovary syndrome (PCOS) are a group in whom obesity is especially common. In these women, there is known to be an increased cardiovascular risk, but again obesity exacerbates the problems of PCOS. Weight-loss appears to be especially beneficial to women with PCOS since it improves fertility and reduces the menstrual irregularity and hirsuties of these patients.

None of these ‘special cases’ deny the relationship between adiposity and its cardiovascular complications, but they do change the slope of the relationship between adiposity and cardiovascular disease risk.

2.4.3 Epidemiology of obesity

Obesity is a major health problem and rates have risen dramatically in recent years. In fact, it has been estimated that worldwide there are now more obese individuals than malnourished, with about 22 million obese children under 5 years old (see Section 2.4.10). In Europe, the overall prevalence figures are around 20% in men and 15–25% in women in 2004. The prevalence has increased by around 40% in most countries since the mid-1990s, perhaps reflecting, at least to some extent, a more sedentary lifestyle. In some developing countries there may also have been a concomitant increase in the consumption of energy or fat, but in Western societies, total energy intake has been falling (see Chapter 1, Section 1.5.3). In the UK, the prevalence of obesity has more than doubled in women and tripled in men since 1980 (see Fig. 2.4). In 2002, 22% of men and 23% of women in England were clinically obese and a further 43% of men and 34% of women were overweight (Fig. 2.4).

Obesity is a major risk factor for a number of pathological disorders, including type 2 diabetes, hypertension and atherosclerosis (Table 2.10). On average, each person whose death could be attributed to obesity lost 9 years of life (National Audit Office, 2001). The economic costs of obesity are also
substantial (Table 2.11). It is estimated that, in 1998, obesity accounted for 18 million days of sickness absence and 30,000 premature deaths in the UK. A conservative estimate is that treating obesity cost the NHS at least £0.5 billion in 1998. The wider costs to the economy in lower productivity and lost output could be a further £2 billion each year (National Audit Office, 2001).

The prevalence and the epidemiology of obesity, with a specific emphasis on trends in the UK, has been dealt with very thoroughly in a previous report of the British Nutrition Foundation Task Force on Obesity, as has the aetiology of obesity (British Nutrition Foundation, 1999a).

2.4.4 Obesity and cardiovascular disease

Obesity is a major cause of increased cardiovascular disease in the general population (Garrison et al., 1996). Fig. 2.3 shows the relationship between obesity and mortality (Allison et al., 1999). Because of the increasing prevalence of overweight and obesity in Western populations, the population attributable risk (see Chapter 1, Section 1.4.3 for definition) from obesity is enormous and increasing. Given that obesity is also the major cause of type 2 diabetes and other co-morbidities, it has been estimated that obesity (with its co-morbidities) has a total impact on cardiovascular disease in the general population approximately equal to that of smoking and perhaps exceeding that of low-density lipoprotein (LDL) cholesterol (Allison et al., 1999). In terms of current secular trends, cardiovascular disease is declining as a result of reduced smoking, but the decline is offset by increasing obesity (Hu et al., 2000a; see Chapter 1, Section 1.4.6).


![Major co-morbidities of obesity.](table)

**Table 2.10** Major co-morbidities of obesity.

<table>
<thead>
<tr>
<th>Co-morbidities principally causing morbidity rather than mortality</th>
<th>Osteoarthrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gall stones</td>
<td></td>
</tr>
<tr>
<td>Bladder dysfunction</td>
<td></td>
</tr>
<tr>
<td>Psychological problems including depression, agoraphobia, etc.</td>
<td></td>
</tr>
<tr>
<td>Polycystic ovary syndrome and other reproductive disorders</td>
<td></td>
</tr>
<tr>
<td>Benign intracranial hypertension</td>
<td></td>
</tr>
<tr>
<td>‘Asthma’, breathlessness and non-specific chest pain</td>
<td></td>
</tr>
<tr>
<td>Increased risk with surgical operations</td>
<td></td>
</tr>
<tr>
<td>Lymphoedema</td>
<td></td>
</tr>
<tr>
<td>Superficial infections such as intertrigo</td>
<td></td>
</tr>
<tr>
<td>Low social status, unemployment and social disadvantage</td>
<td></td>
</tr>
<tr>
<td>Low levels of physical fitness</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Co-morbidities indirectly causing mortality, mostly via cardiovascular disease</th>
<th>Obstructive sleep apnoea and other nocturnal hypoventilation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty liver (a.k.a. non-alcoholic steatohepatitis, NASH)</td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td></td>
</tr>
<tr>
<td>Dyslipidaemia (hypertriglyceridaemia, low HDL-cholesterol, small, dense LDL)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
</tr>
<tr>
<td>Thromboembolic disease</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Co-morbidities directly causing mortality</th>
<th>Cardiovascular disease including cardiomyopathies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity-related cancers such as colonic, uterine, ovarian, gall-bladder</td>
<td></td>
</tr>
</tbody>
</table>

HDL: high-density lipoprotein; LDL: low-density lipoprotein.
Although there is little doubt that an increase in BMI or percentage ideal body weight (%IBW) are powerful univariate predictors of morbidity, some workers suggest that visceral obesity is more closely related to excess morbidity. As indicated in Section 2.4.2, visceral obesity is usually measured by the waist/hip ratio or waist measurement. One interpretation of the link between morbidity and high waist/hip ratio is that visceral fat has especially ‘bad’ metabolic actions, such as increasing the flux of non-esterified fatty acids (NEFA) to the liver. Other workers point to data showing that general adiposity is as dangerous as accumulation in any single region. This second school of thought points out that high waist/hip ratio is strongly linked to low physical activity, to recent weight gain and to high alcohol intake (Seidell & Bouchard, 1997). Studies correcting for these factors will be required before the suggestion can be accepted that visceral fat per se has some adverse metabolic effect. This is not to suggest that a large waist circumference is not important; it may be important as an index of physical inactivity (see Section 2.7).

The relationship between body weight and mortality is also complicated by smoking (Allison et al., 1999) (Fig. 2.3). Smoking is associated with reduced obesity. Thus if smokers are included in the data, the BMI versus mortality relationship is relatively diluted. If only non-smokers are considered, then there is a clearly increased mortality in obese subjects. These relationships are shown in Fig. 2.3, which indicates relationships between mortality and BMI in all subjects and in non-smokers only.

### 2.4.5 Cardiovascular disease and other co-morbidities of obesity

Some of the co-morbidities of obesity are strong risk factors for cardiovascular disease. As indicated in Table 2.10 and Fig. 2.5, obstructive sleep apnoea, dyslipidaemia, hypertension and type 2 diabetes mellitus each predispose to cardiovascular disease. The effect of obesity on cardiovascular disease may be largely explained by these other factors (e.g. hypertension) and the effect of obesity per se over and above these factors has not been properly quantified.

#### (i) Sleep apnoea and cardiovascular disease

Obesity is strongly associated with obstructive sleep apnoea and related causes of nocturnal hypoventilation. These conditions strongly predispose to cardiovascular disease. The specific mortality ratios of subjects with sleep apnoea and related nocturnal hypoventilations are increased two- to threefold. The mechanisms for this include increased arrhythmias, heart failure and thromboembolism. The hypoxia of sleep apnoea also provokes a stress response and hence elements of the insulin resistance syndrome including hypertension.

#### (ii) Obesity-induced diabetes and cardiovascular disease

Obesity is commonly complicated by type 2 diabetes mellitus. The induction of insulin resistance by obesity is thought to be the major cause of type 2 diabetes in this situation (see Section 2.3). In epidemiological studies it has long been shown that subjects with mild elevations of blood glucose (who might nowadays be classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT); see Table 2.13) have increased cardiovascular mortality, but many of those analyses did not try to dissect the independent effects of obesity and hyperglycaemia on cardiovascular mortality. In humans (whether obese or not), diabetes mellitus is associated with an increase in cardiovascular mortality of about

### Table 2.11 Economic cost of obesity in selected countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year</th>
<th>Obesity (BMI)</th>
<th>Direct costs</th>
<th>National health care costs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>1986</td>
<td>&gt;29</td>
<td>US$ 39.3 billion</td>
<td>5.5</td>
</tr>
<tr>
<td>USA</td>
<td>1988</td>
<td>&gt;29</td>
<td>US$ 44.6 billion</td>
<td>7.8</td>
</tr>
<tr>
<td>Canada</td>
<td>1997</td>
<td>&gt;27</td>
<td>CDN$ 1.8 billion</td>
<td>2.4</td>
</tr>
<tr>
<td>Australia</td>
<td>1989/90</td>
<td>&gt;30</td>
<td>AS 464 million</td>
<td>&gt;2</td>
</tr>
<tr>
<td>The Netherlands</td>
<td>1981–1989</td>
<td>&gt;25</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>France</td>
<td>1992</td>
<td>&gt;27</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

threefold in men and about fivefold in women (Fuller et al., 1996).

(iii) **Obesity-related hypertension**

(see also Section 2.6)

Obesity is strongly associated with hypertension. About 55% of patients in hypertension clinics are obese and about 50% of morbidly obese patients are hypertensive (Sharma et al., 1999). Having said that, there remains a problem of misdiagnosis of hypertension in obese subjects because of inappropriately sized cuffs, which leads to some patients being mislabelled as hypertensive.

The cause of hypertension in obesity is not fully understood, but several features of obesity are potentially capable of raising blood pressure (Table 2.12). The increased body mass of obesity requires an increase in oxygen consumption and cardiac output. In simple obesity, both oxygen consumption and cardiac output typically increase in proportion to body surface area (Saltzman & Benotti, 1997). The increase in cardiac output is achieved by several mechanisms including increased pre-load and increased cardiovascular sympathetic drive. Blood volume is increased in obesity in proportion to the increased body mass. Because blood volume increases with obesity proportionally more than does cardiac output, even simple obesity is a volume expanded state (Saltzman & Benotti, 1997). This hypervolaemia increases pre-load on the heart. Hormonal regulation of blood volume by atrial natriuretic peptide may be abnormal in obesity. Adipose tissue converts cortisone to cortisol and the expanded adipose tissue mass of obesity may contribute to subtle changes in glucocorticoids that contribute to hypertension. Adipose tissue is believed to secrete factors such as angiotensinogen directly linked to increased hypertension, and leptin may also have a direct effect in inducing hypertension.
Metabolic Syndrome

Table 2.12  Factors contributing to hypertension in obesity and/or insulin resistance.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors arising from adipose tissue</td>
<td></td>
</tr>
<tr>
<td>(relevant especially to obesity)</td>
<td></td>
</tr>
<tr>
<td>Increased cortisone to cortisol conversion</td>
<td>Will increase circulating volume</td>
</tr>
<tr>
<td>Increased leptin secretion</td>
<td>May have various effects to increase blood pressure</td>
</tr>
<tr>
<td>Increased angiotensin release</td>
<td>Raises blood pressure by constricting blood vessels</td>
</tr>
<tr>
<td>Factors arising from central (hypothalamic) abnormalities (relevant to both obesity and insulin resistance)</td>
<td>Raises blood pressure by constricting blood vessels and stimulating the heart</td>
</tr>
<tr>
<td>Increased sympathetic nervous drive to vasculature</td>
<td>Increases blood volume</td>
</tr>
<tr>
<td>Hypothalamic-pituitary-adrenal axis activation</td>
<td></td>
</tr>
<tr>
<td>Factors arising from other co-morbidities (relevant especially to obesity)</td>
<td>May have various effects to increase blood pressure</td>
</tr>
<tr>
<td>Hyperinsulinaemia</td>
<td>Shortage of oxygen raises blood pressure by reflexes</td>
</tr>
<tr>
<td>Hypoxia, especially at night (e.g. obstructive sleep apnoea)</td>
<td>Relevant to both obesity and insulin resistance</td>
</tr>
<tr>
<td>Uncertain mechanism</td>
<td>This will put extra strain on the heart and raise blood pressure</td>
</tr>
<tr>
<td>Endothelial dysfunction (see Chapter 4)</td>
<td>Raises blood pressure by constricting blood vessels</td>
</tr>
<tr>
<td>Increased blood volume</td>
<td></td>
</tr>
<tr>
<td>Increased atrial natriuretic factor (ANF)</td>
<td></td>
</tr>
<tr>
<td>Factors epidemiologically linked with obesity</td>
<td></td>
</tr>
<tr>
<td>Increased salt intake</td>
<td></td>
</tr>
<tr>
<td>Lack of physical fitness</td>
<td></td>
</tr>
<tr>
<td>Psychosocial stress</td>
<td></td>
</tr>
<tr>
<td>Artefactual (relevant especially to obesity)</td>
<td></td>
</tr>
<tr>
<td>Use of undersized manometer cuffs that misread blood pressure</td>
<td></td>
</tr>
</tbody>
</table>

(Mohamed-Ali et al., 1998). Sympathetic nervous system controlled vascular tone is increased in obesity, as reflected by increased urinary noradrenaline secretion, by increased heart rate, and by reduced heart rate variability (Esler & Kaye, 1998). Endothelial function, as determined by post-ischaemic vasodilatation and intimal-media thickness, is abnormal in obesity (Rocchini, 1998) (see Chapter 4). Whether the endothelial abnormalities are attributable to the endocrine or sympathetic nervous system abnormalities mentioned above is not clear. In epidemiological studies, obese patients tend to have high salt intakes (He et al., 1999) and low levels of physical fitness, which are each independently linked to hypertension (Blair & Brodney, 1999).

In summary, it would appear that obese subjects are prone to increased hypertension, but the mechanism(s) of this tendency is not clearly understood. It is likely that there are several of these mechanisms underlying the hypertension of obese subjects. Whether the mechanisms are the same in different obese patients remains to be established.

2.4.6 Clinical manifestations of obesity-related cardiovascular disease

Cardiovascular disease in obese subjects may have a variety of presentations. These include:
- atherosclerotic coronary disease
- cardiomyopathies and heart failure
- arrhythmias and sudden death
- venous thromboembolic disease
- stroke.

Clearly these presentations are not mutually exclusive; many patients may have more than one of these presentations. Atherosclerosis is common in obese subjects and can be shown frequently at post-mortem (Saltzman & Benotti, 1997). However, perhaps because of coexisting cardiomyopathy, or coexisting type 2 diabetes, or perhaps because of low levels of physical exercise, morbidly obese patients often do not present with a ‘classic’ history of exertional angina.

As for the other clinical presentations of heart disease in obesity, the frequent presence of underlying
co-morbidities, such as type 2 diabetes, hypertension, and nocturnal hypoventilation, may contribute to the clinical picture. In particular, diabetes may lead to ‘silent’ ischaemia as well as predisposing to arrhythmias, heart failure and stroke.

Multiple factors contribute to accelerated atherosclerosis in obesity. They are indicated in Fig. 2.5, but may be grouped into those related to insulin resistance, those related to a thrombogenic tendency and those related to pro-inflammatory cytokines (see Chapter 9).

2.4.7 Obesity as a cause of insulin resistance

Amongst the mechanisms causing insulin resistance (listed in Table 2.7) there are several that are caused or exacerbated by obesity, notably:

- increased lipid fuel availability
- sympathetic and hypothalamic pituitary adrenal axis (HPAA) activation
- increased release of adipokines (see Chapter 9).

Each of these mechanisms improves with weight loss. Perhaps the most important mechanism linking obesity and insulin resistance is insulin’s action as an anti-lipolytic hormone. A failure of insulin’s anti-lipolytic action in obesity allows an increase in circulation concentrations of NEFA. Perhaps because of the high NEFA flux, obesity is associated with increased lipid deposition within the liver hepatocytes (known as ‘fatty liver’ or non-alcoholic steatohepatosis, NASH). Fatty liver is even more strongly linked to insulin resistance and to abnormalities of VLDL metabolism than is whole body adiposity.

It has been recognised for several decades that obesity causes multiple abnormalities of lipid metabolism. The increase in the adipose tissue mass may be directly relevant to these abnormalities (Frayn & Coppack, 1992). Excess NEFA release from adipose tissue increases the rate of supply of NEFA into the circulation ($R_a$ NEFA). This appears to cause the liver to increase VLDL secretion. Kissedah et al. (1976) showed there was a strong relationship between $R_a$ NEFA and the hepatic production rate of VLDL.

Furthermore, in postprandial conditions, obese subjects show marked abnormalities of lipid metabolism, especially after a high-fat load. Adipose tissue plays an important role in postprandial lipid metabolism. The enzyme lipoprotein lipase (LPL) is crucial in this situation, and adipose tissue is one of the major (perhaps even the most important) sites of its synthesis. LPL activity is rate-limiting for clearance of circulating lipids. In the postprandial state circulating lipids include chylomicrons from exogenous lipid and endogenous VLDL. Studies have shown that in lean subjects, local clearance of circulating triglyceride increases during the postprandial period (Coppack et al., 1992).

Hypertriglyceridaemia is strongly associated with low HDL-cholesterol concentrations (see Section 2.3.2), and adipose tissue LPL may play a direct role in this link. LPL acts on circulating lipoproteins to remove triglyceride from the particle. However, as triglyceride is removed from the core of the particle, there appear to be concomitant changes in the surface components. It has long been hypothesised that triglyceride clearance from VLDL particles and chylomicrons is linked to accumulation of cholesterol in HDL particles, presumably by direct transfer of surface cholesterol from VLDL and chylomicrons. By mechanisms which are not yet fully understood, the hypertriglyceridaemia/low HDL dyslipidaemia and its associated slow turnover of the VLDL pool alter the conformation of low-density lipoprotein (LDL) in the circulation. Subjects with this dyslipidaemia (including most obese individuals) may have normal total amounts of LDL, but the particles are small, dense LDL enriched with triglyceride. Low HDL, small, dense LDL, hypertriglyceridaemia forms a well-recognised lipid pattern which has been labelled the atherogenic lipoprotein profile (Austin et al., 1990; see Chapter 3, Section 3.2).

Thus, compared to lean subjects, obese subjects show a reduced LPL action as judged by triglyceride clearance, and also by interparticle transfer of apolipoprotein A-I and cholesterol. These defects in interparticle transfer may contribute directly to the formation of atherogenic remnants. This is the most likely candidate mechanism for the dyslipidaemia of obesity, which is especially relevant when fat loads are consumed.

Other aspects of the insulin resistance syndrome in obesity include hyperinsulinaemia provoked by hyperglycaemia causing increased sympathetic nervous system activity and activation of the hypothalamic-pituitary adrenal axis which contributes to the hypertension part of the syndrome (see Section 2.4.5 and Tables 2.7 and 2.12).

Secretion of adipokines is increasingly recognised as a mechanism whereby adiposity induces other
metabolic disorders. Adipose tissue was previously considered to be a rather passive tissue acting merely as a disposal site for excess lipid. However, as discussed in Chapter 9, it is now clear that adipose tissue is a metabolically and endocrinologically sophisticated tissue. Of particular note is the ability of adipose tissue to generate its own differentiation factors. The visceral and subcutaneous adipose tissue depots also differ in terms of activity and secretory function.

The links between obesity and adipokines are discussed more fully in Chapter 9. The quantitative importance of these pro-inflammatory cytokines to the risk of atherosclerotic heart disease remains to be established, and, as yet, there are no therapeutic consequences that have come from their recognition.

2.4.8 Prothrombotic tendency in obesity
(see also Chapter 6)

There is a well recognised increased tendency to thrombosis in obesity (Blaszyk et al., 1999). This may manifest as venous thrombosis such as deep venous thrombosis (DVT), venous insufficiency, superficial thrombophlebitis and pulmonary emboli. Obesity is a strong risk factor for each of these problems. Venous insufficiency following DVT is a considerable source of morbidity in obesity and accounts for a huge health economic burden. Fatal pulmonary emboli are an important and often preventable problem that is particularly seen in obese subjects during their post-operative course. The low level of physical fitness seen in obese subjects may well mask non-fatal pulmonary emboli and lead to a delay in presentation and diagnosis. Venous problems in obesity are caused by hydrostatic problems related to increased intra-abdominal pressure, by immobility and by changes in the clotting tendency seen in obesity.

The changes in the clotting systems in obesity include increased plasma concentrations of fibrinogen, factors VII and VIII, von Willebrand factor and several other clotting factors (see Chapter 6). Conversely, there are reduced concentrations of anti-thrombotic factors such as plasminogen-activator inhibitor-1 (PAI-1). In epidemiological studies, concentrations of these pro- and anti-thrombotic factors show correlations (positive and negative, respectively) with cardiovascular disease.

The mechanisms for these changes in pro- and anti-thrombotic factor concentrations are not clear. Some workers suggest they are responding to triglyceride concentration; others point to correlations with waist/hip ratio. However, as with several other issues in obesity and cardiovascular disease, deconvoluting these co-segregating factors is difficult and at present we recognise the correlations but are less sure of the causes.

2.4.9 Weight cycling

Weight fluctuations occur in both obese and lean humans. A number of harmful effects have been postulated to occur with weight cycling, including increased risk of insulin resistance and type 2 diabetes, and increase in risk factors for cardiovascular disease. Alterations in food intake and dietary preferences, including increased energy intake during weight regain and an increased preference for dietary fat, have been described in some studies in both humans and experimental animals. There is evidence that palatable foods are preferred after deprivation. These alterations in food intake and preferences and food efficiency could lead to increasing body weight and changes in body composition, including an increased percentage body fat and altered fat distribution, reflecting a shift to increased visceral fat and its associated diseases. Increasing visceral obesity has been put forward as an explanation for the correlation between weight loss or weight cycling and cardiovascular disease and overall mortality seen in some epidemiological studies. There is much debate as to the true effects of weight cycling on health, partly because definitions of weight cycling have not been standardised: the magnitude of weight change, the number and duration of the cycles, may affect the outcomes of weight cycling (British Nutrition Foundation, 1999a).

2.4.10 Childhood obesity

Currently, obesity is one of the most common health problems among children and adolescents, with documented increases in prevalence in many countries including the UK (see Chapter 11, Section 11.3 and Chapter 13, Section 13.6). There is some uncertainty about how best to measure obesity in children. These problems notwithstanding, it appears that early onset obesity is a risk factor for obesity and its comorbidities and mortality later in life. Although the cause of obesity in children is similar to that of adults...
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(i.e. an imbalance between energy intake and energy expenditure), it is not known if total body fat or body fat distribution is the main factor responsible for the co-morbidities. In particular, a specific role for the intra-abdominal adipose tissue in childhood, independently from that of total body fat, on morbidity risk in adulthood has not yet been demonstrated. Likewise, the relationships between adult obesity and (i) adiposity at any early age (including during intrauterine growth, see Chapter 10) or (ii) change in adiposity in childhood, both require further study. In addition, varying biological responses in different racial/ethnic groups may contribute differently to overweight, obesity and their co-morbidities. Although differences in gene–nutrient interactions may contribute, the roles of cultural and socioeconomic variables still need to be determined to understand these disparities.

(i) Measuring obesity in children

As for adults, BMI has limitations as an index of adiposity because it does not distinguish between fat and lean mass (see Section 2.4.2). An additional problem in children is that BMI is influenced by height, with tall children being categorised as overweight or obese more easily (at a smaller level of discrepancy between weight and height centiles) than short children (Mulligan & Voss, 1999). However, BMI has been advocated as a practical measure of adiposity for population studies and clinical monitoring, and has performed marginally better than other weight/height indices compared with skinfold measurements and dual energy X-ray absorptiometry (DEXA) in a US population (Mei et al., 2002). The UK reference standards for BMI are based on cross-sectional data from 11 surveys of children in England, Scotland and Wales between 1984 and 1990. An ‘international’ standard has also been created, using pooled data from surveys in Brazil, the UK, Hong Kong, The Netherlands, Singapore and the USA. It defines cut-off levels of BMI in childhood which correspond to the adult cut-off values of 25 kg/m² for overweight and 30 kg/m² for obesity (Cole et al., 2000). Although an attractive instrument, and currently the only one allowing prevalence rates of overweight and obesity to be compared across international boundaries, this standard has been criticised, and as its authors freely admit, needs further evaluation before entering routine use.

(ii) Obesity in children: secular trends

The prevalence of obesity is increasing among children in the UK and worldwide (see Chapter 13, Section 13.6.2) The highest rates of childhood obesity are observed in the USA and eastern and southern European countries. The data suggest an accelerating phenomenon, with the steepest increases occurring since the early 1990s, and an upward shift in BMI for the entire population, not just a longer tail at the upper extremes. The prevalence of obesity among 4–11-year-old children in England and Scotland rose over a period of 20 years to 1.7% and 2.1% (boys) and 2.6% and 3.2% (girls), respectively, in 1994 (Chinn & Rona, 2001; Fig. 2.6). Among children aged less than 4 years in the Wirral (north-west England), 24% were overweight and 9% obese in 1998 compared with 15% and 5% in 1989 (Bundred et al., 2001). Mean BMI has increased in 11–16-year-old UK children by around 2 kg/m² and waist circumference by 5–6 cm between 1977 and 1997 (McCarthy et al., 2003).

(iii) Relationship of childhood obesity to adult cardiovascular risk

More research is needed in children into the development of ‘hidden’ fat (visceral, intra-abdominal, intramuscular and intrahepatic) and ways to measure it in epidemiological studies. Intra-abdominal fat imaged using computed tomography (CT) and DEXA, and intramuscular fat imaged by nuclear magnetic resonance (NMR), are related to insulin concentrations in children (Huang, 2002; Sinha et al., 2002). As in adults, intra-abdominal fat correlates with dyslipidaemia and glucose intolerance in obese adolescents (Goran, 1998). It is not yet clear whether these relationships are independent of total body fat. Small studies correlating anthropometric measurements with CT and DEXA suggest that waist/hip ratio, waist circumference and trunk/extremity skinfold ratios are not good indices of intra-abdominal fat in children, whereas individual central (truncal) skinfold measurements have some value (Goran, 1998).
children dying from non-cardiovascular causes have shown that higher BMI and higher levels of these risk markers are associated with increased atherosclerotic changes (fatty streaks and fibrous plaques) in the coronary vessels (Berenson, 2002). There is a tendency for obese children to become obese adults (Dietz, 1998b), but the majority of obese adults were not obese as children. In the 1946 UK birth cohort, correlations between BMI at the age of 36 years and weight for height at 11 years were 0.45 and 0.51 for men and women, respectively. Among those categorised as obese at 36 years, 51% of men and 24% of women were overweight at 11 years, and 10% of men and 30% were obese at 11 years (Braddon et al., 1986).

BMI and other measures of body fat (e.g. skinfolds) increase from birth until the end of infancy (around 1 year), then fall, reaching a trough in mid-childhood, which generally occurred between the ages of 3 and 8 years. She reported that a younger age at rebound predicted an increased risk of adolescent obesity. An association between early adiposity rebound and adult obesity has since been shown in other populations (Braddock et al., 1986). BMI and other measures of body fat (e.g. skinfolds) increase from birth until the end of infancy (around 1 year), then fall, reaching a trough in mid-childhood, which generally occurred between the ages of 3 and 8 years. She reported that a younger age at rebound predicted an increased risk of adolescent obesity. An association between early adiposity rebound and adult obesity has since been shown in other populations (Braddock et al., 1986).

A number of studies have related obesity in childhood to adult morbidity and mortality. Men and women who had a higher weight or BMI as children are at increased risk of cardiovascular disease (Must et al., 1992; Gunnell, 1998; Eriksson et al., 2001). Typical hazard ratios are around 2.0 for those in the highest, compared to those in the second quartile of childhood BMI (Gunnell, 1998). Similarly, higher weight or BMI in childhood and adolescence predicts an increased risk of adult type 2 diabetes (Colditz et al., 1990; Dietz, 1998b; Forsen et al., 2000; Eriksson et al., 2003) and insulin resistance syndrome (Vanhala et al., 1998; Srinivasan et al., 2002). There is also a higher risk of type 2 diabetes in men and women who had an earlier adiposity rebound (Bhargava et al., 2003; Eriksson et al., 2003).

One interpretation of these findings is that efforts to prevent adult disease should focus on the detection and treatment of obese children. However, this may be missing the point. Abraham et al. (1971) showed in US men that mortality from hypertensive vascular disease was increased in those who were heaviest as adults, but at any level of adult weight, mortality was highest in those who had the lowest childhood weight. In a Finnish study, diabetes, hypertension and CHD were increased in those who had a higher BMI at 11 years, but at any 11-year BMI the risk was highest in those with the lowest BMI in infancy and a low birthweight (Eriksson et al., 2001; Barker et al., 2002). The adverse effects of childhood BMI are, therefore, conditioned by what went before and what goes after. Increased risk of adult disease appears to be associated with an upward change in BMI during and after childhood, more strongly than...
with a high absolute BMI at any single age. This has implications for prevention of disease because the detection of children moving upward across BMI centiles would require serial measurements, and a change in current policy regarding growth monitoring in children; current UK recommendations are for only a single childhood measurement of height and weight, at school entry (Hall & Elliman, 2003) (see Chapter 13, Section 13.6.2 for further discussion of policies to tackle childhood obesity).

2.4.11 Obesity and ageing

Dramatic changes in fat mass and distribution occur throughout the life span. It is thought that the risks of adiposity vary at different ages. Sizes of fat depots peak in middle-age and decline substantially in advanced old age. There is a significant loss of subcutaneous fat predisposing to the development of pressure sores, thermal instability and cosmetic changes. However, while total body fat is reduced, percentage body fat remains unchanged or even increases in old age because of fat redistribution, with fat content being increased in bone marrow, muscle and other sites outside fat depots. Also, more fat is lost from subcutaneous than visceral fat depots after middle-age, with this increase in visceral fat being associated with increased risk of atherosclerosis and type 2 diabetes. Thus, in old age there is less fat in depots not associated with cardiovascular disease and more fat in depots associated with vascular disease.

2.4.12 Reversibility of adiposity-induced abnormalities

The comments about weight cycling (see Section 2.4.9) notwithstanding, it is apparent that the adverse consequences of adiposity are at least partially reversible with weight loss (gall stones being the exception). The effects of obesity on type 2 diabetes and glucose intolerance are almost completely reversible, whilst others such as effects on blood pressure may be only partially or temporarily reversible. Selective mobilisation of visceral adipose tissue in response to a weight loss programme has been noted among viscerally obese patients, this reduction in visceral adipose tissue being associated with improvements in the lipoprotein–lipid profile and insulin sensitivity.

Different complications of obesity improve to varying degrees with weight loss. In the Swedish Obesity Study, patients who underwent bariatric surgery had $20 \pm 16$ kg weight loss, whilst those treated conventionally gained $0.7 \pm 12$ kg over 8 years of follow-up. In the surgical group, diabetes incidence was reduced by more than 96% at 2 years and around 80% at 8 years. Incidence of all obesity-related risk factors and disorders (including hypertension) improved by about 60% at 2 years, the benefits being associated with weight loss *per se* rather than surgery. However, by 8 years most, if not all, the difference in blood pressure between the surgical and conventional groups had been lost and there was no significant improvement in overall mortality (Sjöström *et al.*, 2001; Torgerson & Sjöström, 2001).

2.5 Diabetes mellitus

Diabetes mellitus is defined as a disorder of glucose metabolism (Table 2.13). It affects around 2–4% of adults in the UK. Diagnostic criteria for diabetes and its classification are indicated in Tables 2.13 and 2.14 (Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997; World Health Organization, 1999). Table 2.13 also defines IGT and IFG, the terms referring to an intermediate metabolic stage that causes increased cardiovascular risk, perhaps almost up to the level of full-blown diabetes mellitus. Although, strictly speaking, the term diabetes also incorporates the condition diabetes insipidus (a disorder of urine production), in this report diabetes will refer solely to diabetes mellitus.

2.5.1 Epidemiology

Around 90% of people with diabetes have the type 2 variety. This is the form most frequently caused by obesity, insulin resistance and low physical fitness. It has been estimated that up to 90% of type 2 diabetes is attributable to increased body weight. Type 2 diabetes is rapidly increasing in prevalence in the UK as a result of increasing obesity and increased numbers of people from ethnically susceptible groups, notably South Asians.

An evolutionary biologist (Diamond, 2003) has argued that diabetes is commonest amongst groups who have recently been exposed to an abundant food supply after centuries of famine. In such groups,
there may be a high prevalence of ‘thrifty genes’ that promote weight gain and hyperinsulinaemia in conditions of abundance. In contrast, in populations who have had good food supply for several generations the process of natural selection will have reduced the prevalence of such genes as these genes become deleterious in food-abundant environments.

Of particular note is the increasing development of type 2 diabetes in children; this condition, which was almost unknown 10 years ago, has rapidly increased in incidence. Approximately 50% of teenagers with newly diagnosed diabetes in some UK clinics have type 2 diabetes.

2.5.2 Prevention of type 2 diabetes

Estimates of the prevalence of type 2 diabetes are shown in Table 2.15. The Finnish Diabetes Prevention Study (Tuomilehto et al., 2001) and the US Diabetes Prevention Program (Knowler et al., 2002) both demonstrated that the incidence of diabetes could be significantly reduced over a period of 3 years.
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In the Finnish study, a programme of lifestyle aimed at reducing body weight, total fat intake, and intake of saturates and increasing dietary fibre and physical activity was implemented in a group of obese subjects (mean BMI 31 kg/m², mean age 55 years) with IGT. Over 3.2 years, 23% of the control group developed type 2 diabetes, compared to 11% in the intervention group; lifestyle modification reduced the conversion to diabetes by 58%.

The US Diabetes Prevention Program studied subjects with either IFG or IGT (mean age 51 years, mean BMI 34 kg/m²) over 2.8 years. In the control group, the incidence of diabetes was 11 per hundred patient years. Subjects given metformin showed a reduced incidence of diabetes at 7.8 per hundred patient years. The third group had a lifestyle modification programme aiming at 7% weight loss and 150 minutes of physical activity per week. In this lifestyle modification group, the incidence of diabetes was 4.8 cases per hundred patient years. Again, lifestyle modification reduced conversion to diabetes by 58%.

As the prevalence of diabetes varies with age, the figures provided are based on the prevalence for the whole population if it had a standardised age distribution. Note the low prevalence in groups of European origin, especially those remaining in Europe, and the high prevalence among Pima Indians and urban New Guineans and among Nauruans today. Also highlighted is the higher prevalence in urban and westernised groups compared with their rural or traditional counterparts.

Source: Data from Diamond (2003), with permission from Nature Publishing Group.

Table 2.15  Age-standardised prevalence of type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Population grouping</th>
<th>Region</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europeans</td>
<td>Britain</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Germany</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Australia (1981)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Australia (2002)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>8</td>
</tr>
<tr>
<td>Native Americans</td>
<td>Chile Mapuche</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>US Hispanic</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>US Pima</td>
<td>50</td>
</tr>
<tr>
<td>Pacific Islanders</td>
<td>Nauru (1952)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Nauru (2002)</td>
<td>41</td>
</tr>
<tr>
<td>New Guineans</td>
<td>Rural</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>37</td>
</tr>
<tr>
<td>Aboriginal Australians</td>
<td>Traditional</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urban</td>
<td>23</td>
</tr>
<tr>
<td>Middle East</td>
<td>Yemen, traditional</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Yemenite, Jews in Israel</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Lebanon, westernised</td>
<td>14</td>
</tr>
<tr>
<td>Black Africans</td>
<td>Rural Tanzania</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Urban South Africa</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>USA</td>
<td>13</td>
</tr>
<tr>
<td>Chinese</td>
<td>Rural China</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urban Singapore</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Urban Taiwan</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Urban Mauritius</td>
<td>13</td>
</tr>
<tr>
<td>Asian Indians</td>
<td>Rural India</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Urban Tanzania</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Urban India</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Urban Singapore</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Urban Mauritius</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Urban Fiji</td>
<td>22</td>
</tr>
</tbody>
</table>

As the prevalence of diabetes varies with age, the figures provided are based on the prevalence for the whole population if it had a standardised age distribution. Note the low prevalence in groups of European origin, especially those remaining in Europe, and the high prevalence among Pima Indians and urban New Guineans and among Nauruans today. Also highlighted is the higher prevalence in urban and westernised groups compared with their rural or traditional counterparts.

Source: Data from Diamond (2003), with permission from Nature Publishing Group.
These studies have confirmed previous work, giving convincing evidence that lifestyle modification can significantly slow the conversion of patients with IGT or IFG to diabetes mellitus. Whether subjects can permanently avoid diabetes by lifestyle changes remains to be determined, as does the ‘dose–response’ curve for weight loss and physical activity, and whether subjects with more normal glucose tolerance would benefit comparably.

### 2.5.3 Diabetes and patterns of cardiovascular disease

Diabetes mellitus causes a unique pattern of problems known as microvascular complications. Diabetes also increases the frequency of macrovascular disease, the relative risk being around two- to threefold for men and three- to fivefold for women (Kannel & McGee, 1979; Morrish et al., 2001) (Table 2.16). Microvascular disease presents clinically as retinopathy, neuropathy and renal disease. Macrovascular disease presents as myocardial infarction, stroke and especially peripheral vascular disease; although there may be some minor differences in the pattern of arteries affected, diabetic macrovascular disease closely resembles that seen with other causes of cardiovascular disease. Anti-hyperglycaemic treatment is proven to reduce microvascular complications, but the trend for improvement in macrovascular disease is less certain.

### 2.6 Hypertension

Hypertension is a common and major cause of stroke and other cardiovascular disease. There are many causes of hypertension, including defined hormonal and genetic syndromes, renal disease and multifactorial racial and familial factors.

In most cases where the mechanism of hypertension is understood, the hypertension can be ascribed to one or more of the following:

- sympathetic activation (related either to psychosocial stress, other physiological stress such as hypoxia, or to the action of drugs such as cocaine)
- hypothalamic-pituitary-adrenal activation
- hyperaldosteronism (primary or secondary), and other abnormalities of steroid metabolism (such as is seen in chronic alcohol intake)
- increased circulating blood volume
- endothelial dysfunction which may lead to changes in the production of local vasodilators such as nitric oxide (NO) and of vasoconstrictors (such as endothelin-1 and vasoactive endothelial growth factor, VEGF). Endothelial function may be often impaired by local cytokine production from inflammatory processes
- reduced arteriolar elasticity (which is seen in normal aging)
- renal disease
- combinations of the above.

There are other less common causes of hypertension, such as hypercalcaemia, raised intracranial pressure, hormonal diseases such as phaeochromocytoma, structural arterial abnormalities (e.g. congenital coarctation of the aorta) and inflammatory conditions of the blood vessels such as polyarteritis nodosa.

**Table 2.16** Factors that may contribute to increased vascular disease in diabetes and obesity.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance and associated dyslipidaemia and hypertension</td>
<td>See Tables 2.4 and 2.12</td>
</tr>
<tr>
<td>Microvascular disease</td>
<td>Damage to capillaries, especially detectable in retina and kidneys</td>
</tr>
<tr>
<td>Increased advanced glycated endproducts</td>
<td>Glucose attaches to proteins and denatures them in a series of irreversible reactions</td>
</tr>
<tr>
<td>Postprandial hyperglycaemia</td>
<td>Not seen in obesity without diabetes</td>
</tr>
<tr>
<td>Postprandial hyperlipidaemia</td>
<td>Not seen in obesity without diabetes</td>
</tr>
<tr>
<td>Increased sorbitol metabolism</td>
<td>Seen in obesity</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>Not seen in obesity without diabetes</td>
</tr>
</tbody>
</table>

There are many causes of hypertension, including defined hormonal and genetic syndromes, renal disease and multifactorial racial and familial factors.
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Of relevance to this report, it should be recalled that several of the factors being discussed in this report contribute to hypertension. These factors (which are mostly discussed elsewhere in this chapter) include:

- Obesity (as discussed in Sections 2.4.4 and 2.4.5, Table 2.10)
- Insulin resistance
- Diabetes mellitus
- Low levels of physical fitness
- Psychosocial stress
- High salt intake
- High alcohol intake.

Many of these factors co-segregate, so it is not entirely clear to what extent the factors are acting independently. However, it is clear that removing these factors will often reduce hypertension.

For information about high blood pressure during childhood see Chapter 13, Section 13.6.4.

2.7 Dietary effects on obesity, insulin resistance, diabetes and hypertension

The diet clearly has major impact upon obesity, insulin resistance, hypertension and diabetes. These aspects are discussed more fully in Chapters 11 and 13.

2.7.1 Diet and obesity

Dietary advice to counter obesity is the subject of a previous British Nutrition Foundation Task Force Report (British Nutrition Foundation, 1999a) and is summarised in Chapter 13.

2.7.2 Diet and insulin resistance

Diet can affect insulin resistance via changes in body weight/obesity and fat distribution. However, of particular relevance to this report is evidence that various dietary changes can influence insulin resistance (see Chapter 11, Section 11.6). Regrettably, most of the diet studies that have achieved clear changes in the diet have been only short-term (usually 3–6 months) and/or animal studies. There is thus a dearth of long-term studies of dietary manipulation. There is also recurrent difficulty in achieving isoenergetic substitution of one dietary component with another.

High-fructose diets, high-fat diets and diets with specific fatty acid composition have each been reported to affect insulin resistance directly. It has been suggested, mostly from animal work, that high-fructose or high-sucrose diets can induce the elements of the insulin resistance syndrome (Wolever, 2000). Fructose (the sugar found in fruit) was initially noted to have an effect of causing hypertriglyceridaemia, but has subsequently been reported to cause hyperinsulinaemia and hypertension. Although most of the animal studies are short-term, the effect can be demonstrated for up to 15 months in dogs. Studies in which fructose is increased in the diet are complicated by the need to ensure that total energy intake remains unchanged, which is difficult to ensure in human studies. Given that fructose intake is generally increasing in Western diets, this is a matter of some consequence. However, the present dearth of longer term, human data led recent reviews to conclude that the case was not yet proven (Daly et al., 1997; Goldberg & Stanner, 2001; Elliott et al., 2002).

In contrast, there is no doubt that high-fat diets cause insulin resistance relative to low-fat diets (Storlien et al., 1986; Peter et al., 1997; Kelly, 2000; Mann, 2000; Riccardi & Rivellese, 2000; Vessby, 2000). The difficulty in this sphere is knowing how much of the defect is attributable to the dietary fat per se and how much to increased total energy intake and consequent obesity. The mechanisms by which a high fat diet may induce insulin resistance are not totally clear (Fig. 2.7), but postulated effects include alterations in the physical properties of cell membranes (e.g. membrane fluidity), alterations in insulin receptor binding or activation, and local effects of muscle triglyceride accumulation.

However, not all dietary fats are equally likely to cause insulin resistance. Both animal and human studies suggest that saturated fatty acids have the most deleterious effects on insulin action (Lovejoy, 1999). Certain monounsaturated fatty acids (e.g. palmitoleic acid) and n-6 polyunsaturates also have adverse effects. Trans fatty acids appear to potentiate insulin secretion, at least in the short-term, to a greater degree than cis fatty acids. This may reflect chronic alterations in insulin sensitivity, although more research is needed to confirm this hypothesis in humans and to establish the time-course of this effect. In contrast, isoenergetic substitution of long chain n-3 polyunsaturates (typically fish oils) for
saturated fatty acids, such as palmitate, have been shown to reduce insulin resistance in some studies (British Nutrition Foundation, 1999a; Riccardi & Rivellese, 2000; Vessby, 2000; see Chapter 13, Table 13.5 for details of sources of these fatty acids).

Conjugated linoleic acid (CLA) refers to a group of isomers of the n-6 polyunsaturated fatty acid, linoleic acid (cis18:2 n-6), that occurs naturally in dairy products. CLA has been shown in rodents to have several beneficial effects including anti-cancer, anti-atherogenic and anti-obesity effects (Pariza et al., 2000). However, the evidence is not always consistent and some studies have shown opposite effects (e.g. pro-atherogenic; Rudel, 1999). Studies in obese humans have shown some loss of abdominal fat with CLA supplementation (Risérus et al., 2001). However, when one of the more prevalent isomers, trans10cis12-18:2, was given to middle-aged insulin resistant men, it caused a significant reduction in insulin sensitivity (Risérus et al., 2002a) along with an increase in markers of inflammation and lipid peroxidation (Risérus et al., 2002b). More studies are needed with CLA in humans before any definite conclusions can be reached.

Excess alcohol intake (see Chapter 13 for current recommendations for alcohol consumption) appears to have multiple metabolic effects, including weight gain and direct damage to the pancreatic beta cells. Alcohol can also contribute to hypertriglyceridaemia and insulin resistance.

Some dietary micronutrients have also been implicated in the insulin resistance syndrome. A deficiency of any of chromium, zinc, or vanadium causes insulin resistance, and appropriate mineral replacement may improve insulin resistance in some people (Anderson, 2000). Vitamin D deficiency may be similar, as the vitamin is necessary for adequate insulin secretion (Boucher, 1998). Carnitine deficiency may also induce insulin resistance in some people. However, chromium, vitamin D and carnitine deficiency are not shown to be common inducers of insulin resistance. As a result, testing for these deficiencies, much less supplementation, is not part of routine screening and treatment of people with insulin resistance syndromes. However, if these deficiencies are identified, then they clearly should be treated on their merits.

2.7.3 Diet and diabetes

Dietary advice for diabetes is summarised in Chapter 11, Section 11.5.

2.7.4 Dietary effects on hypertension

Hypertension is related to salt intake and the relevant dietary recommendations are summarised in Chapters 11 and 13.

2.8 Physical activity

Physical activity means all forms of physical movement including activities of daily life and exercise (Table 2.17). Exercise is usually taken to mean those forms of physical activity undertaken either as recreation or with the intention of improving health. Training indicates regular exercise taken either for sporting or health-improving purposes. Inactivity or
low physical activity indicates a generally sedentary lifestyle.

Fitness is the product of training and can be measured by the speed of completing an activity (e.g. running a determined distance). For scientific purposes, fitness is usually measured as aerobic fitness measured by \( \dot{V}O_2 \)max.

Increased physical activity changes body composition, decreasing body fat content and increasing muscle mass. Different sorts of physical activity can have different effects on body composition; for example, endurance training such as long distance running tends to reduce body fat, aerobic conditioning is particularly able to increase cardiovascular fitness and maximum work rate, whilst resistance training such as isometric or weight-training, tends to increase muscle bulk. Whilst it is generally recognised that physical activity can change body composition, the reverse is also true, with obesity leading to reduced physical activity through a variety of mechanisms.

The effects on cardiac risk of lack of physical fitness \textit{per se} and adiposity \textit{per se} are particularly difficult to distinguish, because of this reciprocal causality. As a consequence, when effects are attributed to one, it is common that the other is contributing.

### 2.8.1 Effects of physical activity on cardiovascular disease

In a general sense, it is clear that increased physical activity has important effects to reduce cardiovascular disease. Physical inactivity is associated with insulin resistance and subsequent diabetes (Kriska \textit{et al.}, 2001). Physical fitness has been demonstrated to be a powerful predictor of low rates of subsequent vascular disease (Sandvik \textit{et al.}, 1993; Hedblad \textit{et al.}, 1997; Wei \textit{et al.}, 1999; Laukkanen \textit{et al.}, 2001). Intervention studies that increase physical activity improve the features of the insulin resistance syndrome and reduce vascular problems (Powell & Pratt, 1996; Wannamethee \textit{et al.}, 1998; Hu \textit{et al.}, 2001), development of diabetes (Wannamethee \textit{et al.}, 2000) and death rates (Paffenbarger \textit{et al.}, 1993; see Chapter 12 for more information about physical activity and cardiovascular disease).

The general conclusions from the exercise literature are that, (a) increased physical activity and/or exercise reduce cardiovascular risk factors, and (b) more intense exercise carried out more often and for longer episodes over a more prolonged period of months reduces cardiovascular risk more than less intense exercise. Although ‘more is better’ in general for cardiovascular benefit, caveats should be made about sudden unaccustomed exercise being potentially dangerous, and some exercise may be positively harmful for joints and muscles. Currently there is a dearth of data comparing different modalities of exercise, e.g. football training versus rowing versus distance running versus weight training in a gym (of comparable intensity, frequency and duration).

Physical activity is difficult to measure reliably in free-living subjects, and indeed some work has suggested that recreational physical activity, rather than...
Metabolic Syndrome

2.9 Genetics

The human genetic material (genome), which is present in most cells, codes for about 30,000 genes. Each gene contains the code for the amino acid structure of a single protein. All the genes are encoded within DNA, which itself is made up of a series of deoxynucleotides. The enormous DNA molecules (and their attached supporting framework) are called chromosomes, and each human has 23 pairs of chromosomes, one of each pair coming from each parent; thus there are potentially two alternative codes (alleles) for each gene on the chromosome. These 30,000 or so genes may also produce ‘splice variants’; this increases the number of protein variants to about 100,000. The structure and function of all these proteins and splice variants depends upon the genetic code within an individual’s DNA.

Some of the risk factors in this report are ‘emerging’ because of secular or temporal trends in which progressive changes in an environment affect a population; for example, the progressive increase in adiposity in Western society (see, e.g., Fig. 2.4). However, within a population at a given time, it is often genetic variations that determine which individuals will be most affected. Genetic factors can mean that one individual will be more obese than another whatever environment they share. Thus the study of newly emerging risk factors may involve both understanding changes in the environment as well as the genes that determine which individuals are likely to be most affected. This process is referred to as a gene–environment interaction.

Susceptibilities to cardiovascular disease and regulation of metabolic events in relation to food intake have been shown to be genetically regulated. The inter-individual variability is likely to depend on differences in the genetic code between people. The genome is variable and within almost every gene a certain degree of genetic variability can be detected, most of which is due to single nucleotide polymorphisms (SNPs), although deletions and insertions of deoxynucleotides are also seen. Rare forms of

Table 2.18 Effect of physical activity on cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Effect of increased physical activity</th>
<th>Effect independent of adiposity and/or insulin resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiposity</td>
<td>Reduced</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Reduced</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Reduced</td>
</tr>
<tr>
<td>Lipid profile (see Chapter 3)</td>
<td>Improved (increased HDL)</td>
</tr>
<tr>
<td>Endothelial function (see Chapter 4)</td>
<td>Improved</td>
</tr>
<tr>
<td>Coagulation-related factors (see Chapter 6)</td>
<td>Improved</td>
</tr>
<tr>
<td>Inflammation (see Chapter 7)</td>
<td>Reduced inflammatory markers</td>
</tr>
<tr>
<td>Adipose derived factors (see Chapter 9)</td>
<td>Improved</td>
</tr>
<tr>
<td>Oxidative stress (see Chapter 5)</td>
<td>Potentially increased in the short-term</td>
</tr>
<tr>
<td>Homocysteine (see Chapter 8)</td>
<td>Not affected</td>
</tr>
</tbody>
</table>

employment-related physical activity, is more closely related to changes in weight.

Perhaps because of the difficulties in measuring physical activity, there has been much debate about the nature of the ‘dose–response’ curve between how much benefit accrues from increasing exercise. Larger increases in physical activity have greater effects on body composition and cardiovascular fitness (Haskell, 1994). However, exercise protocols involving frequent high-intensity exercise have high drop-out rates unless intensive support and encouragement are provided.

Physical training has multiple effects upon various tissues. Skeletal muscle mass increases, with changes in muscle fibre type, increased capillary density, increased glycogen content, redistributed intramyocytic triglyceride and induction of various enzymes related to oxidative fuel metabolism. Cardiac muscle may hypertrophy with similar vascular and enzymatic changes. Adipose tissue mass declines, but there is an increased ability to mobilise and utilise lipid fuel (cf. carbohydrate fuels). Physical activity has multiple beneficial effects on emerging cardiovascular risk factors. Some of these are indicated in Table 2.18. They are discussed in more detail in subsequent chapters, and the role of physical activity in cardiovascular disease prevention is dealt with in detail in Chapter 12.
Cardiovascular Disease

Diet and nutritional effects on cardiovascular disease can largely be seen in the context of gene–environment interactions. Metabolic stress, such as obesity or an unbalanced diet, may reveal dysfunction in a susceptible gene which contains variants with different functional properties. For example, the genetic defects causing familial hypercholesterolaemia have minimal clinical impact in environments with low-fat diets such as rural China. Other examples of this are given throughout this book. In a more direct way, genes and diet can interact when some dietary component switches on genes; for example, certain fatty acids or their derivatives activate genes involved with adipocyte differentiation and behaviour.

2.10 Summary

Some common concepts have been introduced which will be elaborated upon in subsequent chapters. One of the messages from this chapter is that the complex of obesity, low physical fitness, insulin resistance and type 2 diabetes forms an inter-related spectrum of problems which underlies a large, and increasing, burden of cardiovascular disease.

Further research needs to help address obesity and low physical fitness within the population, and to focus on which elements most directly predispose to cardiovascular disease. Subsequent chapters will reiterate the ways in which insulin resistance and obesity relate to emerging risk factors for heart disease. The practical steps to ameliorate these problems are outlined in Chapters 11, 12 and 13.

2.11 Key points

- Insulin resistance, or the relative inability of insulin to facilitate the disposal of glucose in tissues, is a risk marker for diabetes and cardiovascular disease.
- Insulin resistance can be caused by several genotypes, by several other environmental conditions and by various pathological processes.
- Insulin resistance can contribute to several different phenotypes.
- Multiple cardiovascular risk factors co-segregate with insulin resistance to form a ‘metabolic’ or ‘insulin resistance’ syndrome.
- Obesity is rapidly increasing and is a major factor in the aetiology of type 2 diabetes, insulin resistance, hypertension and cardiovascular disease.
- Diabetes is increasing as a consequence of increased obesity and/or low physical activity.
- Obesity, insulin resistance, low physical activity and diabetes are major contributors to many of the emerging risk factors discussed in later chapters.
- The main clinical message is that to avoid these risk factors, people should stay slim and be physically active throughout life.
2.12 Key references


Lipid-related cardiovascular risk factors have attracted enormous attention over the past years, and consensus documents have been produced to implement treatment and preventive strategies. Essentially, this applies to the conventional risk factors such as high plasma total and low-density lipoprotein (LDL) cholesterol, low plasma high-density lipoprotein (HDLLDL) cholesterol and elevated plasma triglycerides (Wood et al., 1988). The related apolipoproteins, apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I), have also been discussed in this context and it has been argued that apoB might serve as an even better risk marker than LDL-cholesterol (Sniderman, 1992; Lamarche et al., 1998; Wallidius et al., 2001; Sniderman et al., 2003). However, issues of laboratory standardisation and the fact that our knowledge of the benefit of cholesterol reduction derives from large-scale studies in which the apolipoproteins have not been employed, restrict the use of the apolipoproteins for this purpose. The principal functions of the major apolipoproteins are described in Table 3.1.

Despite the well-documented benefit in terms of cardiovascular risk reduction after lowering these conventional lipid-related factors, they only predict a fraction of overall cardiovascular disease (see Chapter 1, Section 1.4.8). Accordingly, there is a search for additional factors that can add to this very incomplete equation. Interestingly, many of the emerging factors are directly related to plasma triglycerides, a conventional risk factor for which it has been inherently difficult to show independent associations with cardiovascular disease. This chapter will discuss postprandial lipaemia, remnant-like particles, small, dense LDL particles, and the distribution of HDL subfractions, which are all intimately related to plasma triglycerides, but on their own may contribute to, or partially explain, the enigmatic behaviour of triglycerides in previous large-scale clinical studies (Fig. 3.1). It is thought that these triglyceride-related factors are directly related to the atherosclerosis process, which cannot be the case for triglycerides as such, as it is the cholesterol content of the triglyceride-rich lipoproteins that can be retained in the arterial wall. Other factors dealt with in this chapter are lipoprotein(a) and free fatty acids. Finally, some comments will be made about the genetic determinants of the plasma lipid response to dietary intervention.

Two general and comprehensive reviews on lipoproteins, nutrition and heart disease have recently been published (Mann, 2002; Schaefer, 2002). Focusing

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-100</td>
<td>(1) Structural protein for VLDL</td>
</tr>
<tr>
<td>(2) Contains ligand to the LDL receptor</td>
<td></td>
</tr>
<tr>
<td>B-48</td>
<td>Structural protein for chylomicrons</td>
</tr>
<tr>
<td>A-1</td>
<td>Enables cholesterol transport from peripheral tissues</td>
</tr>
<tr>
<td>E</td>
<td>Contains ligands to the LDL receptor and remnant receptors</td>
</tr>
</tbody>
</table>

LDL: low-density lipoprotein; VLDL: very-low-density lipoprotein.
on the novel lipid-related risk factors mentioned above, this chapter will discuss the extent to which they can be modified by diet and nutrition. A summary of the relationships between cardiovascular disease, diet and the novel lipid-related risk factors is shown in Table 3.2.

Table 3.3 lists the most common method(s) of assessment for the emerging lipid-related factors described.

### 3.1 Atherogenic lipoproteins

A number of factors determine whether a cholesterol-containing lipoprotein particle resident in plasma has atherogenic properties. The size of the particle determines the ease by which the endothelium can be penetrated; small particles are more likely to be deposited in the arterial wall than large particles. The binding to the subendothelial matrix is also dependent on size, in that the smaller particles bind more avidly to proteoglycans (Anber et al., 1996, 1997). The apoB protein, present as one molecule per lipoprotein particle, seems to be crucial. Firstly, lipoprotein particles without apoB are not atherogenic; secondly, apoB has multiple proteoglycan binding domains which enhance the retention of the particle in the subendothelial matrix (Skålen et al., 2002). Finally, physicochemical and compositional characteristics, such as resistance factors against oxidative stress, are likely to be important in reducing the modification of lipoprotein particles. Oxidative modification of apoB-containing lipoproteins converts them to a palatable substrate for macrophage uptake and foam cell formation, the mechanisms of which are described in detail in Chapter 5. Important factors other than those intrinsic to the lipoprotein particle are endothelial function (see Chapter 4) and the regulation of the vascular inflammatory responses (see Chapter 7). Obviously, in most cases, and in most segments of arteries, the influx and retention of plasma cholesterol is balanced by a cholesterol removal process that operates in the opposite direction, i.e. a ‘reverse cholesterol transport’ pathway involving the non-atherogenic lipoprotein HDL. The regulation of this process is poorly defined and the plasma concentration of HDL is probably only reflecting some aspects of the activity of this system. Table 3.4 compares some characteristics of the major atherogenic lipoproteins in plasma, most of which will be discussed in the following sections.

### 3.2 Postprandial lipaemia – atherogenic lipoprotein phenotype

We spend most of our lives in the postprandial (non-fasting) state, but assessment of cardiovascular risk is traditionally based on measurements made in...
Cardiovascular Disease

Table 3.2  Summary of the relationship between cardiovascular disease, diet and novel lipid-related risk factors.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Strength of association with CHD</th>
<th>Effect on other risk factor pathways</th>
<th>Modulation by dietary factors</th>
<th>Evidence of effect on cardiovascular disease with dietary changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postprandial hypertriglyceridaemia</td>
<td>++</td>
<td>Endothelial function</td>
<td>Effectively reduced by n-3 fatty acids</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coagulation/haemostasis</td>
<td>Modulated by dietary CHO content</td>
<td></td>
</tr>
<tr>
<td>Remnant-like particles</td>
<td>++</td>
<td>Endothelial function</td>
<td>Effectively reduced by n-3 fish oils</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Modulated by dietary CHO content</td>
<td></td>
</tr>
<tr>
<td>Small, dense LDL</td>
<td>+++</td>
<td>Endothelial function</td>
<td>Effectively reduced by n-3 fish oils</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Modulated by dietary CHO content</td>
<td></td>
</tr>
<tr>
<td>HDL subfractions</td>
<td>++</td>
<td>Antioxidant protection</td>
<td>Effectively reduced by n-3 fish oils</td>
<td>(+)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Modulated by dietary CHO content</td>
<td></td>
</tr>
<tr>
<td>NEFA</td>
<td>(+)</td>
<td>Endothelial function</td>
<td>High CHO, in particular high sucrose, diet reduces</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>diurnal NEFA</td>
<td></td>
</tr>
<tr>
<td>Lp(a)</td>
<td>++</td>
<td>Haemostasis?</td>
<td>Trans fatty acids may increase</td>
<td>0</td>
</tr>
</tbody>
</table>

+++; very strong association; ++, strong association; +, some association; (+), weak association; 0, strength of association unknown.

CHO: carbohydrate; HDL: high-density lipoprotein; LDL: low-density lipoprotein; Lp(a): lipoprotein(a); NEFA: non-esterified fatty acid.

Table 3.3  Methods used to determine lipid-related cardiovascular risk factors.

<table>
<thead>
<tr>
<th>Lipid-related factor</th>
<th>Common method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postprandial lipaemia</td>
<td>Repeated plasma measurements of triglycerides after a standardised oral provocation of fat or a mixed meal</td>
</tr>
<tr>
<td>Remnant-like particles</td>
<td>Immunoseparation of lipoproteins in plasma in combination with enzymatic measurement of the cholesterol concentration</td>
</tr>
<tr>
<td>Small, dense LDL</td>
<td>Ultracentrifugation or polyacrylamide gradient gel electrophoresis or NMR spectroscopy of plasma</td>
</tr>
<tr>
<td>HDL subfractions</td>
<td>Ultracentrifugation or polyacrylamide gradient gel electrophoresis or NMR spectroscopy of plasma</td>
</tr>
<tr>
<td>NEFA</td>
<td>Enzymatic methods available for routine biochemistry</td>
</tr>
<tr>
<td>Lipoprotein(a)</td>
<td>ELISA</td>
</tr>
</tbody>
</table>

ELISA: enzyme-linked immunosorbent assay; HDL: high-density lipoprotein; LDL: low-density lipoprotein; NEFA: non-esterified fatty acid; NMR: nuclear magnetic resonance.
Lipid-Related Factors

fasted blood samples. It has been argued that post-prandial lipid and lipoprotein levels in plasma would better reflect the diurnal pattern. In addition, post-prandial lipaemia is a state in which atherogenic lipoproteins are generated, such as chylomicron and very-low-density lipoprotein (VLDL) remnants. Furthermore, the enhanced triglyceride flux through plasma, in particular if it is repeated and exaggerated several times a day, week after week, is a driving force for modification of otherwise stable lipoproteins such as LDL. The formation of small, dense LDL, which is likely to be the truly atherogenic LDL particle, is critically dependent on the presence of triglyceride-rich lipoproteins. HDL-cholesterol is also depleted in the presence of triglyceride-rich lipoproteins, and if the lipolytic removal of post-prandial lipoproteins is sluggish, fewer building blocks for de novo synthesis of HDL components are formed. Pathologically elevated postprandial lipaemia should therefore be seen in the context of the atherogenic lipoprotein phenotype (ALP), which comprises an elevated plasma triglyceride level, low HDL-cholesterol and an abundance of small, dense LDL particles (Fig. 3.2). In essence, a healthy lipoprotein transport system keeps the cholesterol in the short inner loop.

Table 3.4  Typical plasma concentrations and approximate residence time in plasma of lipoproteins suggested to have atherogenic properties.

<table>
<thead>
<tr>
<th>Lipoprotein particle</th>
<th>Typical concentration</th>
<th>Residence time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ApoB (mg/l)</td>
<td>Cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Small, dense LDL</td>
<td>50–250</td>
<td>0.2–1.0</td>
</tr>
<tr>
<td>IDL and VLDL remnants</td>
<td>20–100</td>
<td>0.05–0.5</td>
</tr>
<tr>
<td>Chylomicron remnants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>2.0–10</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>Large</td>
<td>0.2–1.0</td>
<td>0.01–0.1</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>1.0–50</td>
<td>0.01–0.5</td>
</tr>
</tbody>
</table>

ApoB: apolipoprotein B; IDL: intermediate-density lipoprotein; LDL: low-density lipoprotein; Lp(a): lipoprotein(a); VLDL: very-low-density lipoprotein.

Fig. 3.2  Major lipoproteins in plasma responsible for cholesterol transport. Very-low-density lipoproteins (VLDL) are produced in the liver to transport triglycerides, cholesterol and fat soluble vitamins from the liver to peripheral tissues. The triglyceride content of the lipoprotein particle is liberated by lipoprotein lipase in muscle and adipose tissue. The triglyceride-poor remnant, called low-density lipoprotein (LDL), retains the cholesterol and is subsequently removed from plasma by LDL receptors, primarily located in the liver. A prolonged residence time of VLDL in plasma due to impaired removal of the triglycerides enhances the cholesterol content of the particle and a VLDL remnant is formed instead of LDL. This particle is potentially atherogenic. A prolonged residence time of LDL due to slow removal via LDL receptors exposes the particle to multiple compositional modification processes by which small, dense LDL is formed. This particle is thought to be highly atherogenic and is poorly recognised by the LDL receptor. Cholesterol in peripheral tissues can be returned to the inner loop by high-density lipoprotein (HDL). In essence, a healthy lipoprotein transport system keeps the cholesterol in the short inner loop.
as described below. Many of the emerging cardiovascular lipid-related risk factors discussed in this chapter are closely metabolically linked and fall under the umbrella of the ALP. In fact, exaggerated postprandial lipaemia, the presence of small, dense LDL, remnant lipoproteins and small and lipid-poor HDL is often seen in the same individual. This dyslipidaemia is also closely linked with an insulin resistant state and abdominal obesity, and has been described as the dyslipidaemia of the metabolic syndrome (see Chapter 2, Section 2.3). Lipoprotein(a), which is described in Section 3.7, has a completely separate regulation.

A large number of case-control studies have shown exaggerated postprandial lipaemia in people with CHD compared with controls (Karpe, 1999). Most often, the findings have remained significant after adjustment for fasting plasma triglycerides, which are normally higher in CHD cases than in healthy control subjects. In addition, cohort studies have consistently shown positive associations between markers of postprandial lipaemia and degree or severity of cardiovascular disease.

The postprandial lipoproteinaemic response after acute fat feeding is highly modifiable by the long-term diet. The most pronounced effects are observed when switching the macronutrient composition from low fat to high fat and when long-chain \( n-3 \) fatty acids become a significant component of the diet.

3.2.1 The effect of low fat diets

Low fat diets that are high in carbohydrate have a tendency to increase fasting plasma triglycerides; acute fat ingestion also raises postprandial triglycerides, particularly when background dietary carbohydrate intake is high (Parks, 2001). The exact mechanism is poorly understood, because both reduced catabolism of triglycerides in peripheral tissues, as well as increased production of triglyceride-rich lipoproteins, have been demonstrated (Parks et al., 1999). The health implications of low fat/high fat diets are discussed further in Chapter 11, Section 11.7. In the context of previous discussion about the relationship between HDL-cholesterol and postprandial lipaemia, HDL is often lowered by low fat diets. These effects may stand out as negative in terms of cardiovascular risk profile; however, they are likely to be more than balanced by the benefits of low fat diets, such as lower total plasma cholesterol levels and reduction in body weight (Price et al., 2000; Yu-Poth et al., 1999).

It should also be stated that most of the studies that have compared high fat, low carbohydrate diets and low fat, high carbohydrate diets, with respect to their relative effects on fasting and postprandial blood lipids, have used extreme levels of fat or carbohydrate intakes over relatively short periods. Most have compared fat intake levels of 45–50% of dietary energy (high fat) with 20–25% of dietary energy (low fat). It is unclear whether potentially adverse effects of high carbohydrate diets on fasting and postprandial triglycerides are seen at more realistic levels of fat and carbohydrate intake.

In addition, although a few long-term studies suggest that the potentially adverse effects of high carbohydrate diets on triglycerides are independent of the type of carbohydrate fed, the data are sparse and diets have generally been poorly defined with respect to carbohydrate quality. There is, therefore, a need for comparative studies investigating the effects of different carbohydrate sources in the long-term diet on the postprandial lipoproteinaemic response. The postprandial hypertriglyceridaemic effect of a low fat diet can be counteracted by moderate physical exercise (Koutsari et al., 2001) or by inclusion of long-chain \( n-3 \) polyunsaturates in the background diet (Roche & Gibney, 1999).

3.2.2 The effect of fatty acid type

The long-chain \( n-3 \) polyunsaturates found in fish oils, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reduce both fasting and postprandial triglyceride concentrations (Harris et al., 1988; Brown & Roberts, 1991; Williams et al., 1992; Zampelas et al., 1994; Agren et al., 1996; Roche & Gibney, 1996; Minihane et al., 2000; Volek et al., 2000). This effect on plasma lipid profiles is likely to be one of the most prominent nutritional modulators of postprandial lipaemia, and it has therefore attracted a lot of attention. However, most studies have used supplements containing relatively high levels of long-chain \( n-3 \) polyunsaturates (2–4 g/day). Few studies have demonstrated triglyceride-lowering effects of long-chain \( n-3 \) polyunsaturates at intake levels of less than 1 g/day. Information about the amount likely to be provided by diet can be found in Chapter 13, Table 13.5. A comprehensive review on this topic has been published by Roche & Gibney.
(2000). Early studies employing stable isotopically labelled substrate have demonstrated marked reduction in both hepatic apoB-100 and triglyceride synthesis and VLDL secretion in subjects following fish oil treatment (Nestel et al., 1984). More recently, prominent induction of adipose tissue lipoprotein lipase messenger RNA (mRNA) and activity has been demonstrated after fish oil administration, suggesting potent effects of these fatty acids on pathways of both VLDL secretion and clearance (Khan et al., 2002). The effects of saturates, monounsaturates and n-6 polyunsaturates are less marked, and not all the potential effects have been studied in detail. Comparison of postprandial responses in subjects habituated to background diets rich in either saturates or n-6 polyunsaturates, has shown postprandial triglyceride responses to be attenuated when the background diet is high in n-6 polyunsaturates (Weintraub et al., 1988; Bergeron & Havel, 1995). Although the triglyceride-lowering effects are not as marked as when long-chain n-3 polyunsaturates are fed, the n-6 fatty acids have been shown to reduce the accumulation of VLDL particles in the postprandial state (Bergeron & Havel, 1995).

Other effects of background diet on postprandial lipoproteins may be more subtle; for example, diets rich in saturates that lead to an increase in LDL- and HDL-cholesterol may induce increased transfer of cholesterol to VLDL and chylomicron remnants in the postprandial state and, by this effect, qualitatively change the nature and potential atherogenicity of the remnants of triglyceride-rich lipoproteins. Several recent studies have used the monoclonal anti-apoB antibody JI-H to solve this problem was to use a compositional-chemical definition based on the density of the lipoprotein species in question. Several recent studies have recently been reviewed elsewhere (Karpe, 2002).

3.3 Remnant-like particles

Triglyceride-rich lipoproteins (TRLs) are heterogeneous and it is unlikely that all forms have similar atherogenic properties. A better identification of the potentially atherogenic subpopulation of TRLs, mostly considered to comprise the slowly metabolised remnants of TRLs, would therefore be useful in clinical and epidemiological studies. It has long been argued that remnants of TRLs are atherogenic, but one of the problems has been that there is no consensuss definition of remnant lipoproteins, nor is there a standardised method to quantify remnant lipoproteins in plasma. Unequivocal demonstration of the role of remnant particles as a risk factor is therefore limited by lack of ability to measure concentrations in large-scale studies. One recent attempt to solve this problem was to use a compositional-biological definition rather than the classic physicochemical definition based on the density of the lipoprotein species in question. Several recent studies have used the monoclonal anti-apoB antibody JI-H
for this purpose (Nakajima et al., 1996). The initial observation showed that the properties of the JI-H antibody exclude binding of chylomicrons and chylomicron remnants. In addition, apoB-100 containing TRLs with an excess of apolipoprotein E (apoE) seem to be excluded from binding to the antibody. This is of interest as abundance of apoE on the surface of lipoproteins is a signature of remnant characteristics. Accordingly, the lipoproteins that are not bound to the JI-H antibody are likely to comprise an estimate of lipoproteins of different origin but with overlapping remnant characteristics. The collective notion is described as remnant-like particles (RLP). Fig. 3.3 describes the principles behind isolation of RLP.

The plasma concentration of RLP cholesterol is closely associated with plasma triglycerides, and it is therefore of importance to find out whether RLP cholesterol adds anything over and above the simple measurement of fasting plasma triglycerides. Two recent studies suggest that this might be the case (Karpe et al., 2001; McNamara et al., 2001), but the quantification of RLP in large-scale prospective intervention studies, preferably with hard endpoints (e.g. cardiovascular disease events), has yet to be done.

It is possible to measure RLP cholesterol directly in plasma, and the assay is therefore applicable for large-scale studies. There is no need for ultracentrifugation; the procedure involves incubation of plasma with the JI-H antibody and an anti-apoA-I antibody bound to Sepharose, with subsequent determination of unbound cholesterol. Frozen plasma can be used, but it has to be stored at −70°C. The two main disadvantages with the RLP methods are that some potentially atherogenic remnants are not readily detected by the antibody exclusion technique, whereas some other minor lipoprotein species containing neither apoB nor apoA-I are included despite the fact that they bear no resemblance to remnant lipoproteins (Marcoux et al., 1998).

RLP cholesterol quantified in fasting plasma is primarily a measure of large-size apoB-100 (VLDL) particles (Campos et al., 1992a) and is thus not directly related to retention of chylomicron remnants. If quantification of RLP cholesterol is attempted in postprandial samples, a certain proportion of the slightly elevated concentration observed derives from cholesterol in apoB-48 lipoproteins (chylomicrons and chylomicron remnants) (Karpe et al., 2001).

RLP cholesterol is elevated in various hyperlipidaemic states (Marcoux et al., 1999) and the level is normally higher in people with CHD than in controls (Marcoux et al., 1999; Twickler et al., 2000; Deighan et al., 2001; Stein et al., 2001). True prospective studies with hard endpoints are lacking. Two recent studies show that the association between RLP cholesterol and CHD or carotid intima media thickness
is independent of LDL-cholesterol and plasma triglycerides (Karpe et al., 2001; McNamara et al., 2001).

There are no long-term dietary intervention studies with measurement of RLP cholesterol, but in a short-term study, RLP cholesterol increased in line with the expected increase in total plasma triglycerides in post-menopausal women at moderate cardiovascular disease risk fed a low fat diet (Koutsari et al., 2001). However, the low fat diet-induced increase in RLP cholesterol was reversed by moderate physical exercise (Koutsari et al., 2001).

RLP cholesterol is reduced by pharmacological lipid agents to the degree that could be expected from the respective drug’s triglyceride-lowering capacity (Karpe, 2002).

### 3.4 Small, dense low-density lipoprotein (LDL)

The formation of small, dense LDL particles is complex and can be seen as a genetic trait, but the major gene(s) responsible remain unknown. Environmental factors also play a major role, in that the plasma triglyceride concentration is a major determinant which is in turn influenced by dietary factors. *In vitro* studies have shown that small, dense LDL particles are formed by sequential exchange of lipids between LDL and triglyceride-rich lipoproteins. The cholesteryl esters contained in the core of the LDL particle are exchanged for triglycerides by the cholesteryl ester transfer protein (CETP). Triglycerides entering the LDL particle are hydrolysed by hepatic lipase and the core volume of the particle is reduced (Fig. 3.4). The formation of small, dense LDL is limited by the availability of triglyceride-rich lipoproteins, as evidenced by the close positive correlation between plasma triglycerides and small, dense LDL. It is assumed that these processes take quite some time and the end product is therefore an aged particle that has lost its defence against free radical attack. The retention in plasma of the particle is partly due to the fact that small, dense LDL has a lower affinity for the LDL receptor than normal buoyant LDL (Nigon et al., 1991). It is thought that a consequence of the altered chemical composition of the small, dense LDL particle is that it more avidly binds to the subendothelial matrix and upon challenge more easily undergoes oxidative modification thereby triggering foam cell formation (Tribble et al., 1992; Chait et al., 1993; Dejager et al., 1993; Anber et al., 1996). The details of these mechanisms are dealt with in Chapter 5.

A large number of case-control studies show strong associations between the small, dense LDL and human atherosclerosis or cardiovascular disease (Crouse et al., 1985; Austin et al., 1988; Tornvall et al., 1991; Campos et al., 1992b; Coresh et al., 1993; Stampfer et al., 1993; Griffin et al., 1994; Gardner et al., 1996; Stampfer et al., 1996; Lamarche et al., 1997; Skoglund-Andersson et al., 1999).

There is an array of different methods by which small, dense LDL can be separated, analysed or quantified. Unfortunately, this has lead to a confusing nomenclature, much of which is dependent on the principles by which the small, dense LDL particles were separated. Analytical ultracentrifugation was the first method to describe the heterogeneity of LDL, and this method defines lipoprotein density in Svedberg flotation units (Sf). Work using preparative ultracentrifugation would define the LDL heterogeneity according to density. Logically, the notion ‘small’ was first introduced when methods were based on separations according to size, i.e. non-denaturing polyacrylamide gradient gel electrophoresis (PAGE). Very often the nomenclature ‘type A/type B pattern’ is used, and this derives exclusively from...
PAGE-based methods (Krauss & Blanche, 1992). All these methods are labour intensive or require very expensive special equipment; none seems immediately suitable for routine clinical use. A method of quantifying plasma concentrations of small, dense LDL that is easy to use and encompasses the experience derived from the density and size separations of LDL would greatly facilitate future clinical biochemical work. One such method might be nuclear magnetic resonance (NMR) spectroscopy which has been claimed to identify subspecies of LDL, although a thorough evaluation against conventional methods of separating and quantifying LDL subclasses has not been made (Otvos et al., 1992, 2002).

The presence of triglyceride-rich lipoproteins is a principal modulator of small, dense LDL; the plasma concentration of the latter is strongly and positively related to the concentration of plasma triglycerides. In fact, all examples in which the triglyceride concentration has been altered to observe a change in the LDL profile are consistent: elevation of triglycerides leads to higher abundance of small, dense LDL; the opposite is observed when triglycerides are lowered, be it with diet or pharmacological agents.

Low fat diets may lead to increased plasma triglyceride concentration; consequently a reduction in LDL size was observed in a study of 105 men switching from a high fat (46%) to a low fat (24%) diet (Dreon et al., 1994). The total LDL-cholesterol concentration was, however, reduced simultaneously, so the net effect on cardiovascular risk is not entirely clear. Women exhibit the same association between the dietary manipulation is combined with other effects of a low fat diet can, however, be reversed if the common apoE polymorphism seems to determine some of the response. The potentially adverse effects of a low fat diet can, however, be reversed if the dietary manipulation is combined with other non-pharmacological efforts to reduce cardiovascular risk. A combined 4-week intervention with a low energy, low fat diet together with physical activity reduced body weight, plasma triglycerides and the number of small, dense LDL particles in a group of obese men with type 2 diabetes (Halle et al., 1999a).

Fish oils, known to alter plasma triglyceride concentrations, have been shown to modulate the LDL fraction. Ingestion of long-chain n-3 fatty acids (EPA and DHA) has repeatedly been shown to lower the concentration of small, dense LDL, although as previously stated, the concentrations used are generally higher than those that might be expected to be provided by the diet (Calabresi et al., 2000; Nordoy et al., 2001; Khan et al., 2002).

Pharmacological agents with a triglyceride-lowering propensity, such as fibrates, may increase the LDL particle size rather substantially (Ruotolo et al., 1998; Vakkilainen et al., 2002). The statins tend to lower the concentration of all LDL subclasses to approximately the same degree, unless a statin with a triglyceride-lowering capacity is used (Pontrelli et al., 2002).

3.5 High-density lipoprotein (HDL) subfractions

In accordance with LDL, HDL can also be divided into subfractions. HDL has a size heterogeneity that is similar to LDL, and within subjects this heterogeneity is correlated, i.e. subjects with small, dense LDL also have small, dense HDL (Krauss et al., 1988), because the lipid components of the two lipoprotein species share common metabolic pathways with shared and co-ordinately regulated gene products (Rainwater et al., 2001). The apolipoprotein composition is more complex in HDL as there are two distinct apolipoproteins, apoA-I and apoA-II, as well as several minor apolipoprotein components such as apoE and apoCs. The main function of apoA-I is to activate lecithin-cholesteryl ester acyl-transferase (LCAT) and to serve as the protein structure for the lipoprotein for primary uptake of tissue cholesterol. This pathway is thought to be of importance for the transport of cholesterol from peripheral tissues back to the liver, and has therefore been denoted the reverse cholesterol pathway (Fig. 3.5). Accordingly, the two main nomenclatures by which HDL is often described are based either on size and/or density, i.e. HDL₁ and HDL₃ (subdivided in to HDL₂a, HDL₂b, HDL₃a, HDL₃b, HDL₃c) (Nichols et al., 1986), or on apolipoprotein composition, i.e. lipoproteins containing either only apoA-I (LpA-I) or both apoA-I and apoA-II (LpA-I-LpA-II) (Alaupovic, 1996).

The presence of the larger HDL subspecies is
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critically dependent on the concurrent presence of low triglyceride concentrations. Both HDL-cholesterol and HDL₂-cholesterol are strongly inversely related to plasma triglyceride concentrations. As a consequence, the HDL-cholesterol concentration is strongly and positively correlated with the HDL₂-cholesterol concentration. This, together with the methodological difficulties involved in accurately assessing the HDL subfraction concentration, may have contributed to the limited number of studies exploring the association between HDL subfractions and cardiovascular disease. The only major cardiovascular endpoint study, the Quebec Cardiovascular Study, concluded that HDL subfractions do not provide additional information on the risk of CHD compared with HDL-cholesterol alone (Lamarche et al., 1997).

Low fat diets either decrease or have no effect on the plasma concentration of HDL₂ (Ehnholm et al., 1984; Williams et al., 1994), whereas physical exercise is a potent intervention by which to raise the HDL₂ concentration (Williams et al., 1994).

3.6 Free fatty acids

Free fatty acids, or non-esterified fatty acids (NEFAs), are rapidly turned over, and the plasma concentration may vary considerably within minutes. The major determinants of the plasma concentration are recent food intake (insulin is an anti-lipolytic hormone that potently induces a reduction in NEFA concentration) and stress (stress hormones are lipolytic and induce mobilisation of NEFAs from adipose tissue).

![Diagram of lipoprotein metabolism](image-url)
In addition, the change in NEFA concentration is much dependent on insulin sensitivity. These rapid modulations would argue against the usefulness of NEFAs as a marker of cardiovascular risk. In favour of the argument would be that NEFAs may have direct effects on cardiovascular function, i.e. endothelial function and heart function, they are directly implicated in glucose/insulin homeostasis, and they are the precursors for the production of potentially atherogenic lipoproteins. The impairment of normal suppression of NEFAs after stimulation with insulin seems to be an early metabolic marker in families with a history of premature myocardial infarction (Kooner et al., 1998).

Few studies have attempted to associate a random fasting measurement of NEFA with cardiovascular disease, and the results are conflicting. In a study of familial risk of cardiovascular disease, it was observed that non-diabetic subjects with elevated NEFA concentration more frequently had parents who had suffered from myocardial infarction and stroke. This association was independent of other conventional cardiovascular risk factors (Carlsson et al., 2000). In the prospective Quebec Cardiovascular Study, it was concluded that the NEFA concentration was associated with increased risk of cardiovascular disease, but this association was not significant after adjustment for other risk factors such as insulin, apoB, triglycerides and HDL-cholesterol (Pirro et al., 2002). The Paris Prospective Study is by far the largest study (22 years of follow-up of 5250 men) to investigate the association between NEFA and cardiovascular disease. The NEFA concentration was independently associated with sudden death, but there was no statistically significant association with myocardial infarction after adjustment for conventional risk factors (Jouven et al., 2001). This finding reinforces the view that NEFA is unlikely to be a useful marker for all-cause cardiovascular disease, largely because of the high degree of variability of plasma levels of NEFA, but may under certain circumstances interfere with cardiac function, possibly through pro-arrhythmogenic effects (Oliver & Opie, 1994).

The plasma concentration of NEFA, in particular the diurnal pattern, is much dependent on the ambient insulin concentration. Few studies have included NEFA measurements after dietary intervention: Raben et al. (2001) compared diurnal metabolic profiles after a 15-day period on a high fat diet, with a similar period on either a high starch or high sucrose diet and found that the high sucrose diet decreased the diurnal plasma concentrations of NEFA significantly more than the other diets.

### 3.7 Lipoprotein(a)

Lipoprotein(a) or Lp(a) is a plasminogen-like glycoprotein attached to apoB by a disulphide bond. As such, most of the apolipoprotein(a) molecules are bound to an LDL-like particle, but due to the apo(a) protein the particle has a different metabolic pathway than LDL. The apolipoprotein(a) protein is heterogeneous and a large number of different variants exist (Scanu, 1992). In essence, the difference is in the number of tandemly repeated protein structures called kringles, which is genetically determined. The main determinant of the plasma concentration of Lp(a) is the production rate (Rader et al., 1994), which is also genetically determined (Boerwinkle et al., 1992). In most populations the plasma concentration is highly skewed and only a small number of people have highly elevated Lp(a) levels.

A large number of studies have shown independent associations between plasma levels of Lp(a) and CHD, but a few studies have failed to detect such an association. Instability of Lp(a) in frozen plasma samples and the very skewed distribution of Lp(a) within populations may have contributed to the disparate results (Kronenberg et al., 1996; Simo et al., 2001). However, two recent meta-analyses including a very large number of studies show an independent and statistical association with cardiovascular disease (Craig et al., 1998; Danesh et al., 2000a).

Lp(a) does not seem to be affected by macronutrient balance, but some reports have indicated an effect of dietary trans fatty acids present in some margarines and hydrogenated oils (and products made from them). Two early reports showed increased Lp(a) concentrations after diets high in trans fatty acids (Nestel et al., 1984; Mensink, 1992) whereas two recent reports either refute the finding or only confirm it in patients with already elevated Lp(a) levels (Chisholm et al., 1996; Clevédecence et al., 1997). For this reason, restricting intake of products containing high levels of trans fatty acids may be beneficial for patients with elevated Lp(a) levels (see Chapter 11, Section 11.8.6).
Lp(a) is surprisingly resistant to pharmacological intervention. Conventional use of statins does not alter Lp(a) concentration, but one report (Carlson et al., 1989) indicates that it may be decreased by nicotinic acid. Lp(a) concentration is reduced by oestrogen and by testosterone, but this is not likely to be an option for treatment, although two studies in which post-menopausal women received hormone-replacement therapy indicate a benefit for patients with elevated Lp(a) levels (see Rosano & Fini (2002) for details).

3.8 Genetic variability and cardiovascular risk

Cardiovascular disease can be described as a polygenic disease much influenced by environmental factors (see Chapter 2). For the overwhelming majority of cases with cardiovascular disease, the genetic component is poorly understood. Essentially, examples of common genetic variants that predispose to cardiovascular disease are few and their respective contribution is small. While there is quite an extensive literature on genotype-specific effects on plasma lipid responses to dietary intervention (Ordovas, 1999), almost all studies have been genotyped retrospectively, which results in problems with statistical power in the rare allele groups and issues of poor baseline matching. The complete paucity of prospective studies makes it difficult to evaluate the relative importance of genetic variability in determining plasma lipid responses to dietary intervention, and the following section will only deal with the apoE gene for which there is some evidence.

The common variation in the apoE (e2/e3/e4) gene is probably the most studied, and has been shown to influence response to dietary changes that may affect cardiovascular risk. It is also the only example of a genetic variant for which the effect of dietary intervention has been studied prospectively. Sarkkinen et al. (1998) recruited carriers of apoE2, apoE3 and apoE4 and investigated their susceptibility to a low fat diet (NCEP-1) and to dietary cholesterol supplementation. Carriers of the apoE4 variant seemed to be hyper-responders. Firstly, they lowered their total plasma cholesterol more than carriers of the apoE3 and E2 variants on a low fat diet (Fig. 3.6). Secondly, they increased their total cholesterol more on cholesterol supplementation, which indicates a genotype-specific susceptibility of cholesterol feeding. It has also been observed that carriers of the apoE2 allele are particularly susceptible to the hypertriglyceridaemic effect of a high sucrose diet (Erkkila et al., 2001).

The apoE4 variant has also been associated with increased risk of cardiovascular disease. In a meta-analysis of 14 observational studies, cardiovascular risk was increased by 26% in apoE4 carriers compared with apoE3 carriers, with no effect in apoE2 carriers (Wilson et al., 1996). Interestingly, carriers of the apoE2 variant had the same risk as homozygous apoE3 carriers despite the inherently lower LDL-cholesterol. This may account for the few apoE2 carriers that develop type III hyperlipidaemia; less than one in ten of homozygous apoE2 carriers develop this severe form of atherogenic hyperlipidaemia. In a recent prospective analysis of the Northwick Park Heart Study, apoE4 carriers had increased risk of CHD, but the effect was restricted to smokers (Humphries et al., 2001).
3.9 Key points

- Conventional lipid-related risk factors include high plasma total and LDL-cholesterol, low plasma HDL-cholesterol and elevated plasma triglycerides.
- Small, dense LDL particles are likely to be the most atherogenic lipid risk factor. These are clearly regulated by diet and nutritional factors (e.g. the amount and type of fat in the diet). At present, the methods by which they can be quantified are complicated, expensive and not for routine clinical use.
- Remnant lipoproteins are formed in the post-prandial state and may add to cardiovascular risk.
- Remnant lipoproteins and small, dense LDL may increase in response to ingestion of low fat diets, but it is uncertain whether the overall effect is detrimental or not. Favourable aspects of low fat diets may balance the potentially negative effects.
- Fish oils reduce fasting and postprandial triglycerides as well as remnant lipoproteins, and this may partly account for the health benefit of regular fish consumption.
- The apoE4 gene variant is related to improved responsiveness to cholesterol-lowering by low fat diets, but also to susceptibility to cholesterol in the diet.

3.10 Recommendations for future research

- A method for the quantification of small, dense LDL for routine clinical use needs to be devised and evaluated.
- Long-term prospective studies need to be done on the effects of low fat diets (coupled with different sources of carbohydrate) on bodyweight stability, diabetes and cardiovascular incidence.
- New genetic markers for dietary responsiveness of lipids and lipoproteins in plasma need to be detected.

3.11 Key references


4 Endothelial Dysfunction

4.1 Introduction

4.1.1 The normal endothelium

The endothelium forms the interface between the artery wall and the constituents of blood. It has been estimated to consist of $10^{13}$ cells, with a total mass of 1.5 kg and a surface area equivalent to a football pitch (Anggard, 1994). The endothelium appears to play a critical role in the regulation of vascular tone, and inhibiting leukocyte adhesion and platelet aggregation, through its release of mediators such as nitric oxide (NO) and prostacyclin (Vane et al., 1990; Moncada & Higgs, 1993; Fig. 4.1). Nitric oxide is derived from L-arginine through the action of the constitutive form of the enzyme nitric oxide synthase (eNOS; Moncada & Higgs, 1993). It inhibits platelet aggregation and adhesion, modulates smooth muscle cell proliferation, attenuates the generation of endothelin, and reduces leukocyte adhesion to the endothelium.

Endothelial dysfunction is thought to presage atherosclerosis, and is characterised by altered permeability barrier function, enhanced adhesion molecule expression, increased leukocyte adhesion and impaired endothelium-dependent vasodilator responses (Kupatt et al., 1996; Scalia et al., 1996; Penn et al., 1997; Rangaswamy et al., 1997). Endothelial dysfunction is also associated with enhanced thrombosis and impaired fibrinolysis.

The vasodilatory function of the endothelium is exploited as the basis of several dynamic tests that 'measure' endothelial function in response to appropriate stimuli, and these are discussed in detail below. There is, however, no universally accepted 'gold standard' measure of endothelial function.

Rather, estimates of different facets of endothelial dysfunction may be obtained indirectly by measuring endothelium-dependent vasodilatation, serum or plasma levels of endothelium-derived regulatory proteins, and possibly by urinary factors such as microalbuminuria (Table 4.1).

Many researchers promote the notion that endothelial dysfunction is likely to be a critical early step in the process of atherogenesis, and as such, endothelial dysfunction is considered to represent an intermediate phenotype for vascular disease (de Caterina, 2000). Its assessment has, therefore, assumed great importance in clinical research, and some methods for its assessment, including both blood measures and dynamic tests, are beginning to show promise in improving risk stratification.

4.1.2 Endothelial cell heterogeneity

It should also be noted that endothelial cells differ in their properties at different anatomical sites in the vascular tree (Thorin & Shreeve, 1988; Vercellotti et al., 1988). To some extent, site-specific variations in shear forces may modulate these properties. Such differences include variations in permeability and cytokine expression. Endothelial heterogeneity may be partially responsible for the characteristic regional distribution of atherosclerotic lesions described in animal models and human post-mortem studies.

4.2 Estimates of endothelial dysfunction

There are three broad categories of endothelial function measures:

(1) endothelial-dependent vasodilatation or 'direct' measures
4.2.1 Endothelium-dependent vasodilatation or ‘direct’ measures

Most methods are based on delivery of vasoactive agents (most commonly acetylcholine (ACh)) to endothelial cells and measuring a reproducible response in some aspect of blood flow. The delivery of acetylcholine tests endothelial function as it requires intact endothelial cells for the generation of nitric oxide in order to bind to muscarinic receptors. It is, therefore, said to be ‘endothelium-dependent’. Vasodilatation is ultimately mediated by the action of nitric oxide on vascular smooth muscle (via the cyclic guanosine monophosphate (cGMP) pathway) and hence sodium nitroprusside (SNP) delivery, a nitric oxide donor, is used as an ‘endothelium-independent’ control. The technique of flow-mediated dilatation differs, however, in that it relies on shear stress to promote nitric oxide release following an interval of ischaemia.

Direct methods can be divided into those examining blood vessels dissected from human or animal tissues, i.e. ex vivo and in vivo techniques.

(i) Ex vivo techniques

The original observation for a vasodilatory function of the endothelium came with organ bath experiments (Furchgott, 1983). When vascular rings were stripped of their endothelium, they were noted to
have an inability to relax in response to acetylcholine, whereas relaxation was clearly evident when endothelium was present (Cherry et al., 1982). The response of vascular endothelium in man can now be assessed by *ex vivo* methods. Briefly, small (200–400 µm diameter) arteries are stripped from buttock biopsies of fat tissue and mounted as ring preparations on stainless steel wires in a small vessel myograph (James et al., 1997). The contraction and relaxation of the vessels in response to various agents in solution is monitored as a change in tension.

(ii) *In vivo* assessment

This group of techniques can be further subdivided into *invasive* and *non-invasive* methods.

(a) Invasive techniques

(1) *Quantitative coronary angiography*. This technique involves direct infusion of vasoactive agents into the coronary blood vessels and monitoring of changes in diameter during recordings taken by standardised quantitative means (Anderson et al., 1995). Much of the original *in vivo* research examining endothelial function employed this technique, but clearly its application is limited to patients with anginal symptoms, and widespread use in subjects without overt symptoms is not possible for ethical reasons.

(2) *Venous-occlusion plethysmography (VOP)*. This is one of the best-established techniques for assessment of endothelial function in peripheral blood vessels and involves assessing forearm blood flow response during intrabrachial infusions of endothelium-dependent and -independent agonists. The procedure, considered by many as the ‘gold standard’, is almost routine in some clinical research centres, although it is relatively invasive even when a small (27-G) needle is used. Briefly a 27-G needle (flushed with heparinised saline) is inserted at a 45° angle under local anaesthesia into the brachial artery of the non-dominant arm, and changes in forearm blood flow between infused and control arms are measured using the technique of bilateral venous-occlusion strain-gauge plethysmography (Chowienczyk et al., 1992). This technique measures changes in diameters of forearms, with an increase being associated with vasodilation of blood vessels and a contraction the reverse.
(b) Non-invasive techniques

(1) **Flow-mediated dilatation (FMD) post ischaemia (large vessels)**. This involves ultrasonic assessment of brachial artery flow (see Fig. 4.2). The brachial artery is of similar size to coronary arteries, and although it is uncommon for the brachial artery to have significant atheroma, brachial responses have been shown to correlate well with responses in the coronary circulation (Anderson *et al.*, 1995). Endothelial function is measured by ‘wall tracking’ of brachial artery dilation in response to increased flow generated by hyperaemia of the arm, hence the name flow-mediated dilatation. Flow-mediated endothelial function is in part mediated by nitric oxide and impaired with classical risk factors for vascular disease (Celermajer *et al.*, 1996; Clarkson *et al.*, 1997; Goodhart & Anderson, 1998; Cuevas *et al.*, 2000; Hashimoto *et al.*, 2000).

FMD has been the most widely used non-invasive measure, but it is technically demanding and requires considerable expertise and expensive equipment for its assessment (Corretti *et al.*, 2002). There remain concerns over its reproducibility.

(2) **Iontophoresis and laser Doppler imaging (microvasculature)**. Skin microvascular function can be assessed using laser Doppler perfusion imaging by iontophoresis of vasoactive agents such as acetylcholine and sodium nitroprusside (Newton *et al.*, 2002). A laser beam that reflects moving blood cells in the skin is used to detect the magnitude of blood flow. Iontophoresis is the use of electric current to drive chemicals into the skin and exploits the fact that most chemicals are charged. Chemicals negatively charged move away from the cathode and positively charged molecules are driven into skin at the positive anode. It is a safe procedure and has been used to measure blood vessel function in children and pregnant women (Ramsay *et al.*, 2002a) successfully and without difficulty. Establishment of the optimal conditions for minimising variability (Ferrell *et al.*, 2002; Ramsay *et al.*, 2002b) has recently improved the robustness of this methodology.

(3) **Pulse wave velocity or pulse wave analysis (large vessels)**. Arterial stiffness is known to be important in the pathophysiology of cardiovascular disease states. There are several ways of assessing it, ranging from examining parameters quantifying the speed of propagation along an arterial segment, namely pulse wave velocity (PWV; Liang *et al.*, 1998), to parameters giving a function of global elasticity behaviour derived from examining the arterial pulse pressure waveform, namely pulse wave analysis (PWA; Wilkinson *et al.*, 2002).

An example of the recording given by a PWV technique is shown in Fig. 4.3. These are simple techniques suitable for larger scale studies and may provide useful surrogate markers of cardiovascular risk (and possibly endothelial dysfunction). Encouraging prospective data from studies using PWV and PWA have recently been published (Guerin *et al.*, 2001; Meaume *et al.*, 2001; Boutouyrie *et al.*, 2002; Safar *et al.*, 2002).

(4) **Capillaroscopy (microvasculature)**. Capillaries play a critical role in cardiovascular function as the point of exchange of nutrients and waste products between the tissues and circulation. Skin capillaries can readily be studied by the technique of capillaroscopy, reviewed in detail by Shore (2000). This technique enables the investigator to assess morphology, density and blood flow velocity. It is also possible to estimate capillary pressure by direct cannulation using glass micropipettes.
There is evidence that endothelial function measured in coronary arteries correlates with endothelial function measured in brachial arteries by VOP and FMD (Anderson et al., 1995). There is also evidence that conditions associated with macrovascular dysfunction, such as diabetes, also demonstrate microvascular dysfunction. However, there is little published data comparing the newer non-invasive techniques with more traditional ‘gold standard’ invasive methods of vascular assessment such as VOP. One of the notable exceptions is work by Wilkinson and colleagues who noted a significant correlation between augmentation index (a measure of PWA) in response to albuterol and the forearm blood flow response to acetylcholine during VOP (Wilkinson et al., 2002). However, the extent of correlation was modest at best with $r = 0.50$, suggesting only about 25% concordance between these measures. Clearly, further studies are required to determine extent of correlation, or otherwise, of different endothelial function techniques.

### 4.2.3 Measurement of blood constituents

As discussed previously, the endothelium elaborates a variety of molecules, some of which are involved in maintaining vascular homeostasis. The blood or urinary concentration of some of these molecules can be readily measured using commercially available kits or research methods. Although a perturbation in the levels of these molecules is used as an indication of endothelial dysfunction or activation, it should be remembered that the endothelium might not be their only source. Altered concentrations may reflect pathology at other anatomical sites, and the clearance of these molecules, through the kidney, reticular endothelial system, or bile, could be an equally important determinant of circulating concentrations as synthesis. Nevertheless, there is some evidence that determination of the first three in the list below may independently predict adverse cardiovascular prognosis (see Table 4.2).

- plasma von Willebrand factor (vWF) (Feng et al., 2000; Jager et al., 2001)
- tissue plasminogen activator antigen (t-PA Ag) (Lowe et al., 2001a)
- soluble adhesion molecules (sVCAM-1, sE-selectin, sICAM-1: data strongest for sICAM-1) (Malik et al., 2002; Ridker et al., 2001a; Rohde et al., 1999)
  - fibronectin
  - thrombomodulin
  - products of nitric oxide metabolism (nitrate)
  - products of eicosanoid metabolism (PGF$_2\alpha$) (Kengatharan et al., 1998).

More prospective data are required before such parameters are routinely incorporated into coronary risk factor stratification protocols. Moreover, further consideration needs to be given to issues of international assay standardisation. Prospective data on fibronectin, thrombomodulin and other proteins proposed to reflect endothelial dysfunction are lacking.

### 4.2.4 Measurement of excreted factors

#### (i) Microalbuminuria

Detectable urinary albumin excretion can be associated with the increased endothelial permeability that accompanies endothelial dysfunction (Shearman & Gosling, 1988). Patients with type 2 diabetes are regularly screened for microalbuminuria; when this
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Table 4.2 Measures of endothelial function showing promise as potential independent predictors of vascular disease.

<table>
<thead>
<tr>
<th>Estimate of endothelial function</th>
<th>Extent of evidence</th>
<th>Limitations to interpretation</th>
<th>Limitations to applicability</th>
<th>Future studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial-dependent vasodilation (see Table 4.3 for details of specific studies)</td>
<td>Prospective studies examining coronary and peripheral vessel function</td>
<td>General lack of consideration of HDL-cholesterol or inflammatory parameters</td>
<td>Most studies performed in high risk populations; extent of prediction beyond classical markers not well examined</td>
<td>More data required on predictive ability of non-invasive methods such PWV, PWA; future studies should include ROC analysis</td>
</tr>
<tr>
<td>Tissue-type plasminogen activator antigen</td>
<td>Meta-analysis of prospective studies</td>
<td>Elevated levels may reflect insulin resistance and inflammation</td>
<td>No universal assay standards</td>
<td>Standardisation; more data on stroke, peripheral vascular disease</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Meta-analysis of prospective studies</td>
<td>Elevated levels may reflect inflammation</td>
<td>No universal assay standards</td>
<td>Standardisation; more data on stroke, peripheral vascular disease</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>Prospective studies</td>
<td>Not always due to endothelial dysfunction</td>
<td>Often seen only in patients with advanced disease</td>
<td>May be better predictor if used in combination with other markers of endothelial dysfunction</td>
</tr>
</tbody>
</table>

HDL: high-density lipoprotein; PWA: pulse wave analysis; PWV: pulse wave velocity; ROC: receiver operating characteristic curves.

is present they are considered to be at markedly elevated risk for cardiovascular disease. Recent data indicate that diabetic patients with microalbuminuria have a reduced endothelial functional reserve compared to patients without it (Dogra et al., 2001; Shand et al., 2002). There is prospective evidence to show that patients with both microalbuminuria and raised von Willebrand factor are at substantially higher risk of vascular disease, but patients with microalbuminuria alone are not (Jager et al., 2001). Thus, microalbuminuria can occur in the presence or absence of generalised endothelial dysfunction. This work shows the usefulness of combining different measures of endothelial function to determine risk. Standardised assays exist for determination of urinary albumin concentration.

(ii) Other urinary measures

Other urinary measures that have been used to gauge the degree of endothelial dysfunction include:

- urinary nitrite and nitrate (Andersen et al., 2002);
- urinary prostanoid metabolites.

The methods for these are much more complex, and relevant prospective data linking their measurements in urine to vascular endpoints are lacking.

4.3 Aetiology of endothelial damage: association with classical and novel risk factors

Effectively, all classical risk factors, and several novel risk factors, are associated with endothelial dysfunction, however these may be measured. Some risk factors are more closely correlated with endothelial dysfunction than others, and the principal associations, with the relevant evidence for each, are discussed below.

4.3.1 Classical risk factors (i.e. parameters incorporated in current risk factor stratification tables)

(i) Hypertension

There are several putative causes of endothelial injury (Puddu et al., 2000). These include the mechanical damage associated with hypertension. It is likely that the deleterious effects of hypertension are exerted by several mechanisms. It is associated with increased
endothelial cell free radical formation, and reduced nitric oxide bioactivity. It also has pro-inflammatory effects on vascular smooth muscle cells.

(ii) Smoking

There is considerable evidence that cigarette smoking can result in both morphological and biochemical disturbances to the endothelium both in vivo and in cell culture systems. Cigarette smoke is a complex mixture, and only a few components have been studied extensively. Nicotine and carbon monoxide appear less damaging than whole smoke, although nicotine up-regulates the expression of several pro-inflammatory and pro-oxidative molecules. Clinical and experimental animal studies suggest that arterial endothelium-dependent acetylcholine relaxation is impaired by smoking (Celermajer et al., 1996; Pittilo, 2000; Wang et al., 2001) and may be attenuated by the antioxidant vitamin C (Heitzer & Munzel, 1996) or smoking cessation (Mays et al., 1998). Wang et al. (2001) suggest that excessive endothelial apoptosis may contribute to cigarette smoke-induced endothelial injury.

(iii) Dyslipidaemia (high cholesterol, low HDL-cholesterol, and high triglyceride)

(see Chapter 3)

Endothelial dysfunction has been described in young children with familial hypercholesterolaemia (Celermajer et al., 1992). These effects may be mediated in part by oxidation products of low-density lipoprotein (LDL; Morel et al., 1983; Steinberg et al., 1997). In experimental animal model studies, the rate at which endothelial dysfunction occurs varies with anatomical site (Stewart-Lee et al., 1994) and may be reversed by the antioxidant vitamin E (Stewart-Lee et al., 1994).

There is evidence that plasma high-density lipoprotein (HDL) cholesterol concentrations are positively related to endothelium-dependent relaxation (O’Connell & Genest, 2002; Spieker et al., 2002), and it has been proposed that the higher HDL-cholesterol concentrations observed in women may be responsible for their observed protection against the adverse effects of hypercholesterolaemia on endothelium-dependent vasodilation (Algottson, 1996; Lee et al., 2002). In patients with type 2 diabetes mellitus, decreased HDL-cholesterol has been shown to be the best predictor of impaired vasodilatation to acetylcholine even after adjustment for all lipid and lipoprotein concentrations and LDL size (Anderson et al., 2001). Endothelial protection by HDL may relate to its antioxidant properties because HDL has been found to protect against LDL oxidation (O’Brien et al., 1997; Toikka et al., 1999). HDL antioxidant actions may be related to its protein components (which bind transition metals) and to two intrinsic antioxidative enzyme systems: platelet activating factor acetylhydrolase and paraoxonase.

Triglyceride-rich lipoproteins and particularly their postprandial remnants are cytotoxic to cultured endothelial cells and can also cross the endothelial barrier and enter the arterial wall, placing them in a position to promote endothelial damage (as reviewed by Sattar et al., 1998). Significantly, in line with the above in vitro data, it has been demonstrated that postprandial hypertriglyceridaemia transiently impairs endothelium-dependent flow-mediated vasodilation (Bae et al., 2001). Furthermore, impairment in post-prandial flow-mediated vasoreactivity correlates with change in 2-hour serum triglyceride concentration (Vogel et al., 1997). Interestingly, pre-treatment with vitamin C and E blocked this effect, supporting a pivotal role for oxidative stress in the link between triglyceride-rich particles and endothelial damage (Plotnick et al., 1997).

(iv) Diabetes

From studies employing a variety of techniques in patients with type 2 diabetes there is now ample evidence for vascular endothelial dysfunction and increased arterial stiffness at several sites, including coronary vessels, brachial arteries and subcutaneous vessels (Tooke & Goh, 1999; Hink et al., 2001). Many aspects of the insulin resistance syndrome may contribute to this dysfunction in patients with diabetes, including elevations in free fatty acids, characteristic lipid changes, obesity and hypertension (see Chapter 2). Additionally, raised glucose concentrations can further damage vascular function.

There is increasing evidence of subtle but significant vascular dysfunction in offspring of patients with type 2 diabetes (Caballero et al., 1999). Using a number of non-invasive techniques, defects in microvascular, macrovascular and autonomic function have been demonstrated, despite there being
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no change in blood pressure and normal glucose concentrations. These findings suggest that vascular dysfunction may be an early feature of the insulin resistance syndrome.

4.3.2 Novel risk factors

(i) Low-grade chronic inflammation

As noted in Chapter 7, there is now considerable evidence implicating low-grade chronic inflammation in the pathogenesis of vascular disease. Therefore, it is of interest that raised C-reactive protein (CRP) concentrations correlate with impaired endothelial nitric oxide production in peripheral arteries, impaired coronary endothelial function, and with elevated pulse pressure (Cleland et al., 2000).

C-reactive protein, or related inflammatory cytokines such as interleukin-6 (IL-6) or tumour necrosis factor-alpha (TNFα), may mediate endothelial dysfunction; Bhagat and Vallance (1999) have reported that infusion of endotoxin or pro-inflammatory cytokines into superficial hand veins of healthy volunteers (provoking a local acute inflammatory response) caused a selective impairment of endothelium-dependent relaxation. Their interpretation that such effects were related to inflammation was strengthened by the observations that the impairment was abolished by prior oral treatment with hydrocortisone or high dose aspirin (Bhagat & Vallance, 1997).

(ii) Obesity, insulin resistance

The association of endothelial dysfunction with obesity (Al Suwaidi et al., 2001; Perticone et al., 2001a) and insulin resistance has been known for some time (Steinberg et al., 1996; Tooke & Goh, 1999). They are both associated with a blunted endothelium-dependent response that is not augmented by euglycaemic hyperinsulinaemia (Hogikyan et al., 1998). In part the endothelial dysfunction may be due to elevated levels of circulating free fatty acids (Steinberg & Lewis, 1997; Steinberg & Baron, 2002).

(iii) Leptin

Interestingly, elevations in plasma leptin concentrations are associated with impaired arterial distensibility as determined by the FMD technique, independent of the metabolic and inflammatory disturbances associated with obesity (Singhal et al., 2002). This observation adds support to the argument that leptin directly, or via its close association with fat mass, gives greater insight into vascular risk than does body mass index (BMI), which is a poorer correlate with fat mass (Wallace et al., 2001).

(iv) Homocysteine

Very high concentrations of homocysteine are toxic to endothelial cells (Harker et al., 1983), and as such may contribute to premature vascular disease seen in patients with homocystinaemia (McCully, 1969; see Chapter 8). This may be due to a free radical mediated mechanism (Upchurch et al., 1997). Recent studies indicate that serum levels within the ‘normal’ range may also confer risk of vascular disease (Verhoef & Stamper, 1995; Nygard et al., 1997; Verhoef et al., 1997). However, the proatherogenic effects of homocysteine appear to be modified by other, as yet unidentified, factors. In man, a methionine load causes a rapid but mild elevation of plasma homocysteine. This is accompanied by an acute deterioration in endothelial responses to acetylcholine (Bellamy et al., 1998; Nappo et al., 1999), an effect reversed by antioxidant vitamins (Raghavveer et al., 2001) and B vitamins (Chao et al., 1999). However, in longer term studies, folic acid and B vitamins did not improve brachial artery vascular responses, although they did reduce blood pressure (Van Dijk et al., 2002).

(v) Low birthweight

Low birthweight is linked to increased risk of cardiovascular disease (Fall et al., 1995a) and type 2 diabetes (Curhan et al., 1996; see Chapter 10). There is some evidence of an association of low birthweight with endothelial dysfunction as measured by FMD (Leeson et al., 1997; Martin et al., 2000a, b).

(vi) Ethnicity

South Asians have an elevated risk of vascular morbidity and mortality in part linked to impaired insulin sensitivity (Cruz et al., 2001), as compared to weight and age-matched Caucasian subjects (see Chapter 1, Section 1.3.3 and Chapter 2, Section 2.4.2). They also have impaired FMD compared to Caucasians, an observation not explained by either classical risk factors or insulin resistance (Chambers et al., 1999a).
4.4 Endothelial dysfunction as an integrated pathway for coronary heart disease (CHD) risk

Due to its strategic site, and for the reasons described above, the endothelium is a target for all risk factors (Gimbrone, 1995). Therefore, measures of endothelial dysfunction may represent an amalgamation of risks attributable to classical and novel risk factor pathways (Fig. 4.4). Classical risk factors appear to explain less than 20% of the variation in endothelium-dependent vasodilatation (Chan et al., 2001). Novel risk factor pathways may, therefore, explain a large amount of variation in endothelial function; as discussed below, evidence to support this suggestion is emerging.

If the endothelium does amalgamate risk, then this emphasises the potential for simple endothelial dysfunction measures to help better predict risk of vascular disease. To be clinically useful, such measures must be both easy to use and predict cardiovascular risk independently, not only of classical risk factors but also of emerging risk factors such as C-reactive protein.

It should be noted that although the endothelium amalgamates risks from all circulating risk factors, its dysfunction is also pivotal in the process of lesion formation by promoting the early and late mechanisms of atherosclerosis. As described above, these include up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, enhanced LDL oxidation, platelet activation, cytokine synthesis and vascular smooth muscle cell proliferation and migration.

4.5 Endothelial function measures as independent predictors of CHD

Until recently, prospective evidence linking endothelial function measures to elevated vascular risk was sparse. However, several recent studies of ‘direct’ measures (Al Suwaidi et al., 2000; Schachinger et al., 2000; Perticone et al., 2001b; Gokce et al., 2002; Halcox et al., 2002; Modena et al., 2002) have generated important support for this proposition (Table 4.3), but more data are needed before potential measures are incorporated into risk factor stratification tables. Future studies must examine
whether measures of endothelial function are independent predictors of coronary events when considered together with the entire range of conventional risk factors and perhaps also other important novel markers, such as C-reactive protein. Where possible, such analyses should include use of receiver operating characteristic curves (ROC). ROC analysis can be used to identify the level where the maximum mean value of sensitivity plus specificity occurs. Moreover, such analyses can help compare the predictive ability of independent variables and various combinations of variables. Finally, more data on the predictive ability of direct endothelial function measures are required in low risk populations, and reproducibility of techniques needs to be better documented and improved.

### 4.6 Relevant genetic studies

Although initial studies supported an association between genes determining nitric oxide availability and endothelial function (and thus vascular risk),

**Table 4.3** Results of prospective studies employing direct endothelial function measures.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Subjects</th>
<th>Technique</th>
<th>Follow-up period</th>
<th>Results</th>
<th>Multivariate analysis</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Suwaidi et al., 2000)</td>
<td>157 with mild CAD</td>
<td>Coronary BF</td>
<td>28 months</td>
<td>6 events in group with severe EF, no events if EF normal or mild</td>
<td>Not done but cases with subsequent events were similar for other baseline risk factors</td>
<td>CRP not included</td>
</tr>
<tr>
<td>(Schachinger et al., 2000)</td>
<td>147 angiography patients</td>
<td>Coronary BF</td>
<td>7.7 years</td>
<td>16 events – cases had significantly impaired responses at baseline</td>
<td>Yes, independent predictor</td>
<td>No HDL, no CRP</td>
</tr>
<tr>
<td>(Heitzer et al., 2001)</td>
<td>281 with CAD</td>
<td>Forearm VOP</td>
<td>4.5 years</td>
<td>91 events – cases had lower baseline responses but greater benefit from vitamin C</td>
<td>Yes, independent predictor but analysis was complex</td>
<td>No CRP, no ROC analyses, large case/control overlap</td>
</tr>
<tr>
<td>(Perticone et al., 2001b)</td>
<td>225 new HBP</td>
<td>Forearm VOP</td>
<td>31.5 months</td>
<td>29 events, risk of event 2.084 higher if response in bottom tertile</td>
<td>Yes, independent but marked sex difference full multivariate analysis not presented</td>
<td>No CRP, no ROC analyses</td>
</tr>
<tr>
<td>(Halcox et al., 2002)</td>
<td>308 angiography patients</td>
<td>Coronary BF</td>
<td>46 months</td>
<td>35 events – individuals had impaired ACh response alone</td>
<td>Yes, independent but retrospective data</td>
<td>No ROC, no CRP, no HDL, BMI significant predictor</td>
</tr>
<tr>
<td>(Modena et al., 2002)</td>
<td>400 women with HBP</td>
<td>FMD repeated at 6 months</td>
<td>67 months</td>
<td>47 events, If FMD improved &gt;10% with anti-hypertensive, 7-fold lower risk of event</td>
<td>Not done</td>
<td>First study to look at response to treatment as predictor of events</td>
</tr>
<tr>
<td>(Gokce et al., 2002)</td>
<td>187 pre-vascular surgery patients</td>
<td>FMD</td>
<td>30 days</td>
<td>45 events, pre-operative EF significantly lower in patients who had subsequent event</td>
<td>Yes</td>
<td>Cut-off point of 8.1% had good sensitivity and −ve predictive value</td>
</tr>
</tbody>
</table>

ACh: Acetylcholine; BF: blood flow; BMI: body mass index; CAD: coronary artery disease; CRP: C-reactive protein; EF: endothelial function; FMD: flow-mediated dilatation; HBP: high blood pressure; HDL: high-density lipoprotein cholesterol; ROC: receiver operating characteristic curves; VOP: venous-occlusion plethysmography; −ve: negative.
more recent studies have largely been unsupportive (Jeerooburkhan et al., 2001). However, the Gln298Asp endothelial nitric oxide synthase gene polymorphism was noted to be associated with differences in endothelial responses to both smoking and n-3 polyunsaturates in healthy young subjects, but interestingly not when the whole group was considered. These findings raise the possibility of genotype-specific prevention strategies in cardiovascular disease (Leibel, 2002).

4.7 Prevention and reversibility of endothelial dysfunction

4.7.1 Lipid-lowering agents

Statins have consistently been shown to improve endothelial function. For example, cholesterol reduction rapidly improved endothelial function after acute coronary syndromes in the RECIFE (Reduction of Cholesterol in Ischaemia and Function of the Endothelium) trial (Dupuis et al., 1999). Endothelial dysfunction in primary hypercholesterolaemia was improved by treatment with atorvastatin or simvastatin plus cholestyramine (Simons et al., 1998). The *fibrate* class of drugs acts primarily to reduce triglyceride concentration and as a result raise HDL-cholesterol levels. Recent evidence shows that such drugs also improve endothelial function in man (Evans et al., 2000; Malik et al., 2002).

4.7.2 ACE inhibitors

There is plentiful evidence demonstrating an improvement in endothelial function in subcutaneous, epicardial, renal and brachial arteries with angiotensin-converting enzyme (ACE) inhibitors. By inhibiting the formation of angiotensin II, ACE inhibitors prevent any damaging effects on endothelial function and vascular smooth muscle cells (Williams et al., 1995; Esper et al., 2000). An increase in the release of nitric oxide under ACE inhibition has a protective effect.

4.7.3 Metformin and thiazolidinediones

Biguanides, of which metformin is the most widely used, are a class of insulin-sensitisers that have been in clinical use for over 50 years. A 12-week trial of metformin compared to placebo in individuals with type 2 diabetes showed improved endothelial-dependent function alone as measured by VOP in the metformin group (Mather et al., 2001).

There is also emerging evidence to indicate thiazolidinediones or peroxisome proliferator-activated receptor-gamma (PPAR) agonists, which appear to be more potent insulin sensitizers than metformin (Hallsten et al., 2002), improve endothelial function, whether determined directly, e.g. by flow-mediated vasodilation (Wang et al., 2004), or indirectly by a reduction in circulating markers of endothelial activation (Sidhu et al., 2003). There is considerable interest, therefore, in determining whether such agents lessen the risk of vascular endpoints in patients with metabolic syndrome and in those with type 2 diabetes.

4.7.4 Weight loss

There is recent robust evidence that a 10% weight loss significantly improves endothelial function in obese women as determined by VOP (Ziccardi et al., 2002).

4.8 Effects of CHD preventative modalities on other risk factor pathways

It is important to note that all of the above modalities not only improve endothelial function measures, but also favourably influence several other vascular risk factor pathways. For example, statins by design reduce lipid levels, but additionally have antioxidant and anti-inflammatory properties (Wassmann et al., 2001). ACE inhibitors are also potently anti-inflammatory, particularly at the level of the vessel wall, whereas metformin, thiazolidinediones, and weight loss enhance insulin sensitivity and simultaneously reduce levels of inflammatory mediators (Haffner, 2002; Ziccardi et al., 2002).

In such cases, it is therefore incorrect to definitively claim that improving endothelial function reduces cardiovascular risk. Rather, improved endothelial function may be secondary to improvements in risk factor pathways ‘up-stream’ in the hierarchy of atherogenic events, and up-stream effects, in turn, may be more important in pathophysiological terms.

Similarly, a reduction in endothelial function cannot be construed as confirmation of a reduction in CHD risk. For example, there is plentiful and generally consistent evidence to show hormone
replacement therapy (HRT) improves endothelial function in the short and medium term, but randomised trials have shown no reduction in CHD risk with HRT and perhaps even an increase in CHD and stroke risk (Beral et al., 2002). In other words, improvements in endothelial function have to be considered in the context of changes of other pathways. For example, if endothelial function improvement is not accompanied by simultaneous improvements in other key pathways but rather deleterious changes in, for example, C-reactive protein or triglyceride levels, the overall CHD risk–benefit balance of specific treatments may be harmful.

4.9 Dietary modulations of endothelial function

There are numerous clinical studies examining the effects of both macro- and micronutrients on endothelial function measures including both ‘direct’ techniques and circulating parameters. The data are too extensive to comprehensively describe herein, but have been reviewed in detail elsewhere (Brown & Hu, 2001). The principal macro- and micronutrients examined in relation to endothelial function are described below.

4.9.1 Macronutrients

(i) Dietary fats

There is now abundant evidence that endothelial-dependent dysfunction occurs soon after a fatty meal, perhaps via a pro-oxidant effect of triglyceride-rich lipoproteins (Plotnick et al., 1997; Vogel et al., 1997). The extent of impairment has been linked to the magnitude of the postprandial triglyceride rise.

(ii) Fish oils

Strong and generally consistent data from in vitro experiments and animal and, most recently, human studies support a beneficial effect of fish oils (rich in n-3 fatty acids) on vascular function (as reviewed by Brown & Hu, 2001). The data are particularly strong for docosahexaenoic acid (DHA). The effect may relate to the incorporation of n-3 fatty acids into cellular phospholipids at the expense of n-6 fatty acids. Interestingly, n-3 fatty acids from plant sources appear to have equivocal effects on CHD risk factors although endpoint studies are lacking (Sanderson et al., 2002a).

(iii) Mediterranean diets

Mediterranean diets are characterised by a high intake of monounsaturates. There is some evidence that a monounsaturate-rich diet improves endothelial function as determined by reduction in circulating markers of endothelial origin, namely, vWF and plasminogen-activator inhibitor-1 (PAI-1).

(iv) Carbohydrate

Acute carbohydrate intake appears to enhance endothelial-dependent function (Ong et al., 1999), an effect probably operating via insulin, since insulin not only has important effects on glucose uptake but has also been shown to have important vasodilating effects (Cleland et al., 1999). However, this is a complex area that needs much more study since a chronically high carbohydrate intake, particularly if rich in refined carbohydrate, can lead to hypertriglyceridaemia and associated postprandial hypertriglyceridaemia, which in turn may impair vascular responses.

(v) Alcohol

There are some human (Teragawa et al., 2002) and supporting in vitro data (Leikert et al., 2002) linking moderate alcohol intake to better endothelial function. Whether this effect is only seen with red wine is controversial. Interestingly, alcohol has also been shown to have anti-inflammatory effects (Mandrekar et al., 2002) (see Chapter 7, Section 7.8.3).

4.9.2 Micronutrients

(i) Dietary antioxidants

There are numerous laboratory, animal and human studies to show beneficial effects of antioxidants on vascular function. Indeed, the majority, though not all, in vivo human studies also suggest a beneficial effect. These findings need to be considered together with the wealth of evidence from randomised trials showing no beneficial effect whatsoever from taking antioxidant supplements (Brown & Hu, 2001; Heart Protection Study Collaborative Group, 2002b) (see Chapter 5, Section 5.10).
(ii) Folate and B vitamins

The relevance of these micronutrients to vascular risk is largely linked to their action to reduce circulating homocysteine levels as detailed in Chapter 8. Homocysteine harms the vascular endothelium in a number of ways, including a direct pro-oxidant effect. There are numerous human studies examining the effects on endothelial function of folic acid alone or together with other B vitamins (Brown & Hu, 2001); the majority of these suggest benefit. A randomised double-blind placebo controlled trial of combined folic acid, vitamins B₁₂ and B₆ on clinical outcomes after percutaneous coronary intervention showed a reduced risk of major vascular events in the treatment group (Schnyder et al., 2002). A reduction in abnormal exercise electrocardiograms (ECGs) and blood pressure has also been reported in a randomised trial (Vermeulen et al., 2000). The results of larger ongoing supplementation trials are awaited with interest.

(iv) L-arginine

As a substrate for nitric oxide, it seems highly plausible that increasing L-arginine intake may enhance endothelial function and reduce vascular risk. There are now more than a dozen studies examining its effects on endothelial function (reviewed by Brown & Hu (2001). Approximately two-thirds of these support a beneficial effect, but a sizeable minority suggest no effect. The Seven Countries Study Research Group examined a potential effect of L-arginine intake on CHD risk but found no association (Feskens et al., 2001).

4.10 Do nutrient-induced endothelial effects correlate with results from endpoint studies?

It is of note that whereas evidence to support a consistent benefit on endothelial function exists for some nutrients, others are associated with both positive and negative effects (Table 4.4).

Table 4.4 Influence of dietary factors on estimates of endothelial function.

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Consistency of endothelial effects</th>
<th>Effects on other risk factor pathways</th>
<th>Evidence for reduction in vascular risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macronutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediterranean diet</td>
<td>+</td>
<td>Insulin sensitivity</td>
<td>Balance of evidence favours reduction in CHD</td>
</tr>
<tr>
<td>Fish oils</td>
<td>++</td>
<td>Anti-inflammatory, anti-thrombotic, lipid-lowering at high doses</td>
<td>Strong evidence favours reduction in fatal CHD</td>
</tr>
<tr>
<td>Reduction in dietary fats</td>
<td>++</td>
<td>Lipids</td>
<td>Reduction in CHD with lowered saturated fatty acid intake</td>
</tr>
<tr>
<td>Alcohol</td>
<td>++</td>
<td>HDL-C, insulin sensitivity, anti-inflammatory</td>
<td>Reduction in CHD with moderate intake</td>
</tr>
<tr>
<td>Micronutrients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid + B-vitamins</td>
<td>+++/−</td>
<td></td>
<td>Preliminary data to suggest reduced restenosis of coronary arteries, fewer CHD events</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>++/−</td>
<td></td>
<td>Not available, but intake not associated with reduced CHD risk</td>
</tr>
<tr>
<td>Antioxidant vitamins taken as supplements</td>
<td>+++/−</td>
<td>Some evidence for anti-inflammatory effect</td>
<td>Majority of evidence suggests lack of effect</td>
</tr>
</tbody>
</table>

+, some supportive evidence; ++, consistent supportive evidence; +++/−, balance of studies supportive; +++/−, majority but not all studies supportive. CHD: coronary heart disease; HDL-C: high-density lipoprotein cholesterol.
of such effects, it can be seen that an improvement in endothelial function should not be taken to infer guarantee of vascular risk reduction. This applies particularly to the area of antioxidants where, although the balance of studies supports an improvement in endothelial function, prospective clinical trials are generally not supportive of CHD risk reduction with antioxidant supplementation. The effects of dietary composition on endothelial function are further complicated by the fact that isoenergetic changes in content are often associated with changes in the balance between fat and carbohydrate, and between types of dietary fat. This may itself cause altered endothelial function (Ong et al., 1999; De Roos et al., 2002).

4.11 Key points

- The endothelium is an active tissue and possesses numerous anti-atherogenic functions in a normal healthy state, including regulation of blood flow in response to metabolic demands, inhibition of blood clotting and prevention of adhesion of inflammatory cells to blood vessel lining.
- Endothelial ‘dysfunction’ can be considered to be present when properties of the endothelium, either in a basal state or after stimulation, have altered in a way that is inappropriate with regard to preservation of normal function.
- All known established and novel risk factor pathways can contribute to endothelial dysfunction and, as such, estimates of endothelial function may represent an amalgamation of risks.
- There is no gold standard measurement. Rather, different aspects of endothelial function can be determined by (i) dynamic tests of its vasodilatory capacity, (ii) measurement of circulating blood constituents released by endothelial cells, or (iii) urinary excretion of specific molecules such as albumin.
- Some of these measures appear to be independent predictors of vascular disease, at least in studies conducted thus far, and are potential candidates for risk factor stratification. Much more information is needed, however, particularly on non-invasive estimates of vasodilatory capacity and with more rigorous assessment of the extent of predictive ability beyond conventional risk parameters.
- Strategies proven to reduce CHD risk improve endothelial function but all also reduce several other risk factors associated with vascular disease. Such observations, therefore, are not consistent with the notion that improving endothelial function directly lessens vascular risk. Rather, improved endothelial function may be secondary to improvements in risk factor pathways ‘upstream’ in the chain of atherogenic events.
- Numerous dietary factors, such as low fat diets and fish oils, improve endothelial function. The strength of evidence for endothelial function improvement varies for differing nutrients and several have effects on other CHD risk factor pathways. Evidence of nutrient benefits on endothelial function measures cannot be used as evidence of their cardioprotective ability. Rather, randomised trials with clinical endpoints are needed.
4.12 Recommendations for future research

Clearly, several areas merit further investigation. These include:

- Improvement in the accuracy and reproducibility of currently available non-invasive measures of endothelial function.
- Assessment of the predictive ability of such measures for vascular endpoints, in particular whether such methods improve risk factor stratification beyond that achievable by classical risk factors and C-reactive protein.
- Assessment of whether improvements in measures of endothelial function predict benefit from certain therapies independently of changes in other risk factors.
- More data on the postprandial effects of dietary fat and carbohydrate on endothelial function and the associated mechanisms.
- More data on assessment of the effects of physical activity on endothelial function.
- More data on the molecular mechanisms underlying effects of therapies or lifestyle measures on endothelial function.

4.13 Key references


5 Oxidative Stress

5.1 Introduction

Oxidative stress is commonly implicated in the aetiology of chronic diseases that have an inflammatory component (Table 5.1). Undoubtedly, this is true of cardiovascular diseases or at least the development of atherosclerosis, where the invasion of inflammatory mediators (monocytes and macrophages) into the developing plaque and the release of reactive oxygen and nitrogen species are considered to be key processes linked with many of the pathological events that ensue (see Chapter 7). Perhaps what is different about atherosclerosis is that this process directly involves one of the principal risk factors in cardiovascular disease, namely the low-density fraction of lipoproteins (LDL; see Chapter 3).

5.2 The normal role of LDL

LDL transports much of the cholesterol from its origin in the liver to the peripheral tissues, where it supplies this sterol for the assembly of cell membranes and, in some cases, the formation of steroid hormones (and bile salts in the liver itself; see Chapter 3). All of these are essential physiological processes. However, LDL also can be found in synovial fluid of patients with rheumatoid arthritis (see Section 5.12). LDL contain not only cholesterol, but also esterified polyunsaturates, acquired from the diet, which are required for biosynthesis of membrane phospholipids and eicosanoids. These polyunsaturates are found in the phospholipid and cholesterol ester fractions in surprisingly large amounts that are disproportionate to normal dietary intake (Table 5.2) and are, in principle, susceptible to oxidative attack by free radicals (see Section 5.3).

Table 5.1 Common diseases in which oxidative damage has been demonstrated.

<table>
<thead>
<tr>
<th>Disease/Condition</th>
<th>Disease/Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atherosclerosis</td>
<td>Parkinson’s disease</td>
</tr>
<tr>
<td>myocardial infarction</td>
<td>Transient spinal cord injury</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Sickle cell anaemia</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>Systemic sclerosis</td>
<td>Acute respiratory distress syndrome</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>Pancreatic cancer</td>
</tr>
<tr>
<td>Inflammatory CNS demyelination</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

CNS: central nervous system.

Table 5.2 Typical fatty acid and antioxidant content of low-density lipoproteins.

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Mean number per mole apolipoprotein B100</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>30</td>
</tr>
<tr>
<td>16:0</td>
<td>280</td>
</tr>
<tr>
<td>16:1</td>
<td>18</td>
</tr>
<tr>
<td>18:0</td>
<td>50</td>
</tr>
<tr>
<td>18:1</td>
<td>170</td>
</tr>
<tr>
<td>18:2</td>
<td>420</td>
</tr>
<tr>
<td>20:4</td>
<td>60</td>
</tr>
<tr>
<td>Total fatty acids</td>
<td>1050</td>
</tr>
<tr>
<td>Total polyunsaturates</td>
<td>495</td>
</tr>
<tr>
<td>Antioxidant</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>6</td>
</tr>
<tr>
<td>γ-Tocopherol</td>
<td>0.3</td>
</tr>
<tr>
<td>β-Carotene</td>
<td>0.2</td>
</tr>
<tr>
<td>Lycopene</td>
<td>0.1</td>
</tr>
<tr>
<td>Ubiquinol</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Source: Reproduced with permission from Esterbauer et al. (1991).
However, LDL also transports a high proportion of the available fat-soluble antioxidants, particularly the tocopherols (vitamin E) and carotenoids (Table 5.2), which are also derived from the diet and which LDL delivers to the tissues. These are normally sufficient to protect the LDL polyunsaturates from oxidation. Furthermore, water-soluble vitamin C can also accept electrons from tocopherol radicals to prevent oxidation and suppress the propagation of fatty acid oxidation.

5.3 Free radicals

These are reactive species that can exist independently with one or more unpaired electrons (Halliwell & Gutteridge, 1999). Possession of an unpaired electron alters the chemical reactivity of an atom or molecule, usually making it more reactive than the corresponding non-radical (the symbol ‘•’ denotes the unpaired electron). Examples include the oxygen free radical superoxide (O$_2^-$) and the hydroxyl radical (•OH). There are also other reactive molecules, which are technically not free radicals (such as hydrogen peroxide or H$_2$O$_2$, and singlet oxygen), although these are often considered collectively with the free radicals (Table 5.3).

Many of the free radicals and non-radicals contain oxygen (these are collectively known as reactive oxygen species). They are constantly being produced in the body as by-products of normal metabolism. The reactivity of these species is very variable. Superoxide anions are relatively weak, but hydroxyl radicals (•OH) are very reactive. They are capable of damaging key macromolecules and can be harmful to the cell. Superoxide anions can be formed in a number of ways, either by enzymic activity in mitochondria, by leakage from the electron transport chain, by the action of NADPH oxidase or from xanthine oxidase (Fig. 5.1). Therefore, cells are constantly producing low levels of superoxide. Since the formation of free radicals is a part of normal metabolism, there are excellent mechanisms for preventing the accumulation of these species or dealing with the products of their oxidative effects.

Superoxide anions can be converted to hydrogen peroxide by the enzyme superoxide dismutase. In turn, this becomes water by further enzymic activity (e.g. catalase, glutathione peroxidase). Other metabolic processes produce hydrogen peroxide directly (e.g. oxidation of certain amino acids). Equally, other mechanisms exist that repair or remove damaged products.

Hydroxyl radicals (•OH) are very unstable and reactive free radicals, and are distinct from the hydroxyl ion (OH$^-$) which is formed abundantly by dissociation of water. Hydroxyl radicals are formed in more exceptional circumstances. The presence of free transition metal ions, such as iron (Fe$^{2+}$) or copper (Cu$^{2+}$), catalyses their formation from hydrogen peroxide (H$_2$O$_2$) by the Fenton reaction.

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{•OH} + \text{OH}^- + \text{Fe}^{3+}$$

For this reason, no more than trace amounts of free metal ions are found in the body (levels are too low to be measured with certainty). Metal ions are bound

<table>
<thead>
<tr>
<th>Table 5.3 Oxygen-centred free radicals and reactive oxygen species.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatomic oxygen</td>
</tr>
<tr>
<td>Superoxide anion</td>
</tr>
<tr>
<td>Hydroxyl radical</td>
</tr>
<tr>
<td>Peroxy radical</td>
</tr>
<tr>
<td>Perhydroxy radical</td>
</tr>
<tr>
<td>Ferryl radical</td>
</tr>
<tr>
<td>Protein carbonyl radical</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Singlet oxygen</td>
</tr>
<tr>
<td>Ozone</td>
</tr>
</tbody>
</table>
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to other proteins, such as haemoglobin, transferrin, ferritin and caeruloplasmin. However, excess free iron can occur, for example, following serial blood transfusions (e.g. iron overload in the treatment of thalassaemia). Under these circumstances, free radical generation exceeds the capacity of the antioxidant defence mechanisms and damage to cellular macromolecules may occur (Table 5.4).

Under normal circumstances, free radicals are produced and there are mechanisms for repair or degradation of the modified macromolecules. Free radicals and other reactive oxygen and nitrogen species (see Section 5.5) appear to be important in the first line defence mechanism against microorganisms. For example, neutrophils form a large amount of superoxide anion via the enzyme NADPH oxidase, which is converted rapidly to hydrogen peroxide. The enzyme myeloperoxidase then catalyses the formation of a known bactericide, hypochlorite (HOCl), from chloride and $\text{H}_2\text{O}_2$:

$$\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \rightarrow \text{HOCl} + \text{H}_2\text{O}$$

Hypochlorite chlorinates tyrosine residues on proteins, but causes little oxidation of lipid.

5.4 Molecular targets for oxidation

In principle, all of the major classes of the components of the body are capable of being oxidised and are summarised below.

5.4.1 Protein oxidation

Oxidation of free thiols is an early event in protein oxidation (Table 5.5). The oxidation of aromatic amino acids is often the more common event after that of thiols. The products vary according to the nature of the oxidant, although some products such as dityrosine may occur with most oxidants. Oxidation of tryptophan also occurs, giving rise to kynurenine, a species readily detected spectrophotometrically. Tyrosine residues are readily oxidised to 1-DOPA causing an autofluorescence, as well as other products outlined in Section 5.4.2. Lysine residues can be subject to oxidative attack or modification to Schiff’s bases in the presence of high concentrations of glucose, which is also an oxidative process. Less specific oxidation products of proteins may also be generated to yield carbonyls by

<table>
<thead>
<tr>
<th>Target</th>
<th>Damage</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>Scission on deoxyribose ring, base damage, strand breaks, cross linkage</td>
<td>Mutations, translation errors, inhibition of protein synthesis</td>
</tr>
<tr>
<td>Protein</td>
<td>Aggregation and cross-linking, fragmentation, breakdown modifications of thiol groups</td>
<td>Modified transport, increased calcium influx, modified enzyme activity</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>Loss of unsaturation of reactive and cytotoxic products</td>
<td>Altered membrane fluidity, permeability effects on membrane bound enzymes</td>
</tr>
</tbody>
</table>

Table 5.4 Effects of free radical damage on cellular components.

<table>
<thead>
<tr>
<th>Active agent</th>
<th>Amino acid</th>
<th>Products formed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several free radicals and oxidants</td>
<td>Peptide bonds</td>
<td>Protein carbonyl</td>
</tr>
<tr>
<td>Several oxidants</td>
<td>Methionine, cysteine</td>
<td>Sulphoxides, sulphenates, sulphoximes</td>
</tr>
<tr>
<td>Hydroperoxides</td>
<td>Aliphatic amino acids</td>
<td>Peptide hydroxides</td>
</tr>
<tr>
<td>Aldehydes, glucose</td>
<td>Lysine</td>
<td>Schiff’s bases</td>
</tr>
<tr>
<td>Several oxidants</td>
<td>Tryptophan</td>
<td>Kynurenine</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>Tryptophan</td>
<td>Nitrotryptophan</td>
</tr>
<tr>
<td>Several oxidants</td>
<td>Phenylalanine</td>
<td>$\alpha$- and $\omega$-tyrosine</td>
</tr>
<tr>
<td>Several oxidants</td>
<td>Phenylalanine</td>
<td>Dityrosine</td>
</tr>
<tr>
<td>Several oxidants</td>
<td>Tyrosine</td>
<td>1-DOPA</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>Tyrosine</td>
<td>3-Nitrotyrosine</td>
</tr>
<tr>
<td>Hypochlorous acid</td>
<td>Tyrosine</td>
<td>Chorotyrosine</td>
</tr>
</tbody>
</table>

Table 5.5 Oxidative modifications of proteins.
oxidation of amino acid side-chains. Protein may also be modified as a consequence of the oxidation of lipids and the formation of Schiff’s bases with the aldehydes (see Section 5.12).

5.4.2 Oxidation of lipids
The oxidation of lipids is evident to the lay person as fats going rancid. This process occurs in man, and there is strong evidence of its occurrence even in healthy people. The primary event in lipid oxidation depends on the abstraction of hydrogen from a double bond of polyunsaturated fatty acids and the redistribution of electrons to form conjugated dienes (Fig. 5.2).

These dienes are highly sensitive to the presence of oxygen and readily form lipid peroxides. The peroxides themselves readily degrade to form alkanes, aldehydes and a host of other minor products including isoprostanes (see Section 5.12.1). The fatty acids do not have to be free, and peroxides of intact phospholipids and cholesterol are formed as they are rich in polyunsaturated fatty acids, which are largely present in these esterified forms. Platelet activating factor (PAF), lysophosphatidylcholine and lysophosphatidic acid are also important potent biologically active products. Many oxides of cholesterol are found, all of which may be genotoxic and cytotoxic to cells.

5.4.3 DNA oxidation
Interest in DNA oxidation products is usually considered to be more relevant to cancer and to the mutations arising from oxidative stress. However, there may be a case for examination of these products.

5.5 Reactive nitrogen species
As well as nitrogen, these molecules usually contain oxygen. The unpaired electrons in the radical species
are normally associated with the nitrogen atom. Reactive nitrogen species are listed in Table 5.6. The central molecule is nitric oxide (NO), which is considered to be a supreme example of a beneficial free radical.

Nitric oxide is formed from the amino acid L-arginine by the action of the enzyme nitric oxide synthase (NOS) of which there are three isoforms. The constitutive enzymes endothelial NOS and neuronal NOS produce small amounts of nitric oxide appropriate to their respective roles as the regulator of vascular tone (and an inhibitor of platelet activation) or as a neurotransmitter (see Chapter 4). In contrast, there is an inducible form of the enzyme, known as iNOS; this synthesises much larger amounts of nitric oxide, often in other cell types such as macrophages in conditions such as infection and inflammation. This nitric oxide is also part of the front line defence mechanisms to kill invading micro-organisms.

Nitric oxide (possibly via the formation of N₂O₃) can also interact with proteins in other ways to form S-nitrosothiols. This pathway may be important for the regulation of metabolism and oxidative phosphorylation in mitochondria. In the plasma, low levels of S-nitrosothiols, which may act as a more stable pool of NO, have been found in healthy individuals (Stamler et al., 2001).

\[ 2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2 \]
\[ \text{NO}_2 + \text{NO} \rightarrow \text{N}_2\text{O}_3 \]

Nitric oxide may act in concert with superoxide anions, also released by macrophages. This reaction leads to the formation of the reactive non-radical peroxynitrite (ONOO⁻) (Fig. 5.3).

Peroxynitrite is protonated to peroxynitrous acid and is rapidly degraded to various products including the hydroxyl radical and the powerful oxidant nitrogen dioxide, also a free radical (Fig. 5.3). These products lead to important modifications of macromolecules (Fig. 5.4). The nitration of tyrosine residues in proteins is used as a fingerprint for the formation of peroxynitrite in tissues, especially in inflammation, although other products are also formed (Fig. 5.5). However, there is emerging evidence that nitration of proteins occurs in normal physiological processes and that it may be reversible, or the nitrated protein may be removed by calcium-activated proteases. Examples of this are spontaneous nitration of proteins in sperm capacitation.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO + O₂⁻</td>
<td>ONOO⁻ → ONOO⁻ (Peroxynitrite)</td>
</tr>
<tr>
<td>Rate constant &gt; 10⁹ mol/s</td>
<td></td>
</tr>
<tr>
<td>ONOO⁻ → OH⁻ + NO₂⁻</td>
<td></td>
</tr>
<tr>
<td>ONOO⁻ → OH⁻ + NO₂⁻</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5.3** Reaction of nitric oxide (NO) to form peroxynitrite (ONOO⁻) and its degradation.

**Fig. 5.4** Reactivity of peroxynitrite with macromolecules.

**Fig. 5.5** Nitration of protein tyrosine residues with peroxynitrite.

### Table 5.6 Nitrogen-centred free radicals and reactive nitrogen species.

<table>
<thead>
<tr>
<th>Radical</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO⁻</td>
<td>Nitric oxide (nitrogen monoxide)</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>Nitrogen dioxide</td>
</tr>
<tr>
<td>ONOO⁻</td>
<td>Peroxynitrite</td>
</tr>
<tr>
<td>N₂O₃</td>
<td>Nitrous oxide</td>
</tr>
<tr>
<td>N₂O₃</td>
<td>Nitrous oxide</td>
</tr>
</tbody>
</table>

**Fig. 5.3** Reaction of nitric oxide (NO) to form peroxynitrite (ONOO⁻) and its degradation.
(Herrero et al., 2001) or nitration, resulting from pulsatile flow in endothelial cells, of signalling proteins such as c-Jun NH₂-terminal kinase (also known as stress-activated protein kinase; Go et al., 1999). It also occurs spontaneously during activation of platelets with collagen (Sabetkar et al., 2002).

It is not certain that all nitration originates as a consequence of the formation of peroxynitrite, and there is evidence that it can be promoted by enzymes. Superoxide dismutase and myeloperoxidase are both capable of nitrating other proteins, the latter only requiring the presence of nitrite and hydrogen peroxide (Sampson et al., 1998). Similarly, haem-containing peroxidase may also perform this function. In the case of myeloperoxidase, it has been shown that the hydrogen peroxide leads to the formation of higher oxygen states of haem and in the presence of nitric oxide produces the nitrogen dioxide radical (NO₂⁻), which is potentially a nitrating species (Abu-Soud & Hazen, 2000). It should be mentioned that peroxynitrite and other oxidants can also give rise to the formation of dityrosine, as well as ortho- and meta-tyrosine from phenylalanine.

5.6 Cardiovascular disease and oxidative reactions

The main risk factors for cardiovascular disease are smoking, hypertension and hyperlipidaemia (i.e. elevated LDL-cholesterol), while high-density lipoproteins (HDL) are beneficial (see Chapter 1, Section 1.4.4). Cigarettes contain approximately 10¹⁵ free radicals per puff and smoking is associated with low levels of plasma ascorbate (vitamin C) and damage to the endothelium of the blood vessels. Smoking has the strongest influence on the risk of early death from cardiovascular disease. Cigarette smoke contains large amounts of nitric oxide, which is mainly converted to the powerful oxidant nitrogen dioxide radical during transfer through the alveoli.

When LDL is persistently exposed to an oxidising environment, oxidation occurs. The presence of transition metal ions, such as copper and iron, catalyses this process, especially in the presence of free radicals or other oxygen and nitrogen-centred free radicals that may be released from cells under inflammatory conditions and as the result of smoking.

The work of Esterbauer and colleagues during the 1980s (Esterbauer et al., 1990) revealed a great deal of information about the chemical processes that occur during the oxidation of LDL in vitro, often simply by exposure of LDL to ferrous or cuprous ions (Fig. 5.6). This led to work on both the oxidation of lipids by a series of propagated free radical reactions and the modification of proteins. Combined with the work of Steinberg and colleagues on oxidation of LDL by long exposure to cells in culture (Witztum & Steinberg, 2001), it was possible to show that LDL could be modified to such an extent that it could not be recognised by its own normal receptor. Instead, oxidised LDL binds strongly to the scavenger receptors of macrophages, following which the lipoproteins are internalised by these cells (see Chapter 3). Many classes of these receptors have been identified. The main function of the macrophages is to remove cell debris from the site of inflammation through receptor-mediated and phagocytic processes. In effect, the receptors recognise the high surface negative charge of dead cells. This is also a feature of oxidatively modified LDL, where the positively charged lysine residues by which the LDL receptor normally recognises the LDL particles have become neutralised by the formation of Schiff’s bases from the aldehydes formed by the oxidation of lipids. Therefore, the macrophages now take up these abnormal lipoproteins, internalise them and ultimately become foam cells stuffed with oxidised lipid (Fig. 5.7). The presence of chain-breaking antioxidant vitamins delays this process, and for this reason they are considered to be beneficial (Fig. 5.6). The main changes involve oxidative breakdown of the lipids to form aldehydes, alkenals and other products, including peroxides, lysophosphatidylycerol and oxides of cholesterol (Table 5.7).

### Table 5.7 Products formed during the oxidation of low-density lipoproteins.

<table>
<thead>
<tr>
<th>Lipid products</th>
<th>Protein modifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjugated dienes</td>
<td>Loss of tryptophan, loss of lysine, oxidation of -SH groups, protein carbonyls</td>
</tr>
<tr>
<td>Lipid peroxides</td>
<td>Formation of adducts between lysine and aldehydes</td>
</tr>
<tr>
<td>Alkenes (ethane, pentane)</td>
<td>Change in electrophoretic mobility of LDL (more negative)</td>
</tr>
<tr>
<td>Aldehydes malonaldehyde 4-HO nonenal Isoprostanes</td>
<td>Cholesterol oxides</td>
</tr>
<tr>
<td>Lysophosphatidylycerol</td>
<td>Lysophosphatidic acid</td>
</tr>
<tr>
<td>PAF</td>
<td></td>
</tr>
<tr>
<td>Cholesterol oxides</td>
<td></td>
</tr>
</tbody>
</table>

PAF: platelet activating factor.
Cardiovascular Disease

5.7 Oxidation of LDL in the artery wall

LDL is a large lipid and protein complex, but can move readily through the endothelium; the concentration of the lipoprotein in the arterial intima may be similar to that in the blood. The basement membrane and the media form a more significant barrier, and the concentrations deeper in the artery wall are one-fiftieth or less than those in the intima.

The site of oxidation of LDL is in the artery wall and this can be detected in atherosclerotic plaque using specific antibodies. Therefore, most of the oxidation must occur in the intima and is often associated with the presence of macrophages. In the process of oxidation, many cytotoxic products are released from the oxidation of lipids (e.g. lipid peroxides, lysophosphatidylcholine, and cholesterol oxides), all of which have profound effects on the cells of the artery wall (Fig. 5.7). Furthermore, the macrophages of the artery wall accumulate these modified LDLs because they are recognised by the scavenger receptors. The macrophages can hydrolyse and catabolise most of the protein and lipid, but not cholesterol, which accumulates either in the free form or as cholesterol esters. Furthermore, the presence of cytotoxic lipid oxidation products damages the lysosomal digestive process, and the cells become enlarged as foam cells and eventually die stuffed with lipid.

The oxidised lipids also cause damage to the endothelium, which expresses more adhesion molecules to attract further macrophages to the region and exacerbates the situation. They also impair the formation of endothelial nitric oxide (see Chapter 4). However, oxidised LDL induces the formation of nitric oxide and superoxide by macrophages, as has been reviewed by Rice-Evans and Bruckdorfer (1995).

5.8 Nature of the oxidising species causing LDL oxidation

Whereas many oxidants are capable of causing oxidation in vitro, the nature of the oxidising species important in vivo is still a matter of discussion.

![Free radical reactions modify the lipids and proteins of LDL. A: ascorbate; ApoB: apolipoprotein B; LDL: low-density lipoprotein; MDA: malondialdehyde; toc: tocopherol.](image-url)
Oxidative Stress

Haem-containing proteins, such as methaemoglobin or metmyoglobin, are also reported to be capable of inducing oxidation. Alternatively, enzymes such as lipoxygenases, which oxidise polyunsaturates, are able to cause these changes (Fig. 5.7). The identity of the specific reactive species will be discussed later, but the actions of superoxide anions alone are very weak. Other enzymic modifications, such as exposure to elastase or cholesterol esterase, may give rise to uptake of LDL by macrophages without extensive oxidation of lipid, although there may be effects on the proteins. It is certainly possible that the oxidising species are not always the same in all atherosclerotic plaques and that a search for a single agent may be futile. There may even be differences at progressive stages of atherosclerosis. In more complex plaques there is often evidence of microthrombi, carrying with them traces of iron, that may then become secondary oxidants, which are different from those that initiated the process.

There is clear evidence of oxidative modification of proteins in atherosclerosis (Aviram, 2000). Using specific antibodies it has been easy to detect the presence of oxidised proteins and LDL. In some cases more specific forms of modification have been determined in which nitrated (Beckman et al., 1994) and chlorinated proteins (Hazen & Heinecke, 1997) have been detected. There is some very recent evidence that chlorination may occur early in atherosclerosis, whereas nitration may be a later event. Little has been achieved in terms of the identification of the modified species, although several types of proteins may be modified (Harrison et al., 2003). Quite separately, the accumulation of lipid oxidation products, such as lysophosphatidylcholine and cholesterol oxides, has been found in the plaques.

Nevertheless, it has been more difficult to demonstrate the presence of oxidative modification outside the atherosclerotic plaque in fluids such as blood and urine, to facilitate diagnosis. Relatively little of this
modified lipid and protein leaks out into the blood or, if it does, the rates are relatively modest so that it can be removed readily from the circulation. It must also be remembered that the mass of the plaque is small relative to the size of the main organs and tissues, which may be able to metabolise or sequester them in other ways. Therefore, the changes in the concentrations of these products will inevitably be small, making their usefulness as markers less apparent.

Nevertheless, there are many reports of increases in ethane expiration, serum and urinary isoprostane concentrations, increased susceptibility of LDL to external oxidising agents, decreases in antioxidants, and increases in plasma lipid peroxides and thiobarbituric acid reactive substances (TBARS; see Section 5.12).

5.9 Cardiovascular disease and the consumption of fruit and vegetables

Much of the emphasis on the dietary aspects of cardiovascular disease relate to the fat hypothesis, which suggests that lipoprotein concentrations are regulated largely by the amount and type of dietary fats consumed (see Chapters 1 and 3). Plant-based diets are also associated with a lower risk of cardiovascular disease. While such diets tend to be lower in fat (particularly saturates), the beneficial health effects have also been widely attributed to the antioxidants found in these foods.

Many years ago it was noted that rates of coronary heart disease (CHD) were higher in areas where fruit and vegetable consumption was lowest (Armstrong & Doll, 1975). More recent studies have also shown that European countries whose populations consume more fruit and vegetables have lower rates of CHD (Rimm et al., 1996; La Vecchia et al., 1998; Simopoulos, 2001), and analyses of trends over time demonstrate an inverse relationship between fruit and vegetable consumption and rates of heart disease (Zatonski et al., 1998). Vegetarians, who generally have higher intakes of all plant foods, exhibit lower rates of CHD (Thorogood, 1997). However, this may be a reflection of healthier dietary or lifestyle patterns rather than any particular dietary constituent, as those who eat more fruit and vegetables tend to smoke less, drink less alcohol, exercise more (Serdula et al., 1996), come from the higher social classes (Thorogood, 1997) and also eat less fat as a proportion of their energy intake. However, these indirect associations do not explain all of the protective effect of high fruit and vegetable consumption, and the presence of directly beneficial dietary constituents such as antioxidants has led to considerable research interest (Buttriss et al., 2002a; Stanner et al., 2004).

Analysis of a number of ecological, case-control and cohort studies showed a strong protective effect of fruit and vegetables for stroke and a weaker protective effect for cardiovascular disease (Ness & Powles, 1997). This has also been supported by subsequent work. For example, Joshipura et al. (1999) followed the diet of over 75 000 nurses and nearly 39 000 male health professionals and noted a 31% reduction in the risk of stroke in the fifth eating the most fruit and vegetables compared with the fifth eating the least. In a meta-analysis of cohort studies investigating the relationship between CHD and markers of fruit and vegetable consumption (both the foods themselves and related nutrients), the risk of CHD was about 15% lower at the 90th than the tenth centile of fruit and vegetable intake (Law & Morris, 1998).

Intervention studies with fruit and vegetables are fewer in number and have often involved several simultaneous dietary interventions. The Lyon Diet Heart Study of patients surviving a first myocardial infarction found that those who followed a ‘Mediterranean’ diet experienced a significant reduction in the occurrence of subsequent cardiac death and non-fatal myocardial infarction over 4 years (Lorgeril et al., 1994, 1998). A fat modified diet, enriched with fruit and vegetables and with moderate physical activity reduced re-infarction and total mortality rates (Singh et al., 1996), and increasing fruit and vegetable intake has also been shown to lower cardiovascular disease risk factors such as blood cholesterol levels and blood pressure (Appel et al., 1997; Jenkins et al., 1997).

There is an interaction between fruit and vegetable intake and smoking, as lower levels of plasma antioxidants are consistently found in smokers (Murata, 1991; Schechtman et al., 1989). This may be in part due to poor diets or the oxidant stress caused by smoking (see Section 5.6). Cigarette smoke contains free radicals, and smoking is associated with low levels of plasma ascorbate and damage to the endothelium of the blood vessels (see Chapter 3). Of all the major risk factors, smoking has the strongest
influence on the risk of early death from cardiovascular disease.

The pioneering work of Gey (1998) and others has shown a north–south gradient in cardiovascular disease risk across Europe, with a high incidence in northern Europe, and an inverse relationship with the plasma concentrations of antioxidant vitamins in these populations, particularly for vitamin E. Other studies have found similar relationships with selenium and flavonoids (Shamberger, 1978; Hertog et al., 1995). These findings have been largely substantiated in case-control studies (Beaglehole et al., 1990; Rimmersma et al., 1990), although such studies are unable to exclude the possibility of changes in nutrient status as a result of onset of disease or associated lifestyle modifications (see Chapter 1). A number of large prospective studies, which provide more convincing evidence of an association (see Chapter 1), have demonstrated decreasing risk of cardiovascular disease with higher dietary intake of antioxidant nutrients (Kardinaal et al., 1993; Rimm et al., 1993; Stampfer et al., 1993; Knekt et al., 1994) and with higher plasma levels of tocopherol and β-carotene (Gaziano & Hennekens, 1993; Christen et al., 2000). The European Prospective Investigation of Cancer (EPIC) study found a relationship between high levels of ascorbate and reduced risk of cardiovascular disease (Khaw et al., 2001), but the levels can also be strongly influenced by other factors such as inflammation (see Chapter 7).

Interestingly, the findings of prospective studies that have investigated the value of vitamin supplements (predominantly in the USA) have been less convincing. In the Nurses’ Health Study, women in the highest (fifth) quintile of vitamin E intake (median intake of 208 IU/day) using supplements of vitamin E, had a 44% lower risk of CHD (after adjustment for confounding factors) compared to those in the lowest quintile of intake (Stampfer et al., 1993). Those in the fourth quintile for dietary intake of vitamin E (an average intake of 17 IU/day), who relied on diet rather than supplements, also demonstrated a lower risk (26% lower than the first quintile). Similar data were found in a cohort of men (Rimm et al., 1993). In the cohort of more than 34 000 post-menopausal women from the Iowa Women’s Health Study, an inverse association was found between dietary vitamin E intake and coronary deaths (Kushi et al., 1996) and deaths from stroke (Yochum et al., 2000) among the women who did not take vitamin supplementation, but vitamin E supplements were not associated with a protective effect on cardiovascular disease deaths (see Section 5.10 for information on intervention studies).

There is growing interest in the role of the plant-derived substances known to be antioxidants (British Nutrition Foundation, 2003a). Intake of antioxidant flavonoids has also been shown to be inversely associated with CHD risk in Dutch men (Hertog et al., 1993). An inverse association between intake of flavonoids, mainly from apples and onions, and cardiovascular disease mortality risk was also reported in a Finnish study (Knekt et al., 1996). However, flavonoid intake was not associated with incidence of a first non-fatal myocardial infarction in a cohort of US male health professionals. The Iowa Women’s Study showed flavonoid intake to be associated with a decreased risk of death from CHD after adjustment for age and energy intake (Yochum et al., 1999). A study within the Iowa cohort found a strong inverse association between CHD and intake of some types of catechins (a subclass of flavonoids), which are known to inhibit peroxynitrite induced oxidations, particularly the water soluble epigallocatechin gallate found in green tea (Pannala et al., 1997). However, the antioxidant effects appear to be restricted to inhibition of nitration of proteins (Schroeder et al., 2001). It should be noted that a significant contribution to dietary polyphenols is made by cocoa products, e.g. chocolate (Arts et al., 1999). Among other properties, increased cocoa consumption appears to inhibit platelet activation (Rein et al., 2000).

The role of selenium in the activity of specific antioxidant enzymes, particularly glutathione peroxidase, has been well established (Holben & Smith, 1999), and it is therefore considered to be an important antioxidant nutrient. In cohort studies, patients with myocardial infarction had low plasma selenium concentrations (Navarro-Alarcon et al., 1999). Nevertheless, some studies show an inverse relationship between plasma selenium concentrations and cardiovascular disease, whereas others show none (Huttunen, 1997). Low levels of selenium have been associated with cardiomyopathy in China, but its importance in cardiovascular disease remains controversial (Neve, 1996). Glutathione peroxidase reduces peroxynitrite to nitrite and may be important in cardiovascular disease in this respect (Sies et al., 1997). Prospective studies investigating low selenium status and heart disease have produced
mixed results: the two studies that found an association (Salonen et al., 1982; Virtamo et al., 1985) were conducted in Finland where selenium intake was very low, one showing a 3.6 fold increase in coronary deaths and a 2.7 fold increase in heart attacks amongst men with low serum selenium levels (<45 µg/l); in populations with higher selenium intakes, no associations were found (Miettinen et al., 1983; Ringstad & Fonnebo, 1987; Salvini et al., 1995). These findings suggest that cardiovascular risk may only be increased by very low selenium status (British Nutrition Foundation, 2001).

5.10 Therapeutic use of antioxidants: evidence from intervention trials

It may have been a gross over-simplification, but it was assumed by many that the simple solution to an oxidative stress was to administer one, or a cocktail of two or three, antioxidants. Despite calls for caution, sales of over-the-counter antioxidants rose sharply. The evidence looked clear and was supported by substantial work with antioxidants in experimental atherosclerosis and animal models. In support of this hypothesis, people in higher socioeconomic classes who suffer lower rates of cardiovascular disease compared to lower socioeconomic groups (see Chapter 1) eat more fresh fruit and vegetables and they smoke less (see Section 5.9), with the net effect of improving their antioxidant status. However, the results of a number of intervention studies have now been published. Benefit from supplementation with alpha-tocopherol in terms of secondary prevention of cardiovascular events (non-fatal myocardial infarction) was demonstrated in one small study, but by no means in all of them (Table 5.8).

The most positive results came from the Cambridge Heart Antioxidant Study (CHAOS), a controlled trial on 2002 heart disease patients with angiographically proven coronary atherosclerosis,

<table>
<thead>
<tr>
<th>Study type</th>
<th>Name</th>
<th>Results</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ecological and cross-sectional studies</td>
<td>–</td>
<td>High intakes of fruit and vegetables related to low risk of cardiovascular disease</td>
<td>e.g. Armstrong &amp; Doll (1975); Rimm et al. (1996); La Vecchia et al. (1998); Simopoulos (2001)</td>
</tr>
<tr>
<td>Cohort studies</td>
<td>–</td>
<td>Low concentrations of plasma antioxidants in patients with high risk of cardiovascular disease</td>
<td>e.g. Gaziano &amp; Hennekens (1993); Christen et al. (2000); Khaw et al. (2001)</td>
</tr>
<tr>
<td>Cross-sectional</td>
<td>WHO-MONICA study</td>
<td>Association of low levels of serum α-tocopherol with high risk of cardiovascular disease</td>
<td>Gey et al. (1991)</td>
</tr>
<tr>
<td>Cohort</td>
<td>US cohorts of health workers</td>
<td>Advantage of high α-tocopherol intake</td>
<td>Rimm et al. (1993); Stampfer et al. (1993)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>US Physicians’ trial</td>
<td>No beneficial effects of β-carotene</td>
<td>Hennekens et al. (1996)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>ATBC study</td>
<td>Male smokers had no benefit from antioxidant therapy (β-carotene or vitamin E). Actual increase in cancer risk</td>
<td>Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group (1994); Tornwall et al. (1999, 2001); Leppala et al. (2000)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>CHAOS</td>
<td>47% decrease in fatal and non-fatal MI with α-tocopherol supplementation</td>
<td>Stephens et al. (1996)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>GISSI trial</td>
<td>No benefit of supplementation with α-tocopherol but fish oils were beneficial</td>
<td>GISSI-Prevenzione Investigators (1999)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>HOPE study</td>
<td>No apparent beneficial effects of antioxidants</td>
<td>HOPE Investigators (2000)</td>
</tr>
<tr>
<td>Intervention trial</td>
<td>MRC/BHF Oxford study</td>
<td>No benefits of α-tocopherol supplementation – statin was effective alone</td>
<td>Heart Protection Study Collaborative Group (2002b)</td>
</tr>
</tbody>
</table>
randomly assigned to receive vitamin E or an inactive placebo (Stephens et al., 1996). The trial continued for almost two years. Vitamin E treatment significantly reduced the risk of cardiovascular disease death and non-fatal myocardial infarction combined. However, the decrease was primarily due to a dramatic reduction in non-fatal myocardial infarction, and cardiovascular disease deaths did not alter significantly. Other smaller trials have also demonstrated beneficial effects of antioxidant supplementation in groups of patients at high risk of oxidative stress (Boaz et al., 2000). In another randomised placebo-controlled trial of 156 men aged 40–59 years with previous coronary artery bypass graft surgery, subjects supplemented with 100 IU or more of vitamin E per day experienced less coronary artery lesion progression than subjects supplemented with less than 100 IU per day (Hodis et al., 1995; Azen et al., 1996).

This is substantiated by a more recent study in which tocopherol supplementation suppressed restenosis in surgically induced atherosclerosis (Fang et al., 2002).

However, most of the other secondary prevention studies, which include many thousands of patients, have shown no protective effect of either single antioxidants nor of tocopherol in combination with beta-carotene or vitamin C, e.g. the GISSI Prevenzione and HOPE studies (GISSI-Prevenzione Investigators, 1999; HOPE Investigators, 2000). For example, the Alpha Tocopherol Beta Carotene Prevention Study (ATBC) demonstrated no effect of beta-carotene or vitamin E supplementation on the incidence of large abdominal aortic aneurysm (Tornwall et al., 2001) or on symptoms and progression of intermittent claudication (Tornwall et al., 1999) amongst men supplemented for an average of 6 years. The vitamin E supplement was associated with a reduction in cerebral infarction, but there was a 50% increase in haemorrhagic stroke mortality (Leppala et al., 2000). The net effect on all strokes was a small decrease in mortality. Supplemental beta-carotene actually increased the incidence of cerebral haemorrhage (Leppala et al., 2000) and led to an increase in deaths from myocardial infarction (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group, 1994). In general, the therapy has been particularly disadvantageous to smokers, for which theoretical explanations can be found. The Beta Carotene and Retinol Efficacy Trial (CARET) was terminated early because subjects receiving a combination of supplements (30 mg beta-carotene, 25 000 IU vitamin A) experienced a 28% increased risk of lung cancer incidence in current, but not former, smokers (Omenn et al., 1996). However, in another trial with beta-carotene (US Physicians) no effects on smokers were observed (Hennekens et al., 1996). A meta-analysis of randomised evidence from published trials before 2002 of beta-carotene supplementation, involving 70 000 people in three large-scale trials in healthy populations, and on vitamin E supplementation in 29 000 patients at high risk of cardiovascular disease in five large-scale trials, failed to confirm any protective effect of these vitamins for cancer or cardiovascular disease (Clarke & Armitage, 2002).

The MRC/BHF Heart Protection Study examined the effects of a cocktail of antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C, and 20 mg beta-carotene) or placebo in 20 536 UK adults aged 40–80 years with coronary disease, other occlusive arterial disease or diabetes mellitus who were supplemented for 5 years (Heart Protection Study Collaborative Group, 2002b). Although this regimen substantially increased blood levels of antioxidant vitamins, it did not produce any significant reductions in the 5-year mortality from vascular disease, cancer or any other major outcome. The protection afforded by treatment with a cholesterol-lowering statin was in stark contrast to the lack of effect of the antioxidants. In the GISSI-Prevenzione trial, dietary fish oils reduced the risk of death, non-fatal myocardial infarction or stroke, but vitamin E supplementation (300 mg daily for 3.5 years) did not provide any benefit (GISSI-Prevenzione Investigators, 1999). However, it must be pointed out that hypercholesterolaemia per se appears to induce LDL oxidation. Hence the effects of vitamin E may be less marked as a result of cholesterol lowering by statins. Therefore, meta-analyses show that supplementation with antioxidants does not provide the benefit that was originally anticipated (Marchioli, 1999; Asplund, 2002; Clarke and Armitage, 2002).

Taking these trials together, the results appear to have dealt a body blow to the oxidation hypothesis. There are several possible explanations for this:

- The habitual diet of many of the subjects investigated is unlikely to have been low in the antioxidant vitamin under investigation, and supplementation may saturate body tissues. In the case of vitamin C most individuals would need 100 mg/day in order...
to reach saturation levels, while higher doses may be required in others.

- Not all antioxidants act with equal activity against different free radicals and free radical reactions. Therefore, the limited antioxidant selection used in the intervention studies may not be appropriate.

- The oxidation process is initiated at an early stage in atherosclerosis. Supplementing people in whom this process has already occurred may be unsuccessful because antioxidants may be unable to reverse existing damage.

- Antioxidants may be unable to access the necrotic core of unstable plaques, which pose the highest risk for cardiovascular disease, in which case late antioxidant therapy would be ineffective. Evidence that antioxidants do have beneficial effects on vasomotor tone (endothelium-dependent relaxation) suggests that the endothelium and intimal regions may be more accessible (see Chapter 4).

- Under certain conditions, for example where free transition metal ions or delocalised, iron-containing proteins are available, antioxidants have pro-oxidant properties and may promote further damage.

- The primary initiating factor in atherosclerosis is either high levels of blood cholesterol or smoking, or both. Oxidation processes play a role in the development of atherogenesis, but not a central one. Consumption of a diet rich in fruit and vegetables and other plant foods typically means that there is a lower consumption of the dietary constituents likely to raise blood cholesterol.

5.11 Oxidative stress and exercise

Although this subject may be slightly peripheral to the main topic, there are a small number of points that should be highlighted. It is universally accepted that regular exercise has an excellent preventative action against cardiovascular disease (see Chapter 12). It increases HDL-cholesterol levels in the plasma, lowers levels of very-low-density lipoprotein (VLDL) cholesterol, increases blood flow, reduces obesity and has many other benefits. Yet there is accumulated evidence that exercise, particularly severe exercise, increases the production of reactive oxygen and nitrogen species (Sen, 2001) which seems to be a negative attribute. This leads to increases in the normal products of oxidation such as isoprostanes and malondialdehyde and protein damage (this has been reviewed by Sacheck and Blumberg (2001)). However, there appears to be an adaptive response in that there are increases in the expression of some antioxidant enzymes, such as mitochondrial superoxide dismutase (Ji, 2002). It appears that this defensive response is attenuated with age (Ji, 2002). There have been many studies in which the effects of antioxidant vitamins have been investigated; not surprisingly, the results are mixed and may depend on factors such as the severity of the exercise and the age of the individual. In general, the benefits of antioxidants such as ascorbate may be only modest (Thompson et al., 2001).

5.12 Measurable factors for oxidative stress in cardiovascular disease

Since the specific effects of oxidative stress are manifested within the tissues of the artery wall, access to this material in living patients would be difficult to say the least. Therefore, measurement of these parameters in readily accessible biological fluids is the only option, but this in itself does lead to some difficulties in interpretation. It would had to be assumed that the products of oxidation had leached out of the tissue into the circulation and that they were not completely removed by other organs as part of the repair and antioxidant mechanisms. At the same time, the markers may also represent the extent of oxidant stress in the whole body and not simply that of the arterial tree. The mass of the atherosclerotic plaque is small compared to that of the body and, therefore, significant amounts would have to exit the tissue for any change to be measurable. Furthermore, such measurements may be complicated by the existence of other diseases that may have oxidant stress as part of their aetiology, for example arthritis where there is a clear role of radical damage that may lead to the release of oxidised products from the synovium. In addition, there may be changes as a result of the ageing process. These may make measurements of generalised oxidative stress difficult to interpret as markers for cardiovascular disease.

As a result, there may be a case for looking at markers that relate directly to the oxidation of LDLs since, according to the hypothesis, this is central to the events that occur in the plaques. Again, LDL oxidation is not unique to atherosclerotic plaques and may also occur in synovial fluid (Dai et al., 1997), or indeed in plasma, in other cases of oxidative stress such as systemic sclerosis (Bruckdorfer et al., 1995).
With this caveat, the remainder of this section outlines the main markers of oxidative stress that can be measured, including those derived from lipids and those resulting in the modification of proteins. In addition, there are the measurements of the antioxidants themselves, which are at least of indirect relevance. Furthermore, there are measurements that test the susceptibility of LDL to oxidation and the presence of antibodies to oxidatively modified LDL (Fig. 5.7; see review by Rice-Evans et al. (1996)). In some circumstances one may select the products of a specific oxidising species (e.g. peroxynitrite).

5.12.1 Measurable products of lipid peroxidation

The measurement of lipid oxidation has been attempted over a number of decades; often the early methods were relatively crude and possibly misleading. Halliwell (2000) has constructed a number of tests which may guide the use of the different techniques for this purpose (Table 5.9); he admits that there is no perfect method, but some are more reliable than others.

Table 5.9 Properties of an ideal assay method to measure lipid peroxidation.

1. It should quantitate a major product(s) of the peroxidation process.
2. The coefficient of variation between multiple runs of the same sample should be very small in comparison with the differences between subjects.
3. It must not be subject to interference by other biomolecules.
4. It must employ chemically robust measurement methods (e.g. mass spectrometry, or HPLC with good identification methods such as diode array or coulometric detectors) or be validated by such methods (e.g. an immunoassay for isoprostanes that is calibrated against, and gives the same results as, mass spectrometry).
5. It must not be confounded by uptake of oxidised lipids present within foods.
6. It should be able to assess both steady-state levels of peroxidation products (i.e. the balance between rates of peroxidation and rates of metabolism/clearance of peroxidation products) and total rates of ongoing lipid peroxidation.
7. The parameter measured should be stable on storage (essential for human epidemiological studies) and should not be formed artifically in stored samples.

HPLC: high performance liquid chromatography.
Source: Reprinted from Halliwell (2000) with permission from the European Society of Cardiology.

(i) Malondialdehyde

One of the most common measurements in the past was that of malondialdehyde (MDA) by the thiobarbituric acid reactive substances (TBARS) test. This has largely been discredited, especially in biological fluids, even when the measurements are made by high performance liquid chromatography (HPLC), rather than by the colorimetric method. There appear to be too many interfering factors to make this a reliable test. However, much lower, submicro-molar concentrations of MDA are detected using gas chromatography–mass spectrometry (GC-MS) methods. This indicates that the problem of interference can be overcome, but some uncertainty remains and there are also problems with stability in frozen stored samples. However, a consortium investigating methods for measurement of oxidative stress in the USA still believes this measurement could be useful (Kadiiska et al., 2000).

Similar approaches can be used to measure 4-hydroxynonenal, a more significant aldehyde produced as a cleavage product from the oxidation of polyunsaturated lipids that may be detected by HPLC (Lang et al., 1985).

(ii) Conjugated dienes

As primary products of lipid oxidation, these would appear to be important substances to measure (Iversen et al., 1985). They absorb readily in the ultraviolet (UV) range, but at wavelengths that are subject to interference by other substances. They also readily break down to other lipid oxidation products. They can be measured more accurately in HPLC and GC-MS, but have not really been convincing as a marker. The most common usage is a dynamic measurement of the oxidation of LDLs in vitro (Esterbauer et al., 1991).

(iii) Lipid peroxides

The measurement of lipid peroxides requires more direct techniques, often using iodimetric assays (El-Saadani et al., 1989). This has been conveniently achieved by the ferrous oxidation–xylene orange (FOX) assay (Jiang et al., 1992), which like the iodine liberation technique gives rise to high levels of peroxides in plasma, which may not be realistic but are useful for measurements in isolated LDL. There are
other methods where lipid peroxide can be measured by HPLC with fluorescence, chemiluminescence (Frei et al., 1988) or MS detection, which yield values of peroxides up to two orders of magnitude. These are distinctly research tools (gold standard) rather than a convenient routine assay. There is a possibility that the results may be confounded by the absorption of lipid peroxides present in dietary fat, especially unsaturated fats that have been used for cooking, and these methods are not in general use.

(iv) Alkane gases
Another possibility is the measurement of small organic molecules, such as ethane and pentane, which are minor components of the oxidation products and are released into the breath (Schaeffer, 1989). These are not easy measurements (capillary GC is used) and do depend on the decomposition of peroxides, which varies between tissues. New technological developments may improve this technique.

(v) Lysophospholipids and cholesterol oxides
Other products such as cholesterol oxides and lysophosphatidylcholine have been considered, but these too are rapidly metabolised and do not accumulate in the blood as they do in atherosclerotic tissue. Nevertheless, these may also prove to be useful markers using HPLC-MS (Sevanian et al., 1994).

(vi) Isoprostanes
By far the most convincing measurement to be made is that of the F2-isoprostanes which again arise from the oxidation of unsaturated fatty acids (Morrow & Roberts, 1997). Several products can be measured with great sensitivity using GC-MS techniques. A key product measured is 8-iso-PGF$_{2\alpha}$ (8-iso-prostaglandin F$_{2\alpha}$) because of its known biological activity (Fig. 5.8), but others are under consideration. Despite the fact that they have a short half-life, they seem to be produced at a steady rate which is increased under oxidative stress and in several diseases. As in the case of the lysophospholipids, there is a considerable literature showing the biological activities of the isoprostanes, which include vasoconstriction (Janssen, 2002) and platelet activation (Minuz et al., 1998).

The isoprostanes can be measured in blood or from a 24-hour urine output, although in the latter case, passage through the kidney leads to the formation of 2,3 dinor iso-prostaglandin F$_{1\alpha}$ (2,3 dinor iso-PF$_{1\alpha}$) which can be measured. The preferred method for measurement of multiple products is HPLC with tandem mass spectrometry (Li et al., 1999).

(vii) Determination of circulating antioxidants
Measurement of plasma or lymphocyte antioxidant concentrations is relatively routine and does give useful information of the nutritional status of individuals. It may also be an index of oxidative stress, since in some inflammatory disorders the ascorbate levels may be low, even though food intake is normal. However, these measurements alone are unlikely to be good direct markers of oxidative stress. The measure of total antioxidant capacity (TAC) of plasma was thought to be a useful adjunct to other measurements (also known as TRAP or TAS assays). Although easy to employ, there is considerable concern over the physiological relevance of these measurements and also the reliability of the data obtained.

(viii) In vitro oxidation of LDL
In the 1980s this method was developed to test the effects of antioxidants on the oxidation of LDL, which could be monitored readily by UV absorption resulting from the formation of conjugated dienes after addition of oxidants such as transition metal ions. Simultaneous measurements of isolated LDL
samples (usually up to 12) could be made in an automated UV spectrophotometer; the method had some potential as a diagnostic tool and is reviewed in detail by Rice-Evans et al. (1996). There is currently greater scepticism about this technique and its physiological significance, although it is still used as an experimental method. There are several parameters that can be obtained from the data, the most frequently used being the lag time before oxidation takes place, which is considered to coincide with the point at which endogenous antioxidants have been consumed. The slopes and maximal changes in conjugated diene formation have also been exploited (Fig. 5.9).

There is evidence that the rates of oxidation may depend on the fatty acid composition of the LDL, as well as the amount of antioxidant present (see Section 5.13). In experimental animals, and in normal and hypercholesterolaemic human subjects, LDL enriched with n-6 polyunsaturates by dietary means appeared to be more readily oxidised than monounsaturates with the same chain length (Parthasarathy et al., 1990; Reaven et al., 1991, 1993).

In conclusion, the general consensus is that measurement of isoprostanes is the best available indicator of lipid oxidation, especially when considering oxidation in the intact individual. However, more convenient, rapid and accurate methods are required for clinical work.

5.12.2 Methods for detection of protein oxidation

(i) Protein carbonyl formation

A major problem with this technique is that it does not distinguish well between oxidation and modification of proteins by glycation and other processes. Simple detection techniques measuring optical absorption at 360 nm following reaction with diphenylhyrazine will be subject to significant interference (for a review see Griffiths et al. (1999)) and are therefore not suitable for use in biological fluids and tissues. More complex chromatographic techniques have been applied, and antibodies can be used to detect the hydrazones in another way. Indeed there is an enzyme-linked immunosorbent assay (ELISA) technique that will give some semiquantitative data that will be of value in biological samples (Buss et al., 1997).

(ii) Protein nitration

Methods of measuring protein nitration have been introduced relatively recently. The most precise of these are by GC-MS of either free nitrotyrosine or nitrotyrosine from nitrated proteins that have been hydrolysed to the constituent amino acids. However, acid hydrolysis was shown to produce artifacts because of the release of nitric oxide from nitrite in acidic conditions (Frost et al., 2000); this has now been overcome by alkaline hydrolysis. Semiquantitative ELISA methods are also available (Khan et al., 1998). The main problem is that the epitopes may be different for nitration sites in different proteins. No antibody specific for nitrotryptophan has yet been described.

(iii) Protein chlorination

Similar GC-MS methods are available for the detection of chlorotyrosine and other chlorination products (Gaut et al., 2002). Although monoclonal antibodies against chlorinated proteins are available (Malle et al., 1995), again the nature of the epitope is uncertain and the antibody does not react to free chlorotyrosine. No ELISA kits have been developed.

(iv) Glycation of proteins

Measurements of protein glycation are usually
associated with diabetes and are linked to the extent of effectiveness of control of diabetes. Glycation is an oxidative process and is therefore included. Measurements are made routinely of electrophoretic shifts in glycated haemoglobin. Quantification of glycation is also possible by determination of fructosamine by HPLC in hydrolysed proteins, and ELISA kits are also commercially available for semiquantitative analysis.

(v) Oxidatively modified lipoproteins

Most of the interest in the oxidation of lipoproteins is centred on events in the atherosclerotic plaque. Oxidatively modified lipoproteins are removed from the circulation by receptors in Kupffer cells and other cells of the reticuloendothelial system. Nevertheless, attempts have been made to detect subtle changes in LDL which may occur, particularly in a subfraction that has an increased electronegativity (Hodis et al., 1994). Oxidised LDL has been detected directly using a sandwich ELISA procedure (Itabe et al., 1996).

(vi) Antibodies to oxidised LDL in the circulation

The oxidatively modified LDL particles are particularly immunogenic, and antibodies to them have been located in the circulation. They have been detected in healthy individuals, but appear to increase in cardiovascular disease (Palinski et al., 1989).

(vii) Paraoxonase

This enzyme, an esterase associated with the HDL fraction, was originally described as an enzyme that hydrolyses organophosphates (see also Chapter 7; Section 7.4.12). HDL may also become oxidised to protect LDL, and may transfer these peroxides to liver cells. However, it has now been shown that paraoxonase 1 (PON1) can also hydrolyse lipid peroxides, protecting both HDL and LDL from oxidation. In transgenic mice, deletion of the PON1 gene leads to increased susceptibility to atherosclerosis. There appears to be considerable polymorphism of the enzyme conveying differing degrees of activity. The occurrence of a specific polymorphism, PON1 192 R, was associated with higher risk of cardiovascular disease in a meta-analysis of all existing studies (Durrington et al., 2001). However, the actual activity of PON1 is always important and is not directly related to the presence of a specific polymorphism, although the polymorphism from PON1(Q) to PON1(R) has been shown to decrease activity in atherosclerotic lesions (Aviram et al., 2000). A complicating factor is that PON activity against LDL peroxides does not correlate directly with PON activity or hydrolysis of HDL peroxides in the different polymorphisms. Nevertheless, this is an area which is worthy of further study, and measurement of the activity of this enzyme and its genotype may be important.

5.12.3 Nucleic acids

There are many measurements for the oxidation of nucleic acids, particularly of DNA. These may be relatively simple, for example the comet assay that can be run with DNA taken from lymphocytes and shows strand breaks indicating potential mutations. This may be relevant to atherosclerosis, if one believes that the proliferative response is due to a mutation in single smooth muscle cells. Other more demanding techniques include liquid chromatography-tandem mass spectrometry (LC-MS-MS) of base oxidation products, such as 8-oxo-2′ deoxyguanosine and 8-oxoguanine (Podmore et al., 2000).

An area of relevance relates to reports that high doses of ascorbate may lead to increases in some of these products in the urine, indicating a potential pro-oxidant effect of this vitamin under certain conditions (Podmore et al., 1998).

5.13 Markers of oxidant stress in cardiovascular disease

It should be made clear at the outset that there are no markers for oxidative stress that have the proven value of key risk factors, such as plasma cholesterol. Whereas cholesterol passes through the plasma en route to the atherosclerotic plaque, oxidation products originate in the plaque and researchers are trying to find traces of them in the plasma or urine. The difficulty is clear. Furthermore, oxidative stress is not unique to atherosclerosis and occurs in many other inflammatory disorders.

There is, however, abundant evidence of products of oxidation in atherosclerotic plaques; for example, fatty acid peroxides, sterol oxides, lysophosphatidylcholine and isoprostanes (Pratico et al., 1997). Traces of oxidised lipids have also been found in the
plasma of patients with cardiovascular disease (Pratico, 1999), as have traces of mildly oxidised LDL (Hodis et al., 1994). The increased presence of auto-antibodies to oxidised LDL and complexes with LDL have also been demonstrated in plasma of such patients (Yla-Herttuala et al., 1994). Increases in oxidised LDL have been demonstrated in patients with cardiovascular disease and diabetes using the sandwich ELISA method (Toshima et al., 2000).

The oxidisability of LDL (lag time) has also been a popular measure, at least experimentally. Early work by Regnstrom et al. (1992) and others established this in patients with unstable angina and confirmed cardiovascular disease. The values also appeared to parallel the coronary scores. A few other studies have not been able to confirm these observations, but there does appear to be some relationship. Again, such changes have also been observed in other diseases as diverse as cystic fibrosis (Winklhofer-Roob et al., 1995) and systemic sclerosis (Bruckdorfer et al., 1995), although in the latter case these patients are also susceptible to large vessel disease. The changes were also observed in smokers (Hininger et al., 1997). Unfortunately, different laboratories use different procedures for this assay, although attempts have been made to standardise it (Rice-Evans et al., 1996).

There is also evidence of modest increases in MDA concentrations in the plasma of patients with unstable angina (Kostner et al., 1997). In patients with acute myocardial infarction concentrations were higher, but returned closer to normal after 2 days (Iqbal et al., 2002). Detection of MDA using GC-MS may reduce the absolute value to an order of magnitude less than the other techniques, indicating interference from other aldehydes. MDA is uniquely formed by oxidative degradation of arachidonic acid, but is also formed during activation of blood platelets as a result of metabolic oxidation of this acid.

The production of ethane and pentane is increased during myocardial infarction, but this also occurs during other inflammatory diseases (Weitz et al., 1991). A more successful approach is the measurement of isoprostanes as they are stable and can be measured with great accuracy by GC-MS. Urinary isoprostanes are increased in conditions that predispose to cardiovascular disease, such as smoking, hypercholesterolaemia and diabetes (Morrow et al., 1995; Davi et al., 1997; Patrono & Fitzgerald, 1997; Wood et al., 2000). Although these compounds may also increase in other inflammatory diseases that may need to be excluded, they are present in atherosclerotic plaque (Pratico, 1999). These observations substantiate much earlier measurements showing that conjugated dienes are increased, although the methodology of the earlier studies is suspect.

Progress in the use of markers for oxidative modifications of proteins in the context of cardiovascular disease has been more limited. There is clear identification of the modifications occurring in atherosclerotic plaque, particularly nitration (Beckman et al., 1994) and chlorination of proteins (Gaut & Heinecke, 2001). However, other products arising from transition metal-catalysed formation of hydroxyl radicals have been found; for example, meta-tyrosine, ortho-tyrosine, 1-DOPA and products of oxidation of aliphatic amino acids (Fu et al., 1998). The presence of protein carbonyls has been described in the past, but this may be influenced by the presence of sugars, DNA and lipids (Dean et al., 1997).

There is less evidence of protein modifications in the plasma, partly because the analytical techniques are just emerging and are technologically much more sophisticated (reviewed by Onorato et al. (1998)). It should now be possible to measure both specific modifications of whole proteins and of individual amino acids (Crow, 1999). Indeed, the latter is less complicated, although it is now possible to avoid the artifacts of protein hydrolysis. Already, increases in nitrotyrosine in patients with coeliac disease (Steege et al., 1998), children with chronic renal failure (Kari et al., 1997), people with diabetes (Ceriello et al., 2001) or acute respiratory distress (Gole et al., 2000) and in smokers (Pignatelli et al., 2001) have been observed using ELISA assays or blotting techniques. One preliminary study did not find any rise in nitrated proteins in patients with unstable angina (K.R. Bruckdorfer, N.J. Bradley & M. Jacobs, unpublished observations). Much can be achieved using MS techniques, and these are eagerly awaited. However, there will be considerable overlap between the effects of arterial disease and other inflammatory disorders, so there is unlikely to be a specific test which is unique to cardiovascular disease. There is also a need to study which individual proteins are modified, and this should now be achievable using modern proteomic methods.

The presence of protein carbonyls in plasma has been studied in conditions related to cardiovascular
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Table 5.10  Key measurements of oxidation products.

<table>
<thead>
<tr>
<th>Product</th>
<th>Method of measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lipid oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>GC-MS</td>
<td>Kadiiska et al. (2000)</td>
</tr>
<tr>
<td>Lipid peroxides</td>
<td>HPLC/Fluorescence</td>
<td>Frei et al. (1988)</td>
</tr>
<tr>
<td>Isoprostanes</td>
<td>GC-MS</td>
<td>Morrow &amp; Roberts (1997)</td>
</tr>
<tr>
<td><strong>Protein oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein carbonyl</td>
<td>ELISA</td>
<td>Buss et al. (1997)</td>
</tr>
<tr>
<td>Tyrosine nitration</td>
<td>Derivatisation/HPLC</td>
<td>Griffiths et al. (1999)</td>
</tr>
<tr>
<td>Tyrosine chlorination</td>
<td>GC-MS</td>
<td>Frost et al. (2000)</td>
</tr>
<tr>
<td>OxLDL</td>
<td>ELISA</td>
<td>Khan et al. (1998)</td>
</tr>
<tr>
<td>Antibodies to oxLDL</td>
<td>Electrophoresis</td>
<td>Gaut et al. (2002)</td>
</tr>
<tr>
<td><strong>DNA oxidation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-Oxo-2'-deoxyguanosine</td>
<td>HPLC-MS-MS</td>
<td>Itabe et al. (1996)</td>
</tr>
</tbody>
</table>

ELISA: enzyme linked immunosorbent assay; GC-MS: gas chromatography – mass spectrometry; HPLC: high performance liquid chromatography; LC-MS-MS: liquid chromatography–tandem mass spectrometry; oxLDL: oxidised low-density lipoprotein cholesterol.

5.14 Relationships of the markers of oxidant stress to general nutrition and antioxidants

There is clear evidence of the presence of oxidative stress, at least in atherosclerotic plaque, by measurable markers either in the affected tissue or, in some instances, in readily obtainable tissues such as plasma and urine. It would therefore seem obvious that dietary antioxidants or foods (particularly oils) that provide a substrate for oxidation would increase or decrease the concentrations of these markers. Consequently many studies have attempted to assess their effects (see Sections 5.9 and 5.10), although it is also clear that these nutrients may have effects on the whole body and not just on the atherosclerotic plaque.

There has always been a concern that dietary fish oils, which are rich in long chain polyunsaturates, may increase the chance of oxidation of LDL (see Chapter 3). No changes were found in the protein carbonyl content of plasma proteins following consumption of fish oils (2.5 g eicosapentaenoic acid and 1.8 g docosahexaenoic acid) (Wander & Du, 2000). In general, it appears that although fish oils can increase oxidation of polyunsaturates this can be suppressed by the addition of α-tocopherol. Inclusion of 400 IU α-tocopherol daily in the diet in addition to fish oils did not influence protein carbonyl determinations (Wander & Du, 2000). Similar measurements were made with other antioxidants or rich dietary

disease. In smokers (Reznick et al., 1992) and in individuals with diabetes (Cakatay et al., 2000) and angina pectoris there are measurable increases in protein carbonyls, although the specificity of the data is doubtful. One important finding is that not all proteins are equally susceptible to oxidation (Shacter et al., 1994). Fibrinogen is particularly susceptible, and this decreases its ability to support platelet activation (Shacter et al., 1995).

The role of PON polymorphisms (see Section 5.12.2) has been studied in patients with cardiovascular disease, but there is confusion in the outcomes of a number of studies (Aviram, 1999). There is considerable variation in the activities of PON1 in the plasma of individuals, irrespective of the particular polymorphism that they carry (Durrington et al., 2001). However, the activity appears to be low in people with diabetes or hypercholesterolaemia and in patients with CHD (Durrington et al., 2001). PON1 activity seems to increase within the developing atherosclerotic plaque, possibly as a protective response (Durrington et al., 2001).

Finally, although there is no really direct evidence linking DNA oxidation damage to atherosclerosis, it has been pointed out that lipid peroxides can form adducts with DNA. These may influence events such as apoptosis and angiogenesis, which are important to the biology of the atherosclerotic plaque (Lee & Blair, 2001).

The main methods used for determination of oxidative stress are summarised in Table 5.10.
sources of antioxidants and generally little effect was found unless, as in one study, the subjects already had a low ascorbate status after 10 weeks (Carty et al., 2000). There was an overall inverse correlation between globin protein carbonyls and plasma ascorbate levels, but plasma thiols were unaltered. Grape-seed extracts containing polyphenols had no influence on protein oxidation in a small study (Young et al., 2000). There appear not to be any studies on oxidative modifications to tyrosine and other aromatic amino acids in human subjects.

There has been a wider range of studies on lipid oxidation. Many early studies reported marked effects of α-tocopherol (vitamin E) on the lag time of oxidation of LDL isolated from normal subjects (Esterbauer et al., 1991). Levels of α-tocopherol intake as low as 75 IU/day are sufficient to reduce lag time as low as 75 IU/day are sufficient to reduce (Esterbauer et al., 1991). Levels of α-tocopherol intake as low as 75 IU/day are sufficient to reduce LDL oxidation (Mabile et al., 1999); this marker (i.e. lag time) can also be increased (positive effect) in people with diabetes as was demonstrated following supplementation of their diet with tomato juice, ascorbate and tocopherol (Upritchard et al., 2000), but is not evident in all studies. For example, vitamin E alone did not increase lag time in diabetics as it did in normal subjects (Astley et al., 1999). The lag time of LDL oxidation has been shown to be increased by extracts of red wine (Nigdikar et al., 1998) either by adding extracts to LDL (in vitro) or by giving wine or extracts to subjects and testing isolated LDL. Reductions in lipid peroxidation by antioxidants, using TBARS or specific aldehydes, have been recorded (Howard et al., 1998; Pfeiffer et al., 1999) as have reductions in cholesterol oxidation products (Porkkala-Sarataho et al., 2000), mainly following administration of combined vitamin supplements. De Rijke et al. (1996) did not find that alcohol-free red wine reduced LDL susceptibility to oxidation.

Measurements of specific analytes such as MDA are inconsistent, but some showed a decrease in these products in the plasma of patients with cystic fibrosis after supplementation with carotenoids (Rust et al., 1998). Many studies have now been conducted on the effects of α-tocopherol and ascorbate on urinary isoprostane metabolites. In normal subjects, reductions of excretion following α-tocopherol supplementation were observed (Davi et al., 1997) using the radio-immunoassay (RIA) method, but not in all studies (Meagher et al., 2001). In some investigations, this treatment was effective in patients with pre-existing illnesses, for example hypercholesterolaemia, or pre-thrombotic states (Pratico, 1999), or diabetes (Davi et al., 1997). However, in smokers no significant effects of α-tocopherol were observed (Patrignani et al., 2000). This was also the case for smokers taking vegetable and/or fruit extracts (Van den Berg et al., 2001); diets rich in fruit and vegetables decrease the excretion of 8-epi-PGF2α. Recent interest in the role of dietary flavonoids as antioxidants did not translate into any effect on isoprostane excretion (O’Reilly et al., 2001), but this study was in healthy individuals. Fish oils may not have a significant effect on isoprostane formation, and in one study a reduction was demonstrated (Mori et al., 2000).

There have been controversial findings that DNA base oxidation products were increased by high doses of ascorbate supplementation (Podmore et al., 1998) or at least that ascorbate altered the ratio of the expected oxidation products especially when given with iron supplements, a common formulation in pharmacy outlets (Proteggente et al., 2000). However, individuals with pre-existing high levels of plasma ascorbate were not affected adversely by iron supplementation (Proteggente et al., 2000).

5.15 Conclusions

There is clear evidence that oxidative stress plays a role in the development of atherosclerotic plaque. This is supported by the fact that diets low in fruit and vegetables increase the risk of disease, although this does not directly point to a role of antioxidants. There is other evidence to suggest that cardiovascular disease is less prevalent in populations where antioxidants are consumed in abundance and where their concentration in the blood is high. This does not mean that the antioxidants can override other powerful risk factors such as smoking, hypertension and hyperlipidaemia. A number of intervention studies with single antioxidants or combinations of them have illustrated this point well.

There is also clear evidence that markers for oxidative stress are useful in the context of cardiovascular disease. Many of the older markers are now considered to be inadequate and newer ones have been developed. In particular, the measurement of isoprostanes for lipid oxidation and specific modifications of proteins should provide more accurate indications of oxidative stress. It has been shown that antioxidants can modify these markers, but this is not the case with all antioxidants or with all types.
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of patients. Many of the intervention trials did not even try to determine whether the formulations they were using would have any effect on these markers in the cohort under study. These optima could be determined with the judicious use of biomarkers (Halliwell, 1999).

Although the antioxidant field has suffered a setback, the oxidative stress hypothesis retains its potency and greater technical sophistication in the detection of these markers is needed to progress this work. As was the case with the cholesterol hypothesis, the gestation time may last some decades.

5.16 Key points

- Oxidative stress is a process which is not unique to atherosclerosis, but plays an important part in its aetiology and explains many of the cellular processes associated with it.
- The stress is generated from a variety of reactive oxygen and nitrogen species and possibly from enzymatic processes.
- There is strong evidence from case-control and prospective human studies of the protective nature of antioxidants, certainly in the form of foods rich in these nutrients, against various forms of cardiovascular disease. However, intervention trials with antioxidant supplements appear to offer little protection over the medium term.
- Several methods are available for the measurement of the oxidation of proteins, lipids and nucleic acids, with varying degrees of sophistication.
- Changes in these measures have been associated with cardiovascular disease or in conditions that predispose to it.
- Diets that are rich in antioxidants or supplements of antioxidants can change these indicators, usually in a beneficial manner. However, this cannot be assumed automatically to parallel a decrease in cardiovascular disease, as the major intervention studies have not shown a beneficial effect of antioxidant supplements on cardiovascular events.
- More work is required to determine the extent to which antioxidants can be effective, or whether it is too late to intervene when atherosclerosis is already well established.

5.17 Recommendations for future research

Future research should include:

- An evaluation of the actual effects of oxidative modification of proteins on cellular and tissue function in different cell types and on tissues as a whole.
- A proteomic approach to establish which proteins are actually modified and what the effects on protein function may be.
- An assessment of the permanence of the changes which occur as a result of oxidative stress in arterial tissue and what mechanisms are important in removal of modified lipids and macromolecules.
- A study of the influence of exogenous dietary antioxidants on oxidative processes in pre-existing atherosclerotic plaque.
- A comparison of the effects of a wide range of dietary antioxidants on oxidative modification of lipids and macromolecules in vivo induced by different reactive oxygen and nitrogen species.
5.18 Key references


The haemostatic or coagulant system is designed primarily to prevent bleeding and haemorrhage following injury to blood vessels, although some of its constituent proteins have additional roles in inflammation, angiogenesis and tissue repair. Staunching of bleeding is achieved in three overlapping phases (Fig. 6.1):

(1) **Primary haemostasis**: adhesion and aggregation of blood platelets at the site of injury. Platelets are anucleated cellular structures, about 1/14 of the size of red blood cells (erythrocytes). About one thousand billion circulate, continually ‘screening’ the vascular endothelium for biochemical signals of injury.

(2) **Secondary haemostasis**: the conversion of the soluble protein fibrinogen to its insoluble product, the fibrin fibril, by activation of the clotting pathway that leads to the generation of the clotting enzyme, thrombin. Thrombin generation from its precursor zymogen, prothrombin, also triggers platelet activation and aggregation.

(3) **Tertiary haemostasis**: fibrin fibrils crosslink with the aid of factor XIII (FXIII), thereby ‘wringing’ the clot of plasma and increasing its firmness by retraction.

The same sequence of events can be triggered by endothelial injury without bleeding, such as occurs with rupture of an atheromatous plaque. The clot

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**Fig. 6.1** The coagulation pathway. Roman numerals represent clotting factors (VII, IX, X, XI, XII) or cofactors (V, VIII). The suffix ‘a’ indicates the activated factor. $F_{1+2}$: prothrombin fragment 1+2; FPA: fibrinopeptide A.
then forms within the lumen of the vessel, when it is known as a thrombus, and the condition as thrombosis. The danger here is partial or complete occlusion of the lumen, obstructing blood flow and perfusion of peripheral tissue with ischaemia and necrosis (cell death).

So fundamental is diet for various aspects of health that the possibility arises of dietary constituents having direct or indirect effects on the haemostatic system that either raise or reduce the risk of serious thrombosis following vessel wall injury. This chapter considers the evidence for such propositions.

### 6.1 The haemostatic system

Coagulation of plasma with the production of clot and serum is an autocatalytic phenomenon, self-limiting in time and space, and involving a complex interplay of numerous reactions with positive and negative feedback loops to provide control. The initiation, evolution, maturation and dissolution of clot and thrombus are under tight regulatory control. In the healthy state the pathway exhibits low level basal ‘steady state’ activity that primes the system, holding it ready for a response within seconds of injury.

Methods of measurement of the haemostatic factors are summarised in Table 6.1. The system can be conceived as comprising:

1. the coagulation proteins (protein zymogens, which can be activated to enzymes) and their cofactors
2. natural anticoagulant proteins and their cofactors
3. inhibitor proteins of the active enzymes in the coagulant and anticoagulant systems
4. the platelet
5. the endothelial cell of the vessel wall.

<table>
<thead>
<tr>
<th>Table 6.1</th>
<th>Methods of measurement of haemostatic factors and markers of haemostatic activity.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clotting factor zymogens</strong></td>
<td>XII, XI, X, IX, VII, prothrombin</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Activated clotting factors/thrombin activity</strong></td>
<td>FXIIa</td>
</tr>
<tr>
<td></td>
<td>Activation peptides</td>
</tr>
<tr>
<td></td>
<td>FXa and thrombin</td>
</tr>
<tr>
<td></td>
<td>FVIIa</td>
</tr>
<tr>
<td></td>
<td>Thrombin–antithrombin complex</td>
</tr>
<tr>
<td></td>
<td>Fibrinopeptide A</td>
</tr>
<tr>
<td><strong>Fibrinolysis</strong></td>
<td>Plasminogen, tPA, PAI–1</td>
</tr>
<tr>
<td></td>
<td>Plasmin–antiplasmin complex</td>
</tr>
<tr>
<td></td>
<td>D-dimer</td>
</tr>
<tr>
<td></td>
<td>Fibrin degradation products</td>
</tr>
<tr>
<td></td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td></td>
<td>Dilute clot lysis time</td>
</tr>
<tr>
<td></td>
<td>Euglobulin clot lysis time</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>Aggregometry</td>
</tr>
<tr>
<td></td>
<td>Beta-thromboglobulin</td>
</tr>
<tr>
<td></td>
<td>Platelet factor 4</td>
</tr>
<tr>
<td><strong>Inhibitors/anticoagulants</strong></td>
<td>Soluble thrombomodulin</td>
</tr>
<tr>
<td></td>
<td>Protein C and protein S</td>
</tr>
<tr>
<td></td>
<td>Proteins C activation peptide</td>
</tr>
<tr>
<td></td>
<td>Antithrombin</td>
</tr>
<tr>
<td></td>
<td>Plasmin–antiplasmin complex</td>
</tr>
<tr>
<td></td>
<td>Plasminogen, tPA, PAI–1</td>
</tr>
</tbody>
</table>

ELISA: enzyme-linked immunosorbent assay; PAI-1: plasminogen activator inhibitor type-1; tPA: tissue plasminogen activator.
These components are of equal significance, and there is considerable sharing of multifunctional proteins such as thrombin, which has more than 20 substrates and plays important roles in all five component systems.

**Points to note**
- The haemostatic system maintains blood in the fluid state, controls bleeding by coagulation, and plays a role in tissue repair.
- The coagulation system involves a ‘cascade’ of events that leads to the formation of a fibrin clot (formed from the precursor fibrinogen by the proteolytic activity of thrombin).

### 6.1.1 The coagulation pathway

As shown in Fig. 6.1, this pathway is generally regarded as consisting of two ‘upstream’ tributaries that feed into a final common pathway leading to thrombin generation and fibrin production. Activity in the system is sustained by reactions at the head of each tributary, one being known as the intrinsic pathway and the other as the extrinsic pathway. It must not be thought that these components of the overall pathway act in isolation, for there are many cross-reactions and back-reactions between them which provide autoregulation.

**Point to note**
- The generation of thrombin can be divided into three phases, the intrinsic and extrinsic pathways that provide alternative routes for the generation of factor X, and the final common pathway that results in thrombin formation.

#### (i) The extrinsic and final common pathways

The main trigger of coagulation is a specific, relatively small protein embedded in the plasma membrane of cells. This protein, called tissue factor, is not normally exposed to blood unless there is vessel wall injury calling for activation of the clotting system as part of the response to injury, which can be traumatic, inflammatory or immunological in character. There is a gradient of tissue factor concentration in the wall of blood vessels, none being expressed in the endothelium until there is injury, while the highest concentration in healthy vessels is found in the tunica adventitia. Cellular tissue factor is the essential activator that initiates coagulation through interactions with a circulating clotting protein called factor VII (FVII). When FVII binds to accessible tissue factor on a pro-coagulant surface it undergoes a single cleavage which converts it to its active enzyme, FVIIa (the ‘a’ suffix indicates the activated state). Cleavage is performed either by autoactivation (where basal levels of FVIIa cleave FVII as part of the complex with tissue factor), or by activated factor IX (FIXa) or activated factor X (FXa). Of interest is the fact that FVIIa, the initiator of extrinsic pathway activity, normally circulates at low concentration in its free state, unlike the other vitamin K-dependent clotting proteases FIXa, FXa, and thrombin.

Coagulation is conceived in terms of conversion of activity in the pathway from a basal or steady state to a ‘cascade’, where the initial stimulus is sufficient to override temporarily the inhibitor and anticoagulant mechanisms to permit one molecule of enzyme to cleave many molecules of its substrate zymogens to active serine proteases, and pro-cofactors to cofactors, before its action is dampened. As this amplification occurs at each step of the pathway, one molecule of FVIIa–tissue factor complex is responsible for many thousands of molecules of thrombin generated from prothrombin that act upon fibrinogen, platelet receptors and other substrates in a time- and concentration-ordered manner to augment coagulation, bring it to completion, prevent premature dissolution of the clot (fibrinolysis), and return the system to its basal state. Thus in secondary haemostasis, FVIIa–tissue factor activates FIX and FX (both substrates appear to compete for the complex in a concentration-dependent manner). Plasma FIXa, with its cofactor von Willebrand factor (vWF)–factor VIIIa (FVIIIa), generated by thrombin’s action on factor VIII, also activates FX. Plasma FXa (the first molecule of the common pathway), with its cofactor factor Va (also generated by thrombin’s action on factor V), then cleaves prothrombin to thrombin. The cleavages of FIX, FX and prothrombin release distinctive activation peptides from the parent zymogens, the concentrations of which can be measured in plasma to provide an estimate of the rates of conversion of zymogen to enzyme (provided renal function is not impaired).
the extrinsic system is essentially the principal initiating pathway of in vivo blood coagulation and involves both blood and vascular elements.

- Haemostasis is initiated by the formation of a complex between tissue factor (TF) exposed as a result of a vessel wall injury, and already activated FVIIa normally present in the circulating blood.

- FVII is activated by FVIIa, FIXa (from the intrinsic system) and FXa (FX can be activated by FIXa).

- FVIIa–tissue factor complex converts FIX to FIXa and FX into FXa on tissue factor bearing cells. FXa then converts prothrombin into thrombin.

- Thrombin activates FVIII, FV, FXI and platelets, and catalyses the conversion of fibrinogen to fibrin. The resultant clot is reinforced by the action of FXIIIa, a crosslinking enzyme.

(ii) The intrinsic pathway and the kallikrein-kinin or contact system

The four proteins of the contact system, factor XII (FXII), factor XI (FXI), prekallikrein (PK) and high molecular weight kininogen (HMWK), play major roles in coagulation, fibrinolysis, inflammation and immune reactions, probably serving to co-ordinate the components in the response to injury in ways that are not yet fully understood. In the classical description of the contact system, the first step appears to be the ordered assembly of these proteins on a negatively charged surface. The forms of the negatively charged surfaces in vivo have not been identified for certain, but several candidates exist. These include collagen, heparans, and free fatty acids. Long-chain saturated fatty acids, such as stearate (C18) and behenate (C22), emulsified with albumin, provide a potent activating surface in vitro, while emulsions of unsaturated fatty acids, such as oleate, are ineffective in this respect (Mitropoulos et al., 1998). As a result of interaction several activation products are produced. Of these, FXIIa and FXIa retain their binding domains and remain attached to the surface, whereas kallikrein and bradykinin lose theirs and are released. Trace amounts of surface-bound FXIIa activate FXII (autoactivation).

However, this perspective of the system is currently under revision. A receptor for HMWK has been described on endothelial cells, platelets and granulocytes. In plasma, most PK and FXI circulate bound to HMWK, and this complex binds to the HMWK receptor. An endothelial cell prolylcarboxypeptidase then appears to activate PK to kallikrein. Kallikrein activates FXII and releases bradykinin from HMWK. Co-operative interactions between the contact factors then generate increased levels of FXIIa, which generates FXIa. Surface bound FXIa then activates FIX, and FIXa without its cofactor vWF–FVIIIa will activate FXII, while with its cofactor it activates FX. Plasma FXa can also activate FXII, provided its natural inhibitors do not prevent this reaction (see Section 6.1.2). Also to be noted at this stage is the important back-activation of FXI by high concentrations of thrombin generated by continuing coagulant activity after the clot or thrombus has formed, described in more detail in Section 6.2.2.

Points to note

- FXII, FXI, PK and HMWK are known as plasma contact proteins which interact upon binding to negatively charged surfaces.

- In the classical description of initiation of activity in the system, FXIIa bound to a negatively charged surface converts PK to kallikrein. Kallikrein digests HMWK to liberate the vasoactive, pro-inflammatory mediator, bradykinin.

- FXIIa (and thrombin via feedback) activates FXI.

- FXIa activates FIX. This provides a pathway for thrombin generation independent of FVII.

- FIXa + [vWF–FVIIIa] activates FX, which feeds into the common pathway already described.

- In the revised perspective of the system, PK is initially activated by an endothelial cell prolylcarboxypeptidase after binding to the cell surface by attachment to HMWK.
6.1.2 Anticoagulant proteins

For the necessary rate of clotting factor generation to occur after tissue injury, a zymogen, a cofactor and a converting enzyme must assemble in an ordered manner on a natural pro-coagulant surface containing the phospholipids phosphatidylserine and phosphatidylethanolamine. The resultant transformation of zymogen to enzyme is suppressed if the converting enzyme is inhibited, the activated cofactor is inactivated, or surface receptors needed for orderly assembly are lost by sequestration. The system comprising thrombomodulin–protein C–protein S is an anticoagulant system whereby FVa and FVIIIa are inactivated, thereby suppressing the generation of thrombin from prothrombin, and FXa from FX, respectively (Fig. 6.2).

A receptor protein for thrombin, called thrombomodulin, is expressed on vascular endothelium at a density of about 100 000 molecules per vascular endothelial cell. Thrombin bound to thrombomodulin becomes anticoagulant in nature by activating protein C. Activated protein C is able to inactivate the cofactors FVa and FVIIIa. On the activated platelet surface, activated protein C cleaves peptide bonds of surface-bound FVa, which serves as a platelet surface receptor for FXa. Similarly, activated protein C can inactivate FVIIIa on surface membranes, thereby suppressing FXa generation by the FIXa–FVIIIa–vWF complex.

Protein S exists in plasma in free form and a form bound to C4b-binding protein, a regulatory component of the classical complement pathway. The free form serves as a cofactor for activated protein C, accelerating the inactivation of FVa and FVIIIa on platelet and endothelial surfaces. Protein S bound to C4b-binding protein retains the ability to react with protein C but not act as a cofactor. Thus the bound form can be considered as a competitive inhibitor of free protein S.

An inherited mutation in FV (Arginine506 to Glutamine) increases substantially the resistance of FVa to inactivation by activated protein C. This mutation, called factor V Leiden, is a relatively common cause of venous thrombosis (see Section 6.9.4). Deficiency of protein S or protein C also predisposes to venous thrombosis.

Activated protein C is slowly inhibited by a specific circulating protein called protein C inhibitor, and also by alpha-1 antitrypsin and alpha-2 antiplasmin.

**Points to note**
- Protein C and protein S are anticoagulant proteins.
- Thrombomodulin ‘modulates’ thrombin so that it can activate protein C.
- Protein S is a cofactor for activated protein C.
- The thrombomodulin–protein C–protein S system inactivates cofactors FVa and FVIIIa, suppressing thrombin and FXa generation, respectively.

6.1.3 Inhibitors of coagulation

Bioregulation of the coagulation pathway is provided by the following circulating and cell-bound proteins:
Antithrombin is the most important of the physiological inhibitors of activated clotting factors, particularly thrombin (Fig. 6.1). An 80% structural or functional deficiency is associated with an increased risk of thrombosis and embolism. Other serine proteases inhibited by antithrombin are FVIIa, FXa, FIXa, FXIa and FXIIa. The drug heparin, and \( \textit{in vivo} \) heparan sulphate proteoglycans exposed on the endothelial surface, accelerate the reaction between thrombin and antithrombin (but much more weakly than other clotting factors), mainly by bringing the reactants into ordered proximity on a surface.

Tissue factor pathway inhibitor (TFPI) is an inhibitor of FXa. When bound with FXa as a bi-molecule, the complex can then inhibit the FVIIa–tissue factor complex, thereby regulating the extrinsic pathway. In plasma, TFPI exists in two forms, one bound to lipoproteins, predominantly low-density lipoprotein (LDL), and the other in free form. The latter form is positively correlated with plasma LDL, triglyceride, fibrinogen and FVII, suggesting that its concentration may rise in situations that threaten an increased risk of arterial thrombosis, though whether it provides protection in this form is not certain.

TFPI is also found bound to the vascular endothelium where it can be released by injection of heparin. Low levels of total \( \textit{i.e.} \) plasma and heparin-releasable TFPI have been reported to be linked to an increased risk of venous thrombosis. Interestingly, heparin-releasable TFPI contains the basic carboxy-terminal tail that is important for coagulation inhibition, suggesting that its active form resides on the endothelium. The same tail region is also important for TFPI’s ability to inhibit the growth of endothelial cells stimulated by fibroblast growth factor. Heparin-releasable TFPI, but not free TFPI, is recognised and bound by the endothelial very low-density lipoprotein (VLDL) receptor.

C1-esterase inhibitor (C1-INH) is the major inhibitor of FXIIa, but it is generally believed that FXIIa is shielded from its inhibitors when bound to a negatively charged surface, including that on circulating lipoproteins. \( \textit{In vitro} \) at 0°C the activity of C1-INH is reduced 15-fold, leaving FXIIa free \( \textit{i.e.} \) unbound to ‘cold-activate’ FVII, but this phenomenon does not occur \( \textit{in vivo} \) at body temperature. Whether surface-bound FXIIa can activate surface-bound FVII \( \textit{in vivo} \) remains unresolved.

Alpha-1 antitrypsin is a serine protease inhibitor with structural similarities to antithrombin. It is an inhibitor principally of FXIa and FXa.

Heparin cofactor II shares similarities with antithrombin and other members of the serine protease inhibitor (serpin) ‘superfamily’. Unlike antithrombin, the only member of the clotting cascade that it inhibits is thrombin. Because it is activated more readily by dermatan sulphate than heparans, and dermatan sulphate is abundant in the extravascular space, heparin cofactor II is thought to be responsible for thrombin control in extracellular fluid. Hereditary deficiency is a rare cause of thrombosis.

Platelet factor XI inhibitor inhibits FXIa, FIXa and thrombin.

Protease nexin II/amyloid beta-protein precursor is a cerebral protein with a potent capacity to inhibit FVIIa, FVII–tissue factor complex, and FXIa. Cerebrovascular deposition of amyloid beta-protein is a major feature of Alzheimer’s disease, and it binds protease nexin II/amyloid beta-protein precursor, possibly contributing thereby to risk of localised cerebral haemorrhage.
Platelets are small anucleate cells formed from megakaryocytes in the bone marrow. Each megakaryocyte releases about 4000 platelets on maturation. The normal platelet count in blood is between $150 \times 10^9$ and $400 \times 10^9/l$, the average platelet lifespan being about 7–10 days. They have a complex internal structure of membranes. Running through the platelet is an open canalicular system comprising invaginated plasma membrane, thus increasing the effective platelet surface many-fold. The membrane phospholipids are involved in surface activation of FX and prothrombin.

In the platelet interior are found numerous granular bodies including dense granules, alpha-granules, lysosomes and glycogen. Dense granules are packed with 5-hydroxytryptamine (5-HT), noradrenaline, adenosine triphosphate (ATP), adenosine diphosphate (ADP) and calcium, all of which have a profound effect on other platelets and vasomotor responses, i.e. contraction and relaxation of the blood vessels. The alpha-granules contain a range of proteins, including growth factors, coagulation proteins and platelet-specific proteins that influence the adhesion of the platelets to each other and to the endothelium, as well as other platelet properties. These proteins include platelet-derived growth factor (PDGF), platelet-derived endothelial growth factor (PDEGF), fibrinogen, vWF, factor V, fibronectin, beta-thromboglobulin, thrombospondin and the heparin antagonist platelet factor 4.

Platelets also have a complex cytoskeleton that is intimately involved in the initial changes in shape associated with platelet activation and also the movement and release of the internal granules and their contents. The platelet plasma membrane has a large number of important glycoproteins on the outer surface. In platelet activation, increased exposure of fibrinogen binding sites is one of the earliest events. On the platelet surface are specific glycoprotein (GP) receptors that react with aggregating factors, clotting factors and inhibitors. These include:

- **GP Ia** – that facilitates adhesion to collagen exposed by trauma
- **GP IIb and IIIa (integrin)** – that attach the platelet to subendothelial vWF; **GP IIb–IIIa** also binds fibrinogen to promote platelet aggregation
- **GP Ib** – that also binds to vWF.

Platelet adhesion to exposed endothelium is promoted by the glycoproteins listed above.

Platelets have a key function in normal haemostasis and are activated by a number of agonists, particularly thrombin, collagen, ADP, and also adrenaline and serotonin, some of which act synergistically in vivo with other agonists. Activation is a result of occupancy by these agonists of receptors on the platelet plasma membrane. The activation of platelets brings about a sequence of events involving shape change, aggregation and the release of granular contents. These rapid changes are controlled by post-receptor events that are still being mapped out. In essence, platelets are activated as a result of the release of agonists from damaged vessel walls, including the exposure of collagen, which induce the sequence of events briefly described below. This results in the adhesion of the platelets to the vessel wall, the release of granule contents, the activation of neighbouring platelets and the formation of a platelet plug, and also a transitory local vasconstriction.

Collagen binding and binding of thrombin to its platelet receptors trigger platelet synthesis of prostaglandins from membrane arachidonic acid. The product, thromboxane $A_2$, suppresses the generation of cyclic adenosine monophosphate (cAMP) from ATP and increases intracellular calcium, which in turn initiates the release reaction, thereby encouraging aggregation of activated platelets. Released thromboxane $A_2$ is also a potent vasoconstrictor. Negatively charged phospholipids, which have ‘flipped’ to the platelet membrane surface during activation support the binding and conversion of FX to FXa by FIXa–FVIIIa, and the conversion of prothrombin to thrombin by bound FXa–FVa. By these means a plug of aggregated platelets incorporating fibrin and entrapped red blood cells and white cells is generated as clot or thrombus.

The extent of the response is limited by the regulatory response of the surrounding endothelium, permitting the blood flow to be restored. The platelets also contribute to the repair mechanism by the release of protein growth factors, specifically PDGF, which leads to smooth muscle proliferation, and PDEGF, which encourages repair of the endothelium. Platelet factor IV, a specific platelet protein, is reported to be a CXC chemokine and may, therefore, have a role in inflammatory responses.
6.1.5 Regulation of platelet activation by the endothelium

The cells lining the vessel lumen normally present an anticoagulant surface to blood, but convert to a coagulant surface when injured physically, immunologically or biochemically. These cells also secrete subendothelial basement membrane, elastin, collagen and fibronectin. Injury upregulates endothelial production of tissue factor, vWF, thrombomodulin, antithrombin, and tissue plasminogen activator (tPA; see Section 6.2.1), so that the cell participates actively in coagulation and fibrinolysis.

In addition to platelet activators, there are factors that inhibit this process, the most important of which are derived from the endothelium. In flowing blood, the lightest of blood cells, the platelets, are thought to flow closest to the cells lining the vasculature and are most exposed to the influence of the endothelium.

The main endothelial factors that determine platelet function are the eicosanoids, particularly prostacyclin (PGI₂), and also nitric oxide (NO) which is derived from L-arginine. In addition, there is adenosine which may be formed as a result of the presence of ecto-nucleotidases on the endothelial surface using ADP or ATP as substrates. These three are powerful inhibitors of platelet activation and, in normal circumstances, may prevent to some extent the formation of platelet aggregates, although not completely as this would impair the normal haemostatic functions of the platelets. They are also potent vasodilators. Prostacyclin biosynthesis may be increased in neighbouring regions following endothelial damage as a protective mechanism that limits the size of the platelet plug. Nitric oxide may be stabilised by the formation of S-nitrosothiols, principally those with thiol groups on proteins such as albumin, which are also potent inhibitors of platelets.

The principal action of nitric oxide is by a non-surface receptor mechanism where the gas diffuses into the platelet and increases intracellular cyclic guanosine monophosphate (cGMP) concentrations by activation of soluble guanylyl cyclase in the platelet cytosol. This activates cGMP-dependent kinases and, in turn, the phosphorylation of key platelet proteins such as vasodilator stimulated phosphoprotein (VASP). This phosphorylation prevents the polymerisation of actin normally stimulated by unphosphorylated VASP during platelet activation. The duration of these effects depends on the presence of phosphodiesterases, which break down the cGMP to 5GMP. In contrast, inhibition of platelets by prostacyclin is cAMP dependent, activating cAMP-dependent kinases that also act on VASP and permit the synergistic interactions of prostacyclin and nitric oxide (see Fig. 6.3).

6.2 The fibrinolytic system

The fibrinolytic system consists of:
- fibrinolytic factors
- anti-fibrinolytic factors.

6.2.1 Fibrinolytic factors

The main regulatory processes of the fibrinolytic system are those that convert the zymogen plasminogen to the enzyme plasmin (Fig. 6.4). This activation is achieved by three different plasminogen activators. The first of these is tPA, which is released into the circulation from vascular endothelial cells stimulated by various agents such as exercise, nicotinic acid and catecholamines. Plasma tPA levels exhibit a distinct circadian rhythm, being lowest in the morning. The mere presence of tPA will not stimulate fibrin...
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dissolution, for it requires to be bound to fibrin which behaves as both a cofactor and substrate. In the absence of fibrin, tPA has a poor affinity for plasminogen. When a fibrin clot is evolving, tPA and plasminogen bind sequentially to fibrin, thereby providing the conditions for rapid generation of plasmin. Furthermore, the original form of plasminogen, called glu (glutamine) plasminogen, is converted to the more activatable form, lys (lysine) plasminogen upon fibrin binding. Effective binding of plasminogen requires access to fibrin’s lysine-binding sites.

Alternative or additional pathways of plasmin generation involve two plasminogen pro-activators, one converted to plasminogen activator by FXIIa and kallikrein, and the other (urokinase type; uPA), which is activated by plasmin, thereby constituting a positive feedback mechanism.

Plasmin can degrade both fibrinogen and fibrin, but only a limited number of accessible lysine sites are available on fibrinogen. A complex array of fibrin and fibrinogen degradation products are generated by the lytic action on free fibrinogen, fibrin monomer and polymeric crosslinked fibrin. The key terminal crosslinked degradation product is known as D-dimer, because it contains two D-domains of fibrinogen joined to one fibrinogen E-domain.

6.2.2 Inhibitors of fibrinolysis

This section considers thrombin activatable fibrinolysis inhibitor (TAFI), plasminogen activator inhibitor type-1 (PAI-1) and alpha-2 antiplasmin.

TAFI (also known as procarboxypeptidase B) is a recently discovered inhibitor of fibrinolysis. It is activated by high concentrations of thrombin generated after clot or thrombus formation. The necessary levels of thrombin are produced by a positive loop in which thrombin activates FXI, and FXIa then augments thrombin generation. The presence of low concentrations of thrombomodulin markedly augments the generation of activated TAFI by thrombin, whereas high concentrations up-regulate fibrinolysis by attenuating thrombin generation via activated protein C and thereby reducing activation of FXI by thrombin. Activated platelets provide a surface for thrombin’s activation of FXI.

PAI-1 is found in plasma and in platelets, from which it is released on activation. In addition to inhibiting tPA and uPA, PAI-1 can inhibit activated protein C, thus increasing thrombin generation, platelet aggregation and fibrin formation. Plasma PAI-1 level peaks in the early morning, declining to its nadir in the early afternoon. The protein is secreted by adipose tissue and endothelial cells; output increases with cell exposure to endotoxin, interleukin-1 and VLDL. PAI-1 is also released from hepatocytes, and increased release has been demonstrated when these cells are exposed to insulin or VLDL. Pro-insulin stimulates PAI-1 release from both hepatic and endothelial cells.

A strong relation exists between plasma insulin, VLDL and PAI-1 levels, and reduction in insulin concentration through exercise and weight loss is associated with a fall in plasma PAI-1 activity and VLDL.

Circulating alpha-2 antiplasmin inhibits plasmin released from clots, but is unable to inhibit surface-bound plasmin. The protein also inhibits activated protein C.

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**Fig. 6.4** Fibrinolysis. The activators of the pathway are factor XIIa, kallikrein, uPA and tPA. Plasmin degrades fibrin to fibrin degradation products (FDPs). Plasminogen activator inhibitor-1 (PAI-1) and antiplasmin are inhibitors of the system.
Points to note

- A fibrin clot is a temporary, emergency measure to prevent immediate blood loss that must subsequently be dissolved.
- For a fibrin clot to dismantle, plasminogen must be activated to plasmin via plasminogen activators such as tPA and uPA.
- Vascular endothelial cells release tPA, and its catalytic activity is enhanced by the presence of fibrin.
- uPA is activated by plasmin. This is an example of a positive feedback loop.
- Plasmin can degrade fibrinogen and fibrin, preventing further coagulation and resulting in clot dissolution, respectively.
- The fibrinolytic process is inhibited by TAFI. PAI-1 inhibits tPA, uPA and activated protein C, thus increasing thrombin generation, platelet aggregation and fibrin formation. Plasma-derived alpha-2 antiplasmin also inhibits activated protein C and specifically inactivates plasmin released from clots.

6.3 The concept of hypercoagulability

There is much confusion of thought and lack of definition underlying the use of the term hypercoagulability. Blood is normally fluid (it will flow), and plasma is a sol or colloidal solution in a fluid state. At times of tissue injury, such as after trauma, surgery and some infections, a local coagulable state is generated as a physiological response. It is difficult to accept, therefore, that trauma or surgery should generate a ‘hypercoagulable’ state, for ‘hyper’ implies ‘too much’ or ‘more than normal’. Some patients may be in a hypercoagulable state for reasons other than trauma or surgery, such as deficiencies of natural anticoagulants and, therefore, be at increased risk of thrombosis at such times, given the additional physiological ‘coagulant’ responses to trauma or surgery. However, in general, most patients are not hypercoagulable before, during or after trauma and surgery.

So what states constitute hypercoagulability? Here it must be said that the term is often used interchangeably with ‘prothrombotic state’ (‘pre’ or ‘prae’ (Latin) means ‘before in time’) or ‘prothrombotic state’ (‘pro’ (Latin) means ‘in favour of’), or ‘pro’ (Greek) means ‘before in time’). Strictly speaking, to coagulate means to convert plasma, a fluid colloidal solution containing fibrinogen, into a fixed clot or gel containing insoluble fibrin. Therefore, only plasma can be in a hypercoagulable state. However, a prothrombotic state would encompass one or more of the following: hypercoagulability, unphysiological activation or sensitivity to activation of platelets, unphysiological conversion or increased sensitivity of conversion of the vascular endothelial cell surface to one supporting coagulation and platelet activation, or hypofibrinolysis (see below), all of which can raise the risk of thrombosis. A prothrombotic state would presumably imply a state that exists for some time preceding actual thrombosis. A prothrombotic state would not necessarily be a prelude to actual thrombosis, just as many people with a high blood cholesterol level do not go on to have a myocardial infarction.

A popular concept of hypercoagulability is one in which biochemical forces supporting coagulation (e.g. exposure of tissue factor) override the forces that promote fibrinolysis (e.g. a reduction in TAFI). Strictly speaking, however, fibrinolysis is not called into play until fibrin has formed, for before such time plasmin has no substrate on which to operate. Thus, the fibrinolytic system can be in an unphysiological ‘hypofibrinolytic state’, when it would contribute to a prothrombotic state, but not a state of hypercoagulability, which precedes fibrin generation. In other words, the prothrombotic state exists when there is an imbalance between coagulability and fibrinolytic capacity that favours fibrin deposition and clot formation (i.e. hinders clot dissolution).

Because thrombin has pivotal roles in coagulation and fibrinolysis, one form of the prothrombotic state could be the result of an imbalance in thrombin’s diverse roles. Since it is the prothrombotic state we wish to avoid, rather than simply the more narrowly defined hypercoagulable state, a clinical and epidemiological measure of this imbalance would serve potentially as a useful tool.

The major circulating marker of the initial phase of thrombin’s action on fibrinogen is a small peptide liberated from fibrinogen, called fibrinopeptide A (FPA). The problems with its measurement are its exquisite sensitivity to a poor venepuncture, and its short life in the circulation (half life about 3 minutes). Thus, a raised level of FPA in a casual blood sample might not be expected unless there was increased
generation in the steady state, or renal clearance was impaired. A marker of hypofibrinolysis would be a decreased concentration of D-dimer, cleaved from crosslinked fibrin by plasmin. However, an index of a low level of activated TAFI (which can be measured) would be useful when assessing the balance of thrombin’s activity on its major substrates. Whether activated TAFI is reduced in the prothrombotic state is as yet uncertain.

Some studies have reported chronically raised levels of FPA in, for example, type 2 diabetes. However, in the Second Northwick Park Heart Study (unpublished), the ratio of FPA to fibrinogen fragment b-beta 1–42, a marker of plasmin’s activity, was not raised in men at high risk of clinical coronary heart disease (CHD). Thus, future work should perhaps focus more on platelet changes and changes to the vascular endothelium as additional causes of common forms of arterial and venous thrombosis.

### Points to note

- The concept of a hypercoaguable state is not well defined, but if used should refer specifically to the coagulability of plasma. Hypercoagulation does not include the state of play of the fibrinolytic system.
- A prothrombotic state is a more useful concept. It implies an imbalance between coagulation and fibrinolysis that favours fibrin deposition and clot formation, e.g. via an increased sensitivity of platelets to activation or an imbalance in thrombin’s diverse roles.
- A prothrombotic state is not necessarily a prelude to a thrombotic event.
- Proposed clinical markers of prothrombosis have included an increased ratio of FPA to D-dimer. (D-dimer levels are frequently increased in the presence of active thrombosis.)

### 6.4 Established haemostatic risk factors for vascular disease

#### 6.4.1 Factor VII

Prospective epidemiological studies have shown that raised concentrations of specific clotting and fibrinolytic factors are predictive of cardiovascular disease (Table 6.2). Plasma FVII coagulant activity (FVIIc; a combination of circulating concentrations of factor VII and factor VIIa) was found to be a marker of risk of fatal (but not non-fatal) CHD in the First Northwick Park Heart Study conducted in the 1970s, but not in the subsequent study undertaken in the 1990s (Meade et al., 1993; Cooper et al., 2000). Why this contrast has occurred is by no means certain, but one possibility is that it reflects a decrease in dietary fat intake over the intervening 20 years (Ministry of Agriculture Fisheries and Food, 1997a; see Section 6.10.3). The latter finding agrees with other studies (Tracy et al., 1999). Thus, raised FVIIc is not invariably a CHD risk factor.

#### 6.4.2 Fibrinogen

Many studies have found plasma fibrinogen concentration to be a strongly positive predictor of CHD (Meade et al., 1993; Heinrich et al., 1994; Tracy et al., 1999; Cooper et al., 2000). The increase of plasma fibrinogen in adults at high risk of CHD could be an expression of the acute phase response accompanying the inflammatory component of CHD, for it is associated with changes in other acute phase proteins, including C-reactive protein (CRP; Kuller et al., 1996; see Chapter 7). The inflammatory cytokine interleukin-6 (IL-6) is known to stimulate the production of the acute phase proteins including fibrinogen, CRP and vWF (Heinrich et al., 1990; Van Deventer et al., 1990). Once elevated, fibrinogen could contribute to a prothrombotic state by its marked effect on blood viscosity. Similarly, vWF, another established CHD risk factor (Meade et al., 1994; Thompson et al., 1995), could be increased by IL-6 stimulation but then influence risk through its role in coagulation as a cofactor for FVIII.

#### 6.4.3 Fibrinolytic factors

In the fibrinolytic system, both tPA antigen and PAI-1 antigen and activity are positive risk factors for CHD (Hamsten et al., 1985; Hamsten et al., 1987; Ridker et al., 1993). Increased levels are believed to be markers of hypofibrinolysis, tPA antigen because it circulates in a complex with the inhibitor. Plasma PAI-1 levels are influenced by IL-6 (Van Deventer et al., 1990). Free PAI-1 antigen concentration has been reported to be a strong predictor of type 2 diabetes (Festa et al., 2002).
Overall, the evidence is not yet strong enough, except perhaps for fibrinogen (Table 6.1), to warrant intervention studies designed to correct raised levels of the haemostatic factors.

### 6.4.4 Platelets

The importance of the involvement of platelets in the early development of atherosclerosis has received differing degrees of support in recent decades. Platelets are sometimes found engulfed within macrophages (Sevitt, 1986), and there is evidence of episodic platelet involvement during plaque development, as indicated by fluorescent antibodies to platelet antigens that identify platelet emboli. Platelet emboli have also been found in the microcirculation of patients who died suddenly. Furthermore, platelet activity is increased after a myocardial infarction – but this could be interpreted as a secondary effect. Platelet activation may be involved in the chemotaxis of monocytes at sites of injury. Atherosclerotic plaques do not have identical pathologies and only a proportion may show platelet involvement in the early stages. The involvement of platelets in the terminal stages of cardiovascular disease and in restenosis may be much more obvious and has received a great deal of attention.

Perhaps the best evidence for the importance of platelets derives from the clear benefit of low-dose aspirin for the suppression of platelet activation by inhibition of cyclo-oxygenase and the prevention of myocardial infarction (Antiplatelet Trialists’ Collaboration, 1994). Cyclo-oxygenase is an enzyme that produces eicosanoids (e.g. the platelet stimulating agent thromboxane A2) from arachidonic acid present in platelet membranes. New platelet inhibitors, such as the purinergic inhibitor clopidogrel that prevents platelet activation by ADP, enhance the power of aspirin (Payne et al., 2002). ADP enhances the activity of other platelet agonists, and is therefore a suitable target for platelet inhibition.

Another area of interest lies in the inhibitors of cyclo-oxygenase-2 (COX-2), an enzyme present only in small amounts in platelets, which in some cases may increase risk of thrombosis because COX-2 is more strongly associated with prostacyclin synthase (prostacyclin is antithrombic by nature), whereas COX-1 in platelets seems to be involved in thromboxane synthase. Also ibuprofen has been shown to impair the inhibitory actions of aspirin on platelets in human subjects (Catella-Lawson & Crofford, 2001).

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**Table 6.2 Established risk factors for cardiovascular disease – strength of the evidence.**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>CHD</th>
<th>Stroke</th>
<th>Statistical independence</th>
<th>Increased with inflammation</th>
<th>Increased in insulin resistance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>(Meade et al., 1993; Heinrich et al., 1994; Folsom et al., 1997; Tracy et al., 1999; Cooper et al., 2000; Festa et al., 2002)</td>
</tr>
<tr>
<td>tPA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>++</td>
<td>++</td>
<td>+ (CHD) + (stroke)</td>
<td>0</td>
<td>+ (antigen)</td>
<td>(Juhan-Vague et al., 1993; Ridker et al., 1993; Lee et al., 1995; Folsom et al., 1997; Thogersen et al., 1998; Kain et al., 2001; Payne et al., 2002)</td>
</tr>
<tr>
<td>PAI-1&lt;sup&gt;2&lt;/sup&gt;</td>
<td>++</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>(Juhan-Vague et al., 1993; Thogersen et al., 1998; Festa et al., 2002; Payne et al., 2002)</td>
</tr>
<tr>
<td>VIIIc/vWF&lt;sup&gt;3&lt;/sup&gt;</td>
<td>+</td>
<td>0</td>
<td>+0 (CHD) +0 (stroke in women)</td>
<td>+</td>
<td>0</td>
<td>(Meade et al., 1994; Folsom et al., 1997; Thogersen et al., 1998; Tracy et al., 1999)</td>
</tr>
<tr>
<td>VIIc&lt;sup&gt;4&lt;/sup&gt;</td>
<td>(+)</td>
<td>0</td>
<td>(+)/0</td>
<td>0</td>
<td>0</td>
<td>(Meade et al., 1993; Tracy et al., 1999; Cooper et al., 2000)</td>
</tr>
<tr>
<td>Platelet</td>
<td>(+)</td>
<td>(+)</td>
<td>↓</td>
<td>0</td>
<td>0</td>
<td>(Tripl et al., 1990; Elwood et al., 2001)</td>
</tr>
</tbody>
</table>

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<sup>1</sup> Tissue plasminogen activator.  
<sup>2</sup> Plasminogen activator inhibitor type 1.  
<sup>3</sup> Factor VIII activity/von Willebrand factor.  
<sup>4</sup> Factor VII activity.  
+++, strong; ++, moderate; +, weak; +0, very weak; (+), one study only; 0, none; ↑↓, direction of sequential activations.
Points to note

- Haemostatic factors positively related to risk of CHD include fibrinogen, vWF and (inconsistently) factor VII activity, tPA antigen and PAI-1 antigen and activity.
- Owing to the exquisite sensitivity of blood platelets to handling, changes in platelet function in those at high risk of CHD, though strongly suspected, are difficult to substantiate.

6.5 Platelet function tests

One of the problems in determining the predictive power of platelet function is to devise adequate and robust tests. Platelets are sensitive cells that deteriorate quickly, and their activity needs to be measured in situ within a short period of removal from the subject. The platelet itself is sensitive to a poor venepuncture, releasing the contents of its granules. Ideally, the initial blood sample is discarded because of activation by the syringe needle and initial local damage. To help preserve the intact platelet, blood should be collected in a special anticoagulant. Many of the tests are ex vivo, and therefore removed from the influence of the endothelium. More sophisticated tests involving catheterisation, e.g. the filtragometer, overcome this problem to some extent. There is some predictive value in the simple measurement of platelet count and size, as well as the presence of platelet microvesicles in plasma (platelet dust). The presence of reticulated platelets and platelet–leukocyte aggregates have also been considered (Rapi et al., 1998; Klinkhardt et al., 2003).

6.5.1 Established tests of platelet function

More commonly, the simple nephelometric (optical/turbidometric) platelet aggregation test in platelet-rich plasma has been employed with the platelets out of contact with other blood cells. This can facilitate agonist dose–response measurements, but because of the pressure of time, often only one or two agonist concentrations are used. Alternatively, the whole blood platelet aggregation test, which measures changes in impedance during activation by agonists, is seen as more physiological, but is also slow. In a study by Elwood et al. (2001), none of the above-mentioned methods showed any predictive power for myocardial infarction, but were better for stroke. Earlier work in a smaller cohort suggested that spontaneous aggregation (without agonists) of platelets in patients with cardiovascular disease was predictive of cardiac events and mortality (Trip et al., 1990).

Other approaches are employed to measure platelet function. One is to measure the amount of platelet-specific proteins released into the plasma. Platelet-poor plasma (prepared by cold centrifugation for 30 minutes at 2000 g in the presence of a cocktail of inhibitors) can be assayed for platelet factor-4 (PF4) and beta-thromboglobulin (both released from alpha-granules), but their circulating half-lives are only 3 minutes and 100 minutes, respectively, and both are raised by impaired renal function. Furthermore, the assays are technically difficult because, here again, activation of platelets during blood sampling needs to be avoided. The predictive qualities of these tests are uncertain and contradictory, perhaps in part because the measurements are influenced by the type of anticoagulant (PF4 acts by binding heparin to increase coagulation and beta-thromboglobulin is influenced by aspirin). However, increased levels have been reported in the diabetic state, and levels of both markers are reduced by the anti-platelet drug, aspirin (Kubisz, 1984).

It has been shown that platelets from patients with acute coronary syndromes produce significantly less nitric oxide (anti-thrombotic) than healthy controls (Loscalzo, 2001). Nitroglycerin also inhibits platelet activity. However, measurement of products of nitric oxide is fraught with difficulty. The main products are nitrate and nitrite, but at least as much is ingested in the diet, making accurate measurement difficult. There is currently much interest in the presence in plasma of nitrosothiols, which are mainly protein based (e.g. S-nitrosoalbumin) and may act as a more stable pool of nitric oxide, from which nitric oxide may be released at the surface of the platelet or transferred onto other nitrosothiols within the platelet. Nitric oxide would normally complex with haemoglobin haem and become biologically inactive. S-nitrosothiols are effective inhibitors of platelet activation. The concentration of these S-nitrosothiols is a matter of some discussion, because they are difficult to measure at low concentrations. Initially reported as being in the low-micromolar range, a concentration sufficient to completely inhibit platelets and causing extensive vasodilatation, concentrations...
in the upper or lower nanomolar range were subsequently reported, depending on the methodology (Frost et al., 2000). Thus, measurement of nitrosothiols is in its infancy. These markers are likely to increase with infections.

6.5.2 Advances in platelet function tests

Measurement of the exposure of platelet-specific proteins on the cell surface, which normally only appear or increase during activation, may prove to be a useful method for indication of platelet activation. Examples are P-selectin, and also certain epitopes of GP IIb–IIIa that seem to be decreased in patients with CHD compared with young healthy controls, but not against age-matched controls (Knight et al., 1997). This procedure again requires care to prevent activation on blood sampling, but glutaraldehyde fixation does permit storage of samples for accurate measurement. European-wide agreed protocols have been established to permit comparisons between laboratories (Schmitz et al., 1998), but expensive equipment makes application to routine analysis difficult. The presence of free P-selectin in the plasma is also a possible measure, but since this protein is also found in the endothelium, its origin may not be specific to the platelet.

Measurement of small molecular weight components from dense granules in platelets is not helpful because the products (e.g. ATP) are found in all cells. The analysis of unique eicosanoids is also a possibility, particularly that of thromboxane A₂, which degrades non-enzymatically to thromboxane B₂ and is measurable in urine and plasma. The main problem is that its formation is very much decreased following the ingestion of aspirin, which is common among patients considered at risk from cardiovascular disease. There have also been suggestions that thromboxane A₂ may also be formed in other tissues. Nevertheless, measurement of thromboxane B₂ by enzyme-linked immunosorbent assay (ELISA) may be a cheap and convenient measure of platelet function if the caveats above are taken into consideration.

As discussed above, the main inhibitors of platelet activity are derived from the endothelium. The measurement of the key eicosanoid, prostacyclin, cannot be achieved directly because of its relatively short half-life. However, its stable metabolic product, prostaglandin 6 keto F₂α, can be measured in blood and urine. The levels are relatively low, and clear predictive associations between urinary output and cardiovascular disease have not been described. However, adverse changes can be detected in smokers, particularly in women taking oral contraceptives (Roy, 1999).

Points to note

- Established platelet function tests, such as aggregometry, have not found a consistent positive relationship between changes in platelet function and risk of CHD.
- More sophisticated tests for platelet activity remain technically difficult and are unsuitable for routine application outside a research laboratory.

6.6 Emerging phenotypic risk factors for CHD

Whereas the associations of diet with the established risk factors for CHD (e.g. obesity, elevated blood cholesterol and hypertension) have been well explored (see Chapters 1, 2, 11 and 13), this is not the case for the haemostatic factors. The evidence to date linking them with CHD and obesity is briefly discussed below. Information about the associations between diet and the haemostatic factors can be found in Section 6.10.

6.6.1 Factor XIIa

Plasma FXIIa is increased in men at high CHD risk (Miller et al., 1997), and is also raised in obesity (Kohler et al., 1998) and in hypertriglyceridaemia (Cooper et al., 2000).

6.6.2 Factor IX and Factor X peptides

The activation peptide of FIX (FIX pep) was found to be raised in men at high risk for CHD in the Second Northwick Park Heart Study (Cooper et al., 2000), suggesting increased turnover of FIX in such subjects. However, the level of FX activation peptide (FX pep) is reduced in men at high risk, indicating that FX turnover is reduced. The meaning of this
6.6.3 Fragment 1+2 (F\textsubscript{1+2})

In the Second Northwick Park Heart Study (Cooper et al., 2000) and in the Atherosclerosis Risk in Communities (ARIC) Study (Folsom et al., 2001), the activation peptide of prothrombin, called fragment 1+2 (F\textsubscript{1+2}) was not associated with CHD risk, but it has been reported to be raised after myocardial infarction (Merlini et al., 1994). In one study (Rugman et al., 1994), but not in another (Woodward et al., 1997), F\textsubscript{1+2} was increased in obesity.

6.6.4 D-dimers

Fibrin D-dimer, a marker of fibrin turnover, is another factor predictive of CHD (Ridker et al., 1994; Cushman et al., 1999; Folsom et al., 2001), and recurrent cardiovascular events (Cortellaro et al., 1993). Raised levels have been reported in obesity (Lee et al., 1995; Cushman et al., 1999).

6.6.5 Others

Associations of antithrombin (curvilinear) (Meade et al., 1991) and free protein S (linear and positive; see Section 6.1.2) with CHD (Rudnicka et al., 2001) have been reported. Antithrombin increased in concentration with adiposity in one study (Woodward et al., 1997) but decreased in another (Conlan et al., 1994). Protein S expression is upregulated in HepG2 cell lines by IL-6 (Hooper et al., 1995). In the ARIC Study, an inverse relation was described between plasma thrombomodulin concentration and the incidence of CHD (Salomaa et al., 1999), while the association of plasminogen with future CHD was positive (Folsom et al., 2001).

**Points to note**
- Emerging haemostatic risk factors for CHD include activated factor XII, prothrombin fragment 1+2, FIX activation peptide and D-dimer.
- Markers of platelet activation linked to CHD risk are being actively sought, including surface proteins exposed upon platelet activation.

6.7 Established and emerging phenotypic risk factors for stroke

The haemophilic states, characterised by severe congenital deficiency of FVIII or FIX, markedly increase the risk of intracranial haemorrhage. Inherited deficiency of antithrombin (see Section 6.1.3), protein C or protein S (see Section 6.1.2) has been reported in cases of stroke, but whether the deficiency was causal is uncertain. Inherited resistance to activated protein C, due to a mutation in FV (FV Leiden; see Section 6.9.4) does not increase the risk of thrombotic stroke (Ridker et al., 1995). High concentrations of fibrinogen are a well established risk factor for thrombotic stroke (Wilhelmsen et al., 1984). Ridker et al. (1994a) have reported that the mean concentration of tPA antigen is higher among men at increased risk of stroke than in those not at risk. Using conventional aggregation tests, changes in platelet function were observed for stroke, but in an unexpected direction; patients had low responses to platelet agonists (Elwood et al., 2001).

**Points to note**
- High fibrinogen concentrations are an established risk factor for thrombotic stroke.
- Other haemostatic factors that might predict the likelihood of stroke are under investigation (*e.g.* tPA antigen).

6.8 Established and emerging phenotypic risk factors for venous thromboembolism

Inherited deficiencies of antithrombin, protein C and protein S (see Sections 6.1.2 and 6.1.3) are well known to predispose to venous thromboembolism, but fortunately they are rare conditions. A poor anticoagulant response due to activated protein C resistance, either as acquired resistance to this enzyme, or inherited as FV Leiden (see Section 6.9.4), is also an established risk factor for venous thromboembolism. Emerging risk factors are a high level of FVIII (Kyrle et al., 2000; see Section 6.1.1) and FIX (Van Hylckama Vlieg et al., 2000), though the mechanisms are uncertain. High pre-operative concentrations of
F$_{1+2}$ and D-dimer are associated with a raised incidence of post-operative venous thromboembolism (Lowe et al., 1999).

Points to note
- Activated protein C resistance is an established risk factor for venous thromboembolism.
- High levels of FVIII and FIX are potential risk factors for venous thromboembolism.

6.9 Genetics and cardiovascular disease risk

Variation or polymorphism is being recognised in a rapidly increasing number of genes coding for proteins in the haemostatic system. Some of these mutations are associated with phenotypic variation and clinical effects. Many are rare, being present in less than 1% of the population or even confined to a small number of families. These will not be considered. The reader should beware of nomenclature in this field. The variation can be described at the level of the gene (e.g. FV G1691A, meaning that mutation replaces the nucleotide guanine at position 1691 in the gene structure with an adenine nucleotide), the phenotypic resultant change in the protein (e.g. FV Arginine506Glutamine, meaning that G1691A results in substitution of an arginine by a glutamine at position 506 in the protein sequence of amino acids), or the origin of its discovery (e.g. FV Leiden, meaning that FV G1691A was first described by a group in Leiden).

6.9.1 Factor VII

Six common polymorphisms associated with FVII levels have so far been described in the gene for FVII. In the promoter, a decanucleotide insertion at position −323 from the start codon is in strong linkage disequilibrium with a single nucleotide polymorphism (SNP), which produces substitution of an arginine at position 353 of the protein with a glutamine (R353Q). There are three further polymorphisms recognised in the FVII promoter region; at −401 (guanine to thymine), −402 (guanine to adenine) and at −670 (adenine to cytosine). The sixth common polymorphism exists in a hypervariable region in intron 7, called HVR4, giving rise to three alleles of different length. Heterozygotes with the decanucleotide insertion at −323, and/or the Q353 variant (represented in 20% of the population) have significantly lower (20–30%) FVII levels than non-carriers. The −670C and the −402 variants are associated with significant elevation of FVII concentration, whereas the −401T allele produces a decrease in plasma FVII. In general, these genetic variations in FVII are not associated with arterial or venous thrombotic diseases including CHD, although a positive association of −670C with CHD has recently been reported from the Second Northwick Park Heart Study (Carew et al., 2003). Furthermore, a recent meta-analysis, which included some large studies that have since been questioned, found that those homozygous for the Q353 allele were afforded protection against cardiovascular disease (Wu & Tsongalis, 2001).

6.9.2 Prothrombin

A common polymorphism in the prothrombin gene, consisting of a substitution in the 3′-untranslated region at nucleotide 20210 of a guanine for an adenine, is associated with an increased prothrombin concentration and raised risk of venous thromboembolism, especially during oral contraceptive therapy (Martinelli et al., 1999). The polymorphism is also associated with cerebral venous thrombosis and abdominal venous thrombosis in infancy. The prevalence of the mutation in the UK is about 2%, and A20210 is accompanied by an increased concentration of plasma F$_{1+2}$, indicating its association with increased thrombin generation (Bauer et al., 2000).

Risk is raised many-fold by the conjoint inheritance of two genetic polymorphisms associated with venous thrombosis, for example prothrombin A20210 and FV Leiden. G20210A is not linked to arterial thrombosis or CHD (Wu & Tsongalis, 2001).

6.9.3 Fibrinogen

Plasma fibrinogen is a 340 kDa glycoprotein comprising three distinct pairs of polypeptides (alpha, beta and gamma) linked by disulphide bonds. These polypeptides are encoded by three genes which form a cluster in chromosome 4. Many polymorphic sites have been identified in these genes, but those subject to most study are located in the beta chain.
These are named according to the restriction enzyme used for detection, the BclI polymorphism in the 3′ region and the HaeIII polymorphism in the 5′ promoter region of the chain. These two polymorphisms are in linkage disequilibrium. The HaeIII variant is associated with an increased fibrinogen concentration in smokers (Behague et al., 1996). Although a high fibrinogen concentration is clearly associated with stroke and CHD, in large studies no obvious link has been found between fibrinogen genotype and arterial disease. With respect to venous thromboembolism, a polymorphism in the coding region of the alpha chain (Threonine312Alanine), which is close to the FXIII crosslinking site at position 328, has created some interest because of reports that Alanine312 is associated with a rigid fibrin gel structure (Muszbek et al., 1996). Homozygotes for the alpha Alanine312 allele are reported to be at increased risk of pulmonary embolism (Carter et al., 2000).

6.9.4 Factor V

Plasma FV is a 300 kDa glycoprotein. A mutation in its gene at nucleotide 1691, which replaces a guanine with an adenine, leads to substitution of a glutamine for an arginine at amino acid 506 of the protein. The Glutamine506 variant (factor V Leiden) is associated with an increased risk of peripheral venous thrombosis (Koster et al., 1993) and of cerebral venous thrombosis, but not CHD (Wu & Tsongalis, 2001). It is normally activated by thrombin, but is more poorly inactivated by activated protein C than is the FV Arginine506 variant. An additional property of the Glutamine506 variant relates to the ability of FV to act as a cofactor in the inactivation of FXIIIa. This anticoagulant function requires preliminary cleavage of FV zymogen by activated protein C, which is impaired in the FV Glutamine506 type. The presence of Glutamine506 is associated with an increase in plasma prothrombin F1+2 (Bauer et al., 2000), indicating increased basal thrombin formation. Risk of recurrent venous thrombosis is greatly augmented by the combined presence of FV Leiden and another risk factor for this disorder (Nowak-Gottl et al., 2001).

Recently, an A4070G polymorphism has been described in the FV gene, in which possession of the G4070 allele is associated with venous thromboembolism and activated protein C resistance (Alhenc-Gelas et al., 1999).

6.9.5 Factor XIII

Plasma FXIII accelerates maturation of the fibrin clot by promoting the formation of shear-resistant and fibrinolysis-resistant crosslinked fibrin molecules. It is composed of two A and two B subunits assembled as a tetramer of 320 kDa. Four common polymorphisms have been described in the A subunit, among them Valine34Leucine, located three amino acids from the thrombin-cleavage activation site at Arginine37–Glycine38. The leucine allele of this polymorphism has been associated with protection against venous thrombosis (Catto et al., 1999), apparently due to increased FXIII activation and enhanced crosslinking of fibrin fibrils. The same variant allele may also protect against myocardial infarction, but promote intracranial haemorrhage.

6.9.6 Endothelial protein C/activated protein C receptor

This small protein is present predominantly on the surface of endothelial cells in large blood vessels where it captures protein C for activation by the thrombin–thrombomodulin complex. A 23 base-pair insertion in exon 3 of the gene has been linked to venous thrombosis (Biguzzi et al., 2001).

6.9.7 Thrombomodulin

A polymorphism in the thrombomodulin gene leads to substitution of a valine for an alanine at amino acid 455. There is one report of an association between this polymorphism and myocardial infarction (Norlund et al., 1997), but this has not been confirmed (Ireland et al., 1997). Recently, a haplotype comprising the rare alleles of two thrombomodulin variants, one in the coding region (A455V) and the other in the promoter region of the gene (−1208–1209 bp) has been reported to contribute to CHD risk in northern Europeans, especially in the obese (Konstantoulas et al., 2004).

6.9.8 Plasminogen activator inhibitor

The PAI-1 gene encodes a 50 kDa protein. A polymorphism of special interest is a 4G/5G insertion/deletion at −675 bp in the promoter region. Many studies have reported that the homozygotic 4G/4G
state is associated with a considerable elevation in plasma PAI-1 concentration (Ye et al., 1995).

Data on the possible relation between this polymorphism and CHD are highly conflicting. Thus, for example, in one large study the 4G allele was associated with a significantly increased risk of myocardial infarction (Margaglioni et al., 1998), while in the large ECTIM study no relation was found (Morange et al., 2000). No associations have been described between the 4G/5G polymorphism and cerebrovascular disease. Recent studies have claimed an additional risk of venous thrombosis in carriers of FV Leiden, who also have a mutation in the PAI-1 promoter at A844G (Morange et al., 2000) or who carry the 4G allele of the 4G/5G polymorphism (Segui et al., 2000).

6.9.9 Platelet membrane GP IIb–IIIa complex
Platelets possess a series of membrane glycoproteins that play crucial roles in their adhesion and aggregation, binding to circulating endothelial and subendothelial ligands such as fibrinogen and vWF. GPIIb is a 150 kDa protein which associates with the 90 kDa protein GPIIIa in the activated platelet. A mutation in GPIIIa results in substitution of a proline for a leucine at position 33. The leucine allele is known as PLA1, whereas the proline allele is called PLA2. The literature on this polymorphism is highly contradictory, though it has been suggested that the PLA2 allele may increase the risk of myocardial infarction in smokers (Ardissino et al., 1999). There is no strong evidence for an association of this polymorphism with cerebrovascular disease.

6.10 Dietary characteristics and haemostasis
This section considers the inter-relations of haemostasis with energy intake, alcohol consumption, dietary fat (quantity and composition), carbohydrate and micronutrients. Its main points are summarised in Table 6.3.

<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Energy (obesity/weight loss)</th>
<th>Alcohol</th>
<th>Fat</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>tPA1</td>
<td>+++</td>
<td>+++</td>
<td>+/0</td>
<td>0</td>
</tr>
<tr>
<td>PAI-12</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>FVIIc/vWF3</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
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<tr>
<td>FVIIIc</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>Platelets</td>
<td>0</td>
<td>↓+++</td>
<td>(SFA ↑) + + (Plant PUFA ↓) + + (Fish oils ↓) +++</td>
<td></td>
</tr>
</tbody>
</table>

1 Tissue plasminogen activator.
2 Plasminogen activator inhibitor type-1.
3 Factor VII activity/von Willebrand factor.
4 Factor VII activity.

+++, strong; ++, moderate; +, weak; +/0, very weak; (+), one study only; 0, none; ↑↓, direction of sequential activations.
6.10.1 Dietary energy

Studies of dietary energy and haemostasis have used adiposity and weight loss as markers of intake. Obesity is well known to be related to a raised fibrinogen concentration (Vorster et al., 1989), increased PAI-1 (Iso et al., 1993), increased plasminogen levels (Licata et al., 1995), and impaired viscosity (Fanari et al., 1993). Waist-to-hip ratio (a measure of central obesity; see Chapter 2) is positively associated with plasma fibrinogen concentration (Krobot et al., 1992; Avellone et al., 1994). PAI-1 expression is detectable in adipose tissue, and transforming growth factor beta (TGF-beta) administration raises PAI-1 mRNA in mice (Lundgren et al., 1996). In childhood, fibrinogen and FVIIc levels are higher in the obese than in the lean (Cook et al., 1999).

Weight loss induced by energy restriction is followed by a reduction in FVIIc (Slabber et al., 1992), a fall in PAI-1 level and activity (Huisveld et al., 1990; Peternel et al., 1994), and a reduction in the circulating tPA/PAI-1 complex (Calles-Escandon et al., 1996). An increase in plasmin–antiplasmin has also been reported (Velthuis-te Wierik et al., 1995). A more recent study of energy restriction confirmed changes in PAI-1 and tPA antigen concentrations and activity (Pilgeram & Pickart, 1968). Together, these results are consistent with an improved fibrinolytic capacity with weight loss.

Reported results on the influence of energy restriction on fibrinogen concentration are conflicting, possibly because any association is determined by the parallel response of fatty acids. Circulating free fatty acids stimulate hepatic fibrinogen output (Pilgeram & Pickart, 1968), and during weight loss fibrinogen’s peaks and troughs tend to mirror those in free fatty acid levels (Vorster et al., 1989). Thus, during rapid weight loss, fibrinogen and free fatty acids levels increase, while during less dramatic declines in weight, both fall.

Point to note
- Obesity is associated with several indicators of a prothrombotic state.

6.10.2 Alcohol

Moderate alcohol intake protects against CHD (Doll et al., 1994), partly through effects on high-density lipoproteins (HDL), but also possibly through effects on haemostasis.

Alcohol intake is inversely related to fibrinogen concentration (Stefanick et al., 1995; Mukamal et al., 2001) and perhaps offers protection in this way, as well as by reducing the concentrations of FVII (Gorinstein et al., 1997; Mukamal et al., 2001) and FVIII (Mukamal et al., 2001). An early epidemiological report found that alcohol consumption raised fibrinolytic activity (Meade et al., 1979). However, more recent studies suggest that while intakes of up to one drink per day do not influence PAI-1 concentration, intakes higher than this increase PAI-1 concentration (Djousse et al., 2000; Mukamal et al., 2001) and activity (Marques-Vidal et al., 1995), and on this account would be expected to reduce fibrinolytic activity (MacCallum et al., 1998). Plasma tPA antigen is also raised in heavy drinkers, which is also consistent with decreased fibrinolytic activity (MacCallum et al., 1998). This is because when a dual rise occurs in PAI-1 and tPA concentration, the rise in the latter is generally considered to be secondary to its formation of an inactivating complex with PAI-1. Consistent with these findings, in beer-drinking men a change to low alcohol beer for four weeks was accompanied by decreases in tPA and PAI-1, while the ratio of tPA/PAI-1 increased (Dimmitt et al., 1998). In contrast to these studies, a short period of alcohol consumption in men with coronary artery disease was reported to produce a reduction in PAI-1 and an increase in FVIIc and FVII antigen (FVIIag) (Gorinstein et al., 1997).

The impact of alcohol on platelet function is complex. Alcohol is recognised as a potent but transitory inhibitor of platelet function. Acute withdrawal may have rebound effects, including the activation of platelets by normal agonists (Neiman, 1988). In the Caerphilly Heart Study, alcohol intake was associated with an increased sensitivity of platelets to aggregation on exposure to thrombin, but with a decreased aggregatory response to ADP and collagen (Renaud et al., 1992). Both red and white wine reduce thrombin-induced platelet aggregation (Struck et al., 1994).

Plasma TFPI appears to be unaffected by alcohol consumption (Dimmitt et al., 1998).
Points to note
- Reports of the effects of acute and chronic alcohol consumption on haemostatic markers have been inconsistent.
- Regular alcohol consumption appears to reduce fibrinogen, FVII and FVIII concentrations.
- Heavy drinking, however, has been associated with reduced fibrinolytic activity.
- The effect of alcohol on platelet function is not clear, but probably varies with the type of beverage taken and the intensity and duration of consumption.

6.10.3 Dietary fat and coagulation

Following early reports of a high FVIIc concentration in men at high risk of CHD (Meade et al., 1986), particular attention has been given to links between FVII levels and dietary fat intake. This interest was raised partly by the epidemiological cross-sectional associations of FVIIc and FVIIag with plasma triglyceride concentration in the non-fasting and fasting states (Constantino et al., 1977; Miller et al., 1985; Mitropoulos et al., 1989).

In a study of six healthy adults, dietary intake was recorded continuously for seven weeks by weighed inventory, while total fat intake was varied. Fasting blood samples were taken at two to three day intervals, giving 20 study days. Plasma FVIIc was related positively to total energy intake, total fat intake, and consumption of saturates, monounsaturates and polyunsaturates in the previous 24 hours. In multivariate analysis, total fat was the main determinant of FVIIc. Importantly, whereas fasting triglyceride concentration was increased on a low fat, high-carbohydrate diet, FVIIc was reduced (Miller et al., 1986). These, and many other studies in which triglyceride and FVIIc did not track together following dietary intervention (Marcckmann et al., 1993a), indicate that total fat intake, rather than blood triglyceride levels, was the primary determinant of FVIIc. Prior exercise will reduce postprandial lipaemia after a standardised fatty meal, but not the accompanying activation of FVII (Gill et al., 2001). The cross-sectional positive association of FVIIc with triglyceride in epidemiology arises simply because habitual fat intake is a major determinant of non-fasting and fasting triglyceride levels. Factor X, prothrombin and fibrinogen were not influenced by manipulation of fat intake.

Improved methods of measurement have shown that the increase in FVIIc after acute dietary fat loads is due to postprandial activation of FVII, rather than an increase in FVIIag levels (Miller et al., 1991; Sulomaa et al., 1993; Silveira et al., 1994). The response is generally observed within 2 to 3 hours, persists for several hours (sometimes beyond return of triglyceride to fasting levels), and exhibits dose–response characteristics (De Grassi et al., 1994), especially in older age. An habitual increase in total fat consumption is, however, generally accompanied by elevations in both FVIIa and FVIIag (Miller et al., 1995).

Numerous studies have examined the possible importance of fat composition for postprandial change in FVIIa levels. A good example is provided by Tholstrup et al. (2003). In this study, 16 young healthy men received five test meals in random order, rich respectively in stearic acid, palmitic acid, oleic acid, linoleic acid and C18:1 trans fatty acid. Eight subjects took a test meal rich in a combination of myristic and palmitic acids. Between study periods the habitual diet was consumed, except in the 48 hours before the meal when a standardised diet was eaten. To optimise matching of test fats with respect to fatty acid composition and location of fatty acids on the glycerol backbone of triglycerides, fats were produced by interesterification of tristearin, tripalmitin and trimyristin, high linoleic acid oil, high oleic acid oil or a fat rich in C18:1 (trans). The meals rich in stearic acid, palmitic acid, or myristic acid caused less increase in FVIIa than the three meals rich in unsaturated fatty acids. Stearic acid produced the least elevation of FVIIa postprandially, in agreement with the report of Sanders et al. (2001). Chain length of the fatty acid, from C14 upwards, appeared to matter less for FVIIa than the presence of an unsaturated bond. In agreement with the report of Sanders et al. (2000), stearic acid subjected to random esterification produced a lower triglyceride response at 4 hours postprandially than other fatty acids. However, apart from one study providing approximately 20% of dietary energy as stearate (Kelly, 2001), fats naturally rich in stearic acid (e.g. butter) have not been shown to have this effect (Oakley et al., 1998). When stearic acid is provided in the background diet in supra-physiological amounts, sufficient to enrich the plasma lipids with this fatty acid, FVIIc...
increased more rapidly postprandially than when supra-physiological amounts of linoleic acid were provided in the background diet (Mitropoulos et al., 1994). This finding may indicate that, provided it is absorbed in sufficient quantity, stearic acid leads to more FVIIa production than unsaturated fatty acids. Moreover, habitual diets with a higher proportion of monounsaturates to saturates, report less activation of FVII in response to test meals (Roche et al., 1998; Larsen et al., 2000; Kelly, 2001). For effects of n-3 polyunsaturate-rich diets on coagulation, see Section 6.10.6.

Medium chain dietary fatty acids, which are absorbed by the portal system rather than by way of chylomicron metabolism, do not cause postprandial FVII activation (Yahia et al., 1995).

The mechanism whereby dietary fat leads to activation of FVII has been thoroughly investigated but remains uncertain. Long-standing observations that, in vitro, saturates support the activation of FXII led to the suggestion that free fatty acids liberated during lipolysis of triglyceride-rich lipoproteins created a negatively charged surface that supported the generation of FXIIa. In vitro, FXIIa has been shown to activate FVII directly, and indirectly by way of FIX. However, normal postprandial activation of FVII occurs in patients with complete factor FXII deficiency, which appears to refute such a mechanism in vivo (Miller et al., 1996). An alternative explanation has been based on the association of vitamin K-dependent clotting factors, such as FVII and FIX, with triglyceride-rich lipoproteins in plasma, the presence of pro-coagulant aminophospholipids as minor components of lipoproteins, and a positive cross-sectional relation between plasma FVIIa and serum phospholipid concentration. In a pure system (consisting of selected reagents only, rather than whole blood or plasma), triglyceride-rich lipoproteins promoted activation of FVII by FXa and the types of lipoprotein particles and their components (e.g. phospholipids) explained some of the differences between lipoprotein fractions in terms of ability to support factor VII activation (Kjalke et al., 2000). Human dietary intervention studies have also illustrated that this mechanism was a determinant of FVIIa activation (Silva et al., 2003). A third novel proposal is that FVII activation follows the shift of pro-coagulant phospholipid such as phosphatidylycerine from the inner leaflet to the outer leaflet of cell-surface membranes, which accompanies reverse cholesterol transport out of cells during postprandial activation of the ATP-binding-cassette transporter A1 (ABCA1). This shift allows the ordered assembly of the vitamin K-dependent clotting factors on the membrane and their interaction (Miller et al., 2002). High-fat meals have been reported to be associated also with activation of pre-kallikrein (Larsen et al., 2000) and FIX (Silveira et al., 1994). Kallikrein can activate FIX. Notably, there is no evidence that postprandial activation of FVII is accompanied by conversion of prothrombin to thrombin (Bladbjerg et al., 2000). Nevertheless, the possibility cannot be excluded that FVIIa generation after fatty meals ‘primes’ the clotting system and thus raises the risk of clinically significant coronary thrombosis in the event of coincident rupture of an atheromatous plaque with exposure of tissue factor to blood.

### Points to note

- An increase in total fat (acute or chronic) causes postprandial activation of FVII.
- A diet that is habitually high in fat is accompanied also by an increase in factor VII antigen.
- A background diet with a higher proportion of polyunsaturates or monounsaturates to saturates may attenuate the acute pro-coagulant effects of fatty meals.
- The underlying mechanism for activation of FVII and its meaning for CHD risk, have not been fully elucidated.

### 6.10.4 Dietary fat and fibrinolysis

In population surveys, PAI-1 antigen and tPA antigen were found to be positively associated with habitual fat consumption and inversely related to carbohydrate intake (Rankinen et al., 1994; Byberg et al., 2001), suggesting that high fat diets suppress fibrinolysis. The relations of PAI-1 antigen with fat intake and tPA with carbohydrate intake were independent of triglyceride concentration (Rankinen et al., 1994). In agreement, a reduced fat intake is reported to decrease plasma PAI-1 (Sundell & Ranby, 1993). Marckmann et al. (1994) reported an increase in fibrinolytic activity (i.e. reduced PAI-1) as measured by the euglobulin clot lysis time, on
switching from a high-fat diet (39% energy) to a low fat diet (28% energy as fat) for two weeks. The effect appeared to persist for at least eight months (Marckmann et al., 1993b).

In a study of acute effects, 8 hours after consumption of 100 g of butter, PAI-1 levels increased and clot lysis was suppressed (Kozima et al., 1993). However, when the response was studied over shorter periods, no effects were observed on PAI-1 or tPA activity. In contrast, a three-day increase in total fat intake was reported to suppress the response of tPA to venous occlusion (Ho et al., 1995).

With regard to dietary fat composition and fibrinolytic activity, there appear to have been relatively few studies to date. The Uppsala Longitudinal Study reported that PAI-1 activity was positively related to habitual monounsaturates and polyunsaturates intake, but not to saturates consumption (Byberg et al., 2001). However, Lopez-Segura et al. (1996) found that a diet enriched in monounsaturates decreased PAI-1 antigen and activity, but that it had no effect on plasminogen, tPA or alpha-2 antiplasmin. Temme et al. (1999), in a study in which healthy men and women took three six-week diets in random order, enriched respectively in lauric acid, palmitic acid and oleic acid, found that the palmitic acid diet was distinguished by higher levels of PAI-1 activity. No significant differences in plasminogen or alpha-2 antiplasmin activity were observed between diets.

In studies of the acute effects of test meals of differing fatty acid composition, Tholstrup et al. (2003) found that 8 hours after the meal, unsaturated fatty acids, irrespective of specific type, were associated with a higher PAI-1 antigen and a lower tPA activity than saturates (although values after all meals were reduced from baseline owing to diurnal variation). Muller et al. (2001) also observed that polyunsaturated-rich test meals produced a lower tPA than did a palmitic acid-rich meal. In vitro studies showed that linoleic acid stimulated the release of PAI-1 from cultured HepG2 cells (Bani et al., 1997).

**Points to note**

- A high fat diet appears to result in suppressed fibrinolysis.
- Studies investigating the chronic and acute effects of dietary fat quality on fibrinolysis are inconsistent and few in number.

### 6.10.5 Dietary fat and platelet function

In other sections of this report, the importance of lipoprotein profile for risk of cardiovascular disease has been stressed (see Chapter 3). The platelet/coagulation cascade is also influenced by the lipoprotein profile and has been reviewed extensively elsewhere (Bruckdorfer, 1999).

It has been reported in animal and human experiments that increases in platelet cholesterol content accompany hypercholesterolaemia. Furthermore, there are many reports that platelet activation may be increased in patients with familial hypercholesterolaemia, probably due to an excess of cholesterol in the platelet membrane. There is continuing controversy about whether more modest rises in LDL influence platelet activity, but there is clear evidence that low levels of oxidation in LDL enhance activity (Meraji et al., 1992) and that lysolecithin acid (LPA), present in oxidized LDL and in atherosclerotic lesions, may be the main agent that promotes stimulation (measured by shape change) through activation of the LPA-receptor on platelets (Stiess et al., 1999).

Higher levels of HDL tend to suppress platelet activation, especially when associated with increased levels of the minor apolipoprotein, apoE. The explanation for this is that receptors for apoE on platelets activate endogenous nitric oxide synthase to release nitric oxide and inhibit platelet activity (Riddell et al., 1997). This may also contribute to the reduction in platelet activity in liver disease where apoE levels are often high (Desai et al., 1989).

It is not surprising that dietary fats, which may alter the composition of the platelet membrane, would affect their function, since many of the events that occur during platelet activation are membrane dependent. In general, animal experiments show that increased consumption of saturates stimulates the activity of platelets, whereas polyunsaturates reduce activity (Hornstra, 2001). Whereas much of this applies to humans as shown from the early work of Renaud, the great difficulty in obtaining reliable data from human platelets has prevented measurements in large-scale epidemiological investigations (American Heart Association, 2001). In contrast to earlier data, it has been shown that diets rich in stearic acid do not increase thrombotic risk factors (including platelet activation) in healthy males (Kelly et al., 1999). Modest changes (7–10% of energy) from
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dietary oleic acid to lauric, myristic or palmitic acids did not seem to influence platelet function when assessed by measurement of ADP-induced whole blood aggregation (Temme et al., 1998). Similarly, trans isomers of polyunsaturates did not influence platelet aggregation or thromboxane formation adversely (Armstrong et al., 2000). Beneficial (Sirtori et al., 1986), detrimental (Mutanen et al., 1992; Turpeinen et al., 1998) and neutral (McDonald et al., 1989, Freese, 1994; Vicario, 1998) effects of mono-unsaturate-rich diets on platelet function have been proposed. A more recent long-term controlled dietary intervention study in young healthy students showed a significant reduction in the ex vivo activation of platelets in subjects on a high monounsaturate diet (Smith et al., 2003). Notably, it is recognised that the predictive value of ex vivo platelet aggregation or thromboxane production is uncertain, and may partly explain discrepancy between laboratories.

However, the lipid and fatty acid composition of platelets appears to be important, as clear changes could be detected in patients (requiring coronary angiography) with cardiovascular disease compared to healthy controls, using high-resolution high-field proton nuclear magnetic resonance spectroscopy as the analytical tool. Increased platelet cholesterol and decreased diacylglycerols, ethanolamine-containing phospholipids and linoleate content were observed in patients’ platelets (Noula et al., 2000). The clearest dietary protective effect of fats on platelet function is that of fish oils (see Section 6.10.6).

6.10.6 Dietary fish oil/supplements and haemostasis

(i) Coagulation

Studies of the effects of fish oil and other marine oils have so far been largely confined to the fasting state. Most have found no effect upon FVII (Sanders et al., 1981; Harris et al., 1988; Roche & Gibney, 1994), although Hornstra (1982) reported changes consistent with FVII activation. Roche and Gibney (1994) found that supplementation with 900 mg of n-3 polyunsaturates as fish oils for 16 weeks had no effect on postprandial FVIIc, whether taken with a low fat or a high-fat diet. When 15 g of olive oil was replaced with 15 g of fish oil (MaxEPA; Seven Seas, Hull), a marked reduction in postprandial lipaemia was not accompanied by any change in postprandial FVII activation (Yahia & Sanders, 1996).

Studies of the effect of dietary n-3 fatty acids on fibrinogen concentration have been highly inconsistent, most reporting no effect (Brox et al., 1983; Harris et al., 1988; Gans et al., 1990), some reporting a reduction in fibrinogen (Radack et al., 1982; Berg Schmidt et al., 1990), and others an increase (Haines et al., 1986).

(ii) Fibrinolysis

Not only linoleic acid (and oleic acid) but also eicosapentaenoic acid (EPA) significantly increase transcription of PAI-1 in cultured endothelial cells (Nilsson et al., 1998). Although not all studies are supportive (Hansen et al., 2000), supplementation of the diet with long-chain n-3 fatty acids or an increased oil-rich fish intake appears to increase PAI-1 levels (Emeis et al., 1989). In the PRIME Study, the erythrocyte content of n-3 fatty acids was inversely related to plasma tPA antigen concentration (Scarabin et al., 2001).

(iii) Platelet function

The effect of fish oil in modulating platelet function (Dyerberg et al., 1978) by attenuation of thromboxane A2 synthesis is now well known, and was clearly demonstrated in the GISSI trial involving men recently recovered from myocardial infarction. Long-term fish oil consumption (1 g/day n-3 polyunsaturated fatty acids) significantly reduced the incidence of deaths from cardiovascular disease, non-fatal events and strokes (GISSI-Prevenzione Investigators, 1999). Although suppression of platelet activation may be important here, fish oils have other beneficial effects as anti-arrhythmics, by lowering serum triglycerides and stimulating endothelial nitric
oxide synthesis (Abeywardena & Head, 2001). DART, another secondary prevention trial, showed that feeding oil-rich fish two or three times a week for two years lowered mortality by 29% (Burr et al., 1989). The effects of plant n-3 fatty acids have also been compared with those of fish oils and found to be less effective as inhibitors of platelet aggregation (Wensing et al., 1999). In a study on vegetarian men, additional dietary alpha-linolenic acid increased the EPA and docosahexaenoic acid (DHA) content in platelet phospholipids, but no concomitant change in platelet function was observed (Li et al., 1999).

Points to note

- Fish oils appear not to activate fasting or postprandial FVII levels.
- Fish oils attenuate thromboxane A2 synthesis and thereby suppress platelet activation.

6.10.7 Dietary carbohydrates

Many studies of the effects of fibre have used diets relatively low in fat and animal protein, making difficult the disentanglement of the actions of fibre per se on the haemostatic system. Fehily et al. (1982) reported in a cross-sectional study that a high intake of cereal fibre was associated with a reduced fibrinogen concentration, but addition of cereal fibre to the diet of healthy adults had no effect on fibrinogen (Fehily et al., 1986). In another study, subjects consumed either rye or wheat bread for four weeks. Rye bread provided twice the fibre content of wheat bread, but no significant differences between diet periods were observed for fibrinogen concentration, FVIIc, prothrombin fragment F1+2, tPA or PAI-1 levels (Turpeinen et al., 2000). Whether soluble non-starch polysaccharide (NSP) has any effect on fibrinogen in humans is uncertain, but two studies reported lowering effects in obese baboons (Venter et al., 1990) and obese rats (Venter et al., 1991). High-fibre diets have been reported to improve fibrinolysis. Thus, oat husk fibre (Sundell & Ranby, 1993) and guar gum (Landin et al., 1992) have been reported to reduce the level of PAI-1. Mehrabian et al. (1990) concluded that a high carbohydrate, low fat diet taken for three weeks reduced plasminogen, tPA and PAI-1. How dietary fibre may influence fibrinolytic factors is uncertain. High-fibre diets are often prescribed with a low dietary fat intake and exercise to improve cardiovascular risk factor status. Lindahl et al. (1999) reported that such a regimen taken for one year reduced weight in obese individuals, and reduced tPA and PAI-1 levels, therefore improving fibrinolytic capacity. However, this improvement could have been due to weight loss rather than high-fibre intake per se.

Point to note

- Diets that result in weight loss, including low fat, high fibre diets, should improve fibrinolytic activity.

6.10.8 Micronutrients

There are miscellaneous reports of associations of vitamin intake and plasma levels with haemostatic status. In the Swedish MONICA study, a high plasma retinol level was accompanied by reduced plasma fibrinogen and tPA levels, and an increased PAI-1 level (Eliasson et al., 1995). Hankey et al. (1996) reported raised PAI-1 activity and FVIIc in patients who had raised retinol levels. Retinoic acid induces the activation of latent transforming growth factor-beta (TGFβ), and simultaneously up-regulates expression of the TGFβ receptor types I and II. The latter responses reduce the concentration of TGFβ needed to exert its effects. Thus retinoic acid modulates the actions of TGFβ on endothelial cells and hepatocytes, suppressing fibrinolytic activity by decreasing tPA expression and increasing PAI-1 expression (Yoshizawa et al., 1998).

Stief et al. (2000) have reported that physiological concentrations of oxidants of the hypochlorous acid and chloramine types are anticoagulant in human plasma. They produce irreversible oxidation of fibrinogen, FV, FVIII and FX, prolonging the prothrombin time and activated partial thromboplastin time. Chloramines also inhibit platelet aggregation, but chlorinated LDL particles activate platelets. These effects are inhibited by ascorbic acid, which protects clotting factors against non-radical oxidants released from activated phagocytes. However, in a randomised, placebo-controlled crossover study,
ascorbic acid supplementation had no effects on platelet performance, tPA, PAI-1, fibrinogen and vWF levels in healthy subjects with low normal ascorbic acid concentrations (Tofler et al., 2000).

Some vitamins could affect haemostasis though an influence on plasma homocysteine levels. Stamler and Slivka (1996) reported that homocysteine acts as an inhibitor of protein C by competing for thrombomodulin. Whether folate and vitamin B₆ alter haemostatic mechanisms by lowering homocysteine levels is not known. Kristensen et al. (1999) found that a moderately raised increase in homocysteine after methionine loading was independently associated with an elevated tPA concentration in young adults who had recovered from an ischaemic stroke. This elevation was accompanied by a low tPA activity and a raised PAI-1, indicating a state of hypofibrinolysis.

In one study of obese type 2 diabetics, vitamin E (600 mg daily for three months) produced a reduction in tPA with no change in PAI-1 (Skrha et al., 1999). In another study, high-dose supplements of vitamin E were followed by decreased expression of platelet adhesion molecules in type 2 diabetes (Ferber et al., 1999).

Other data tend to support an effect of vitamin E on platelet function (Steiner, 1999). There are clear anti-aggregatory effects of vitamin E in vitro, the mechanisms of which may not relate to the antioxidant action of alpha-tocopherol but rather to direct membrane effects through transduction mechanisms leading to inhibition of protein kinase C (Steiner et al., 1997).

The problem with many of the studies on human subjects is that measurements were made with single doses of platelet agonist or antagonist so that wide intra-individual variations in platelet activity masked any effects. Calzada et al. (1997) showed that supplementation with 300 mg alpha-tocopherol per day for eight weeks modestly, but significantly, reduced the responsiveness of platelets to ADP and arachidonic acid (but not collagen) and increased sensitivity to the platelet inhibitor PGE₁. In the same study, ascorbate had an even more modest non-significant effect, as was the case for beta-carotene. In a follow-up study, it was shown that doses of natural alpha-tocopherol, as low as 75 IU/day, were sufficient to provide the same effects in terms of aggregation, whereas secretion of ATP from platelets was further decreased as the dose increased to 400 IU (Mabile et al., 1999). Whether these changes were sufficient to change the prothrombotic challenge provided by an atherosclerotic plaque is difficult to determine. The current evidence has been reviewed elsewhere (Schoene, 2001). In much earlier work, patients with verified cardiovascular disease, with increased levels of plasma lipid peroxides, showed improvements in platelet function with a cocktail of antioxidants (Salonen et al., 1991).

Despite the rising interest in non-vitamin antioxidants such as flavonoids (British Nutrition Foundation, 2003a), there have not been many studies on their effects, outside the well known inhibitory actions of genistein, which is a profound inhibitor of tyrosine protein kinases, important for platelet activation. Epigallocatechin gallate, an important antioxidant (a flavan-3-ol) in green tea and chocolate, has been shown to inhibit platelet aggregation by a range of agonists (Kang et al., 1999), but at concentrations well above those which would normally be found in the blood of tea drinkers. However, this flavonoid also inhibits nitration of proteins by peroxynitrite in platelets at even lower concentrations (2 µmol/l) and may have specific effects that are not yet fully understood (Sabetkar et al., 2002). Nitration of platelet proteins is a reversible process, which has not yet been related to platelet function.

Although ascorbate has little direct effect on platelet activation, there is compelling evidence that it does increase nitric oxide formation in the endothelium (Heller, 2001). There are many articles that show that infusion or ingestion of large doses of antioxidants can reverse impairment of endothelium-dependent relaxation. Plasma ascorbate levels and those of other antioxidants are strongly reduced by smoking, which may have both direct and indirect effects on platelet activity. A meta-analysis of several past studies showed a pattern of increased platelet aggregation immediately after inhalation, followed by a decreased responsiveness in periods between cigarettes (Smith et al., 1998), possibly as a down-regulation response to the initial activation. Recent work shows that there is a decrease in the capability of platelets from smokers to form thromboxane A₂ (TXA₂), which may explain why smokers seem to be more sensitive to aspirin (Weber et al., 2000). The presence of activated platelets in smokers is confirmed by flow cytometric measurements of exposure of surface glycoproteins and that of P-selectin (Nair et al., 2001).
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Points to note
- Studies suggest a potential role for retinol, vitamin C and folate/vitamin B₆ in haemostasis, but much more research is required before dietary intervention trials are warranted.
- The role for a beneficial effect of vitamin E, particularly on platelet function is somewhat stronger. However, the efficacy of vitamin E supplementation on thrombosis risk is unknown.

6.10.9 Miscellaneous foods
Capsicum (Visudhiphan et al., 1982), chillies (Glatzel & Ruberg-Schweer, 1967), garlic (Resch & Ernst, 1995), and green tea (Ali & Afzal, 1987) have been reported to increase fibrinolytic activity, but the possible mechanisms are uncertain. Garlic also has a profound effect on platelet function (Srivastava & Tyagi, 1993). The effects of these foods on haemostasis, when eaten as part of a balanced diet, are uncertain.

6.10.10 Diet and genotype
Although there have been an increasing number of studies examining the effects of genetic polymorphism on the blood lipid responses to diet in humans, very few studies of this type have been reported on haemostatic responses. Sanders et al. (1999) found that the Arginine353Glutamine polymorphism in the FVII gene had no effect on postprandial activation of FVII after an oleate-rich test meal. Loktionov et al. (1998) examined the effect of black tea consumption on PAI-1 activity: in individuals carrying the apolipoprotein E2 allele, but not in those of the E3/E3 or E3/E4 genotypes, tea appeared to cause a reduction in PAI-1 activity. Employing a very high saturates test meal (130 g), Byrne et al. (1998) found an increase in PAI-1 activity of marginal statistical significance 8 hours postprandially in men possessing the 4G allele for the PAI-1 genotype, as compared to 5G homozygotes, but no genotype effect on PAI-1 antigen level. More studies of this type are certain to be undertaken in the future.

Points to note
- A number of polymorphisms in genes coding for haemostatic proteins have been identified.
- Studies investigating the influence of diet on haemostatic genetic polymorphisms are very scarce.

6.11 Key points
- Blood coagulation is a complex autocatalytic process involving cellular elements and soluble proteins, self-limiting in time and space, with control through numerous positive and negative feedback loops. Initiation, evolution, maturation and eventual dissolution of the clot or thrombus involves coagulation enzymes (serine proteases) and their cofactors, anticoagulant proteins and cofactors, inhibitory proteins, blood platelets and vascular endothelial cells.
- In addition to prevention of bleeding, the constituent elements have roles in inflammation, angiogenesis and tissue repair.
- The prothrombotic state can be regarded as an imbalance between coagulant and fibrinolytic capacity favouring fibrin deposition and survival. It comprises one or more of hypercoagu-

lability of plasma, unphysiological activation or sensitivity to activation of platelets and/or endothelial cells, and hypofibrinolysis.
- Haemostatic factors positively related to risk of CHD include fibrinogen, von Willebrand factor and (inconsistently) factor VII activity. Plasma tPA antigen and PAI-1 antigen and activity are also positively associated with CHD risk. Changes in platelet function in those at high risk of CHD, though strongly suspected, are difficult to substantiate for methodological reasons.
- Emerging haemostatic risk factors for CHD include activated factor XII, prothrombin fragment 1+2, factor IX activation peptide and D-dimer. Markers of platelet activation linked to CHD risk are being actively sought.
including surface proteins exposed upon platelet activation.

- Genetic variants of many haemostatic factors are being increasingly recognised, some of which are related to venous thrombosis. Variants associated with risk of CHD have yet to be definitely established.
- Obesity is associated with several indicators of a prothrombotic state.
- Reports of effects of acute and chronic alcohol consumption on haemostatic mechanisms have been inconsistent. Regular alcohol consumption appears to reduce the level of fibrinogen and some other clotting factors, and also reduce fibrinolytic activity. The impact of alcohol intake on platelet function has not been comprehensively investigated, but may vary with the type of beverage taken and the intensity and duration of consumption.
- Fatty meals cause postprandial activation of factor VII, although the underlying mechanism and its meaning for CHD risk have not been fully elucidated. A high fat diet is accompanied also by an increase in factor VII antigen, together with evidence for suppressed fibrinolysis.
- Low levels of oxidised LDL enhance platelet activity, whereas high levels of HDL tend to suppress platelet activation.
- Dietary saturates increase platelet activity, whereas polyunsaturates have the opposite effect.
- Fish oils attenuate thromboxane $A_2$ synthesis and thereby suppress platelet activation.
- An increasing number of genetic polymorphisms are being described in the haemostatic system, but studies of their potential relevance for responses to diet are in their infancy.

### 6.12 Recommendations for future research

Areas where more data are needed to clarify the effects of diet on established haemostatic risk factors are apparent throughout the text. However, several general comments can be made.

- As the term implies, more studies are needed of the emerging haemostatic risk factors to establish their importance for cardiovascular disease. Should the evidence strengthen the case for causal association, studies would then be justified on the effects of dietary energy (including obesity and exercise), alcohol, dietary fat, carbohydrate and micronutrient intake on the level and activity of these new risk factors. Should the effects of diet appear important, a case could then be made to examine the importance of genotype for the responses to diet.
- Dietary energy intake as indicated by body fat, alcohol consumption, saturates, polyunsaturates including fish oils, and various micronutrients have all been linked to cardiovascular disease. Our understanding of the importance of these dietary characteristics for the status of the haemostatic system remains patchy, and research is needed to provide a more comprehensive picture. Prothrombin $F_{1+2}$, a marker of the action of FXa on prothrombin; fibrinopeptide A, released by the action of thrombin on fibrinogen; and D-dimer, an end-product of plasmin activity on crosslinked fibrin, are useful markers of pro-coagulant and fibrinolytic activity, and together provide an assessment of haemostatic balance. They and other markers of haemostatic activity could be studied in, for example, childhood and adult obesity, or in relation to acute and habitual alcohol consumption. More studies of dietary fat and haemostasis are needed, but in studies of acute effects it is important to control the diet in the days beforehand and to use an isoenergetic low fat control meal, in order to avoid confusion of meal-effects with diurnal fluctuations.
- Many reported associations between dietary factors and haemostatic factors are unexplained mechanistically. For example, is excess body fat associated with an increased plasma fibrinogen concentration because of increased production or decreased clearance of fibrinogen?
- Most of our time is spent in the postprandial state. Thus it remains important to examine not only the effects of the habitual diet on basal levels of haemostatic factors, but also to assess
the acute effects of specified meals on post-prandial levels. The possibility of interaction between the characteristics of the habitual diet and the last meal, with respect to the postprandial state of the haemostatic system, is worth bearing in mind in future research.

- The importance of fish oils for prevention of cardiovascular disease is increasingly appreciated. Further studies are warranted of the effects of dietary $n$-3 long-chain polyunsaturates on established and emerging haemostatic risk factors. Studies should compare the responses to physiological and supra-physiological doses of these fatty acids.
- Of particular importance is the need to overcome the impasse to research due to the lack of tests of platelet behaviour in vivo that are relevant for cardiovascular disease. There is an urgent requirement for tests that are relatively insensitive to the quality of venepuncture, reproducible and capable of being performed on large numbers of samples inexpensively.

### 6.13 Key references

7
Inflammation-Related Factors

7.1 Introduction

Inflammation is an intrinsic element of atherogenesis through all stages of its development, from initiation, through progression to its thrombotic complications (Ross, 1999). There are a number of putative markers of persistent, low-grade inflammation, which have been studied as potential predictors of risk of future cardiovascular or peripheral vascular disease (PVD). For this approach to be valid requires long-term circulating concentrations to be reasonably stable and show a similar intra-individual variability as other established risk factors, such as blood cholesterol. Importantly, high-sensitivity assays have recently been used to detect what is presumably low-grade inflammation that would previously have been undetected, providing a valid tool for the assessment of subclinical inflammation as a potential new marker of vascular risk.

7.2 The immune system

The immune system consists of a network of lymphoid organs, cells, humoral factors and cytokines, which are co-ordinated for host defence. There are two functional divisions within the immune system – the innate (or natural) immune system and the acquired (specific/adaptive) immune system. The innate immune system consists of granulocytes, monocytes, macrophages and natural killer cells, which do not specifically recognise foreign pathogens, but are involved in surveillance and phagocytosis (engulfment and destruction of foreign material). Lymphocytes are involved in specific immunity, and are able to recognise and react with specific antigens to mediate their removal. All cells of the immune system originate in the bone marrow and are found circulating in the bloodstream, organised into lymphoid organs (such as the thymus, spleen and lymph nodes) and dispersed in tissues throughout the body.

Communication within the acquired immune system and between the acquired and innate immune systems is brought about by direct cell-to-cell contact involving adhesion molecules and by the production of chemical messengers. The key messengers in immune responses are cytokines. Although there are a large number of cytokines, tumour necrosis factor-alpha (TNFα), interleukin-1 (IL-1) and interleukin-6 (IL-6), which are produced by monocytes and macrophages, activate a number of cell functions that define their central role in immune responses (Fig. 7.1). These cytokines increase vascular permeability and expression of adhesion molecules by endothelial cells in the arterial wall, allowing the infiltration of granulocytes and lymphocytes into sites of injury and infection where they assist with the elimination of pathogens. They also mediate a metabolic response to infections, inducing fever and loss of appetite, the mobilisation of protein from skeletal muscle and the mobilisation of fat from adipose tissue (see Chapter 9). The liver is an important target for the action of the cytokines; it responds to their signals by synthesising 'acute phase proteins', such as C-reactive protein (CRP) and fibrinogen, which are required for repair and recovery. TNF, IL-1 and IL-6 are described as 'inflammatory' cytokines, since they bring about the effects of systemic inflammation, which is the body’s response to infection or injury and is typified by redness, swelling, heat and pain. Although inflammation is an integral part of the innate immune response, failure to resolve the response or chronic overproduction can be
detrimental and has been implicated in the aetiology of inflammatory diseases, such as rheumatoid arthritis, psoriasis and inflammatory bowel disease.

### 7.3 Inflammation and atherosclerosis

Atherosclerosis bears many of the hallmarks of a chronic inflammatory disease (Ross, 1999). It is characterised by accumulation of monocytes and lymphocytes through all stages of its pathogenesis, beginning with the formation of fatty streaks underlying the endothelium of large arteries. The infiltration of monocytes and lymphocytes occurs as a result of the secretion of chemoattractant molecules such as monocyte chemoattractant protein-1 (MCP-1) and the expression of adhesion molecules by endothelial cells lining the artery wall in a manner identical to that observed in an inflammatory response to an infection. Several stimuli for the inflammatory response in atherosclerosis have been proposed, including oxidised low-density lipoprotein (LDL), homocysteine, free radicals and infectious microorganisms (see Chapters 5 and 8). However, the nature of the immune response towards these stimuli is not clear. For example, while oxidised LDL induces immune cell activation and inflammation, it is not a strong stimulus for antibody production (Stemme et al., 1995). On the other hand, antibodies against oxidised LDL have been found in individuals with established coronary heart disease (CHD) (Stemme, 2001). While the precise inflammatory nature of oxidised LDL is not entirely clear, it is accepted that monocytes that have infiltrated the arterial intima and differentiated into macrophages take up oxidised LDL through scavenger receptors in an unregulated manner, accumulating large amounts of cholesterol and becoming foam cells (see Chapter 1, Fig. 1.1). Although homeostatic responses exist to remove cholesterol from macrophages, they progressively fail in atherosclerosis, and when the macrophages eventually die, through necrosis (uncontrolled cell death) and apoptosis (programmed cell death), the lipid is deposited within the core of the developing plaque (Fig. 7.2). Cytokines secreted by both lymphocytes and macrophages within the plaque exert pro- and anti-atherogenic effects on components of the vessel wall. Smooth muscle cells migrate from the medial portion of the arterial wall towards the intima and secrete extracellular matrix proteins that form a fibrous cap (see Chapter 1, Fig. 1.1 and Fig. 7.2). The cap separates the highly thrombogenic contents of the plaque lipid core from the potent coagulation system contained within the circulating blood (see Chapter 1, Fig. 1.1 and Fig. 7.2). Analysis of advanced human plaques suggests that they undergo repetitive cycles of microhaemorrhage and thrombosis, which predominantly occurs at the shoulder regions (Fig. 7.2). Matrix metalloproteinases secreted by macrophages degrade extracellular matrix proteins and contribute to the weakening of the fibrous cap, which can lead to plaque rupture (Fig. 7.2). The resulting thrombosis can lead to a fatal occlusion of the artery.
While much of the inflammatory activity in atherosclerosis is located in the arterial intima, there is compelling evidence to suggest that it is reflected by a persistent, low-grade inflammation in the circulation. This chronic, low-grade inflammation is likely to be the result of a ‘spilling over’ of inflammatory molecules (cytokines secreted by monocytes and soluble adhesion molecules shed from the surface of endothelial cells) from the vessel wall into the circulation, where they subsequently act on the liver to induce the secretion of acute phase proteins, including CRP, fibrinogen and serum amyloid A (Fig. 7.3). Both the inflammatory mediators (cytokines and soluble adhesion molecules) and the acute phase proteins have been studied as potential predictors of risk of future cardiovascular disease or PVD. Since they are functionally related, there are strong associations between plasma levels of some of these molecules. The following sections assess the potential for individual markers of the inflammatory response to predict risk of vascular disease and the degree to which they are independent risk factors.

**7.4 Risk factors**

**7.4.1 C-reactive protein (CRP)**

CRP is an acute phase reactant synthesised by the liver and regulated principally by the cytokine IL-6. Its plasma levels increase dramatically (100-fold or more) in response to bacterial infections, physical trauma and other inflammatory conditions (Tracy, 1998). In the majority of individuals, values of circulating CRP concentrations were found to be below...
Inflammation-Related Factors

the detection limit of most routine assays (<1.0 mg/dl). However, the use of the new ‘high sensitivity’ CRP (hsCRP) assays has allowed the assessment of subclinical levels in ‘healthy’ individuals. It has been argued that levels of markers of inflammation vary too greatly over time to allow accurate prediction of risk. However, as long as measurements are not made within 2–3 weeks of an acute infection, hsCRP levels are stable over long periods in most individuals (Ridker et al., 1999). The exception to this are individuals with clinically apparent inflammatory conditions, such as rheumatoid arthritis, who are likely to have elevated levels of hsCRP well into the clinical range (>1.5 mg/dl); clearly the diagnostic value of hsCRP for vascular risk will be of limited value in these patients (Ridker, 2001).

High serum concentrations of CRP correlate with the presence of subclinical cardiovascular disease and the risk of acute cardiovascular events. Several large scale, prospective epidemiological studies have shown that the plasma level of hsCRP is a strong independent predictor of future myocardial infarction (MI), stroke and PVD among individuals without known cardiovascular disease (Kuller et al., 1996; Tracy et al., 1997; Ridker et al., 1998, 2000; Danesh et al., 2000b; Koenig et al., 1999; Mendall et al., 2000). For example, in a cohort of 22 000 apparently healthy middle-aged men, those with baseline levels of hsCRP in the highest quartile were reported to have a two-fold increase in risk of stroke or PVD and a three-fold increase in risk of MI (Ridker et al., 1997, 1998). The effects were independent of all other lipid and non-lipid risk factors, and were apparent in both smokers and non-smokers. The epidemiological data from a number of study populations support this observation (Fig. 7.4). CRP was identified as an independent prospective cardiovascular disease risk factor in the higher risk middle-aged men of the Multiple Risk Factor Intervention Trial (MRFIT) (Kuller et al., 1996), in elderly patients in the Cardiovascular Health Study (Tracy et al., 1997), in post-menopausal women in the Women’s Health Study (Ridker et al., 1998, 2000) and in three European cohorts (Koenig et al., 1999; Danesh et al., 2000b; Roivainen et al., 2000). Some studies have suggested that the addition of hsCRP to lipid screening improves the estimation of vascular risk over the use of lipid screening alone, since hsCRP has been shown to be a significant predictor of risk even in individuals with normal LDL-cholesterol levels (Danesh et al., 2000b). This is pertinent, since a high proportion of MIs occur in individuals with normal plasma lipid levels. It is interesting to note in this context that the relationship between LDL-cholesterol and hsCRP is weak, which has led to the suggestion that hyperlipidaemia and enhanced inflammation are separate but interactive processes (Ridker et al., 2001c). Furthermore, treatment with the statin group of cholesterol-lowering drugs significantly reduces CRP levels, an...
**Table 7.1** Distribution of hsCRP among apparently healthy American men and women. Normal values for CRP range from 0.01 mg/dl to 0.8 mg/dl. Individuals with chronic inflammatory conditions have CRP levels above 1 mg/dl; for example, patients with rheumatoid arthritis have CRP levels of approximately 1.5 mg/dl, reflecting ongoing chronic inflammation in the joints.

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<tr>
<th>Quintile</th>
<th>Range (mg/dl)</th>
<th>Risk estimate</th>
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<tbody>
<tr>
<td>1</td>
<td>0.01–0.069</td>
<td>Low</td>
</tr>
<tr>
<td>2</td>
<td>0.07–0.11</td>
<td>Mild</td>
</tr>
<tr>
<td>3</td>
<td>0.12–0.19</td>
<td>Moderate</td>
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<tr>
<td>4</td>
<td>0.20–0.38</td>
<td>High</td>
</tr>
<tr>
<td>5</td>
<td>&gt;0.38</td>
<td>Highest</td>
</tr>
</tbody>
</table>


The utility of hsCRP in clinical practice requires estimates of risk across a spectrum of hsCRP levels (Ridker, 2001). A quintile approach using a population distribution of hsCRP based on analysis of more than 5000 subjects has been suggested by Ridker *et al.* (2001; see Table 7.1).

The use of this approach can be refined to include estimates of relative risk derived from the determination of lipid and hsCRP measurements, as illustrated in Fig. 7.5. In this model, individuals with levels of both hsCRP and total cholesterol:high-density lipoprotein (HDL) cholesterol (TC:HDL-C) in the highest quintiles represent a very high-risk group. The model also accommodates the prognostic value of hsCRP in traditionally low-risk groups, since individuals in a low quintile for TC:HDL-C and a high quintile for hsCRP can be identified and assigned an appropriate global risk estimate (Ridker *et al.*, 2001c).

Circulating CRP levels are associated positively with a number of ‘classical’ cardiovascular risk factors, such as age, body mass, systolic blood pressure, smoking, and negatively with others, such as physical activity and HDL-cholesterol. Elevated levels of serum CRP are also associated with a number of features of the insulin resistance syndrome (Lemieux *et al.*, 2001; see Section 2.3.2), and weight loss has been demonstrated to be associated with decreased circulating concentrations of CRP (Heilbronn *et al.*, 2001). This may be related to the fact that adipocytes secrete IL-6, an important regulator of hepatic CRP production (see Chapter 9). Diabetes mellitus is a strong determinant of macrovascular disease (see Section 2.5), and type 2 diabetic patients have been observed to have increased levels of hsCRP, suggesting the presence of low grade systemic inflammation (Ford, 1999). Multivariate analysis of prospective data from the West of Scotland Coronary Prevention Study demonstrated that CRP can predict the

![Fig. 7.5](image-url) Relative risk of first coronary event among apparently healthy men (left) and women (right) associated with different hsCRP concentrations and TC:HDL-C ratios. CRP: C-reactive protein; TC:HDL-C: total cholesterol to high-density lipoprotein cholesterol ratio. Reproduced from Ridker (2001) with permission of Lippincott, Williams and Wilkins.
Inflammation-Related Factors

The development of type 2 diabetes in middle-aged men independently of established risk factors, with the highest quintile (CRP > 0.42 mg/dl) being associated with a greater than three-fold risk relative to the lowest quintile (Freeman et al., 2002). However, in a separate (smaller) study, the association of CRP with incident diabetes was attenuated after adjustment for body mass index (BMI), waist circumference or insulin sensitivity (Festa et al., 2002).

Serum CRP levels are also associated with plasma D-dimer concentrations, an indicator of activation of the coagulation system (see Chapter 6). However, coronary risk appears to be independently determined by levels of CRP and D-dimer (Lowe et al., 2001b), and although these markers of inflammation and fibrin turnover show modest association with each other, they probably have interactive associations with risk of cardiovascular disease.

It is unclear at present whether CRP is simply a marker of the inflammatory process associated with atherosclerosis, or if it plays an aetiological role in atherogenesis (Ferns, 2001). It is possible that both are partially true and that serum concentrations of CRP (as well as other acute phase proteins) reflect the inflammatory response to atherosclerotic damage, but in addition enhance clot formation, lipid oxidation and cell activation (Tracy, 1998). Although the pathophysiological role of CRP is unclear, it may be involved in complement activation and the regulation of tissue factor expression. It has also been suggested that CRP may be involved in monocyte chemotaxis, adhesion molecule upregulation and LDL uptake (Zwaka et al., 2001).

No intervention has yet been evaluated for its ability to specifically reduce CRP; indeed there is no direct evidence as yet that reduction of circulating CRP levels would necessarily result in a lowering of the risk of a cardiovascular event. However, both aspirin and statins have been shown to reduce the risk of a cardiovascular event to a greater degree in the presence of elevated hsCRP, suggesting that alleviation of the inflammatory component of cardiovascular disease may provide additional benefits to other treatments (Ridker et al., 1997; Albert et al., 2001).

It is also important to note that although hsCRP screening may be of value in primary prevention, it may not be very useful in secondary prevention. Episodes of acute ischaemia can substantially increase levels of hsCRP. Moreover, other parameters, such as ventricular function and infarct size, are likely to be of key importance to outcomes in these patients.

7.4.2 Fibrinogen

Fibrinogen is the precursor of fibrin and plays a dual role in atherosclerosis as the major clotting factor in the blood (see Chapter 6) and a pro-inflammatory molecule. Fibrinogen is synthesised in the liver and, like CRP, is an acute phase reactant, whose circulating levels can fluctuate enormously during acute responses to tissue damage or infection. Plasma levels of fibrinogen, are known to be regulated by genetic and lifestyle factors. Patients with cardiovascular disease have significantly raised levels of fibrinogen, and the presence of PVD increases this further (Erren et al., 1999). Levels of circulating fibrinogen are closely correlated with those of CRP and serum amyloid A (Erren et al., 1999). Univariate analysis of a large, nested case-control study has reported that plasma levels of total cholesterol, LDL-cholesterol, triglycerides, apoB100, fibrinogen, CRP and the TC:HDL-C ratio were all significantly higher among men who subsequently developed PVD compared with those who did not (Ridker et al., 2001b). In multivariate analysis, CRP was the strongest non-lipid predictor, but increasing concentrations of fibrinogen were also able to predict risk (Ridker et al., 2001b). However, the intercorrelation between the two inflammatory markers is very strong, implying that their effects are not additive and that either one or the other could improve the predictive value of standard lipid screening (Ridker et al., 2001b). As a result, some investigators have favoured the use of CRP (Tracy, 1998; Rifai & Ridker, 2001), while others have favoured fibrinogen (Ernst & Koenig, 1997; Montalescot et al., 1998). Since CRP is the stronger univariate predictor of risk (Ridker et al., 2001b), it is possible that it will have greater predictive power in global assessment strategies.

7.4.3 Serum amyloid A

Serum amyloid A (SAA) is a precursor of amyloid A protein and comprises both constitutive (apoSAA1, apoSAA2) and acute phase (apoSAA4) isoforms. The serum amyloid A proteins are a family of inflammatory apolipoproteins with a high affinity for HDL, and their production by the liver and other...
tissues is thought to be induced by IL-1 and IL-6. Their role in lipid metabolism is unclear, although they may be involved in HDL trafficking. A small number of studies have investigated the association between SAA and the incidence of CHD (Haverkate et al., 1997; Ridker et al., 1998, 2000; Danesh et al., 2000b). Taken together, these studies show that a comparison of individuals with values in the top third with those in the bottom third gives a combined risk ratio of 1.6 for CHD. However, further studies are required to determine whether the association is independent of possible confounders.

### 7.4.4 Albumin

Some plasma proteins are described as ‘negative’ acute phase reactants because their concentrations decline during an inflammatory response. Albumin is an example and appears to be inversely related to CHD risk. In the National Health and Nutrition Examination Survey (NHANES I), there was a significant reduction in risk associated with serum albumin concentrations above 4.4 g/dl (Gillum & Makuc, 1992). In the MRFIT, albumin concentrations greater than 4.7 g/dl were associated with an odds ratio of 0.45 for subsequent CHD-related death or MI compared with values below 4.4 g/dl (Kuller et al., 1991). A meta-analysis of eight prospective studies demonstrated that individuals with serum albumin levels in the bottom third compared with the top third had a combined CHD risk ratio of 1.5 (95% CI: 1.3 to 1.7) (Danesh et al., 1998). There is some suggestion that the relationship is only present in smokers, but this is unclear, since there appears to be no correlation between albumin levels and either years of cigarette smoking or numbers of cigarettes smoked per day (Nelson et al., 2000). The reduced albumin may be indicative of a generalised inflammatory response, although it has also been proposed that it may reflect a generalised increase in vascular permeability, it may affect platelet aggregation and it may act as an antioxidant by scavenging peroxyl radicals (Nelson et al., 2000).

### 7.4.5 Leukocyte count

An association between leukocyte count and cardiovascular disease was first identified as early as the 1960s, and prospective studies have consistently supported this. For example, the Framingham Study reported that a rise of $1 \times 10^9$ cells/l was associated with a 32% increase in CHD risk in men (Kanel et al., 1992). A five-year prospective study of 4860 males aged 45–63 years suggested that the majority of the risk associated with leukocyte count was contributed by neutrophils, which produced a relative risk of 3.5 for the highest quintile of values compared with the lowest (Sweetnam et al., 1997). Eosinophils also demonstrated a significant association with CHD. However, it is not clear why these particular cell types are associated with CHD since, although they are involved in inflammatory response, neither neutrophils nor eosinophils are thought to contribute to plaque formation in atherosclerosis.

A meta-analysis of seven large prospective studies has illustrated the importance of the consideration of confounding factors, particularly smoking, on the apparent association of leukocyte count with CHD (Danesh et al., 1998). This meta-analysis indicated a combined risk ratio of 1.4 (95% CI: 1.3 to 1.5) when the upper third of total leukocyte counts was compared with the lower third, after adjustment for smoking.

### 7.4.6 Erythrocyte sedimentation rate (ESR)

ESR primarily reflects an increase in plasma fibrinogen concentration, through its effect on plasma viscosity, and is therefore an indirect assessment of inflammatory status. Although probably inferior to CRP in terms of sensitivity, its measurement is rapid and inexpensive, and at least one study has demonstrated a relationship between ESR and CHD mortality (Erikssen et al., 2000). However, ESR is strongly related to other risk factors, particularly fibrinogen, and it is not clear whether it contributes any additional risk over that of fibrinogen.

### 7.4.7 Circulating immune complexes

Multivalent interactions between antigen and antibody can result in the formation of immune complexes. If such complexes form or become trapped within tissues, they can initiate an inflammatory reaction. Immune complexes may be deposited in vessel walls virtually anywhere in the body and this does not appear to be determined by the source of the antigen. As a result, immune complex-mediated diseases tend to be systemic, with little or no specificity for a particular antigen located in a particular tissue.
It is likely that antigen–antibody complexes are produced during many immune responses, but are of pathologic significance only if their quantity, structure, clearance or local functional and anatomic properties are such that abnormally large amounts are deposited in tissues. Importantly, it is thought that immune complexes bind to inflammatory cells and stimulate local secretion of cytokines and vasoactive mediators, which cause increased vascular permeability and enhanced deposition of immune complexes in vessel walls, which may be involved in atherosclerosis.

It has been reported that precocious (early) myocardial infarction (MI) occurs in individuals with inflammatory disorders, such as systemic lupus erythematosus and rheumatoid arthritis (Erhardt et al., 1989; Manzi et al., 1997), which are characterised by chronic immune complex formation (as well as many other features of chronic inflammation); a prospective study of 257 men aged 50–70 years demonstrated that levels of circulating immune complexes were able to predict subsequent MI (Mustafa et al., 2000). The same authors have suggested that circulating immune complexes induced by food proteins may be involved in precocious MI (Mustafa et al., 2001). However, both studies were relatively small and the methods employed to assess levels of circulating immune complexes are too cumbersome for use on a large scale or for screening. The argument for a role of circulating immune complexes in atherosclerosis is also weakened by the fact that one of the hallmarks of immune complex-mediated diseases is that cellular infiltrates are composed predominantly of neutrophils, which is not the case in atherosclerosis. On the other hand, complexes of proteins may be involved in precocious MI (Mustafa et al., 1999) and reported to induce activation of monocytes, suggesting a potential role in foam cell formation (Huang et al., 1999). Thus, while immune complexes are believed to be involved in vasculitis and arteritis, their role in atherosclerosis is unclear.

### 7.4.8 Cytokines, growth factors and other secreted products

The hepatic synthesis of CRP is largely under the control of the pro-inflammatory cytokine, IL-6. Leukocytes are thought to be an important source of circulating IL-6, although it has been estimated that as much as one-third of total circulating IL-6 can originate from adipose tissue, depending on the degree of adiposity (Yudkin et al., 2000). Like many of the inflammatory markers, IL-6 levels are affected by infections, tissue injury, trauma and by smoking. In fact, other pro-inflammatory cytokines, such as IL-1 and TNFα, also promote the hepatic acute phase response, but their role appears to be less significant than that of IL-6. However, a close relationship exists between circulating concentrations of CRP, IL-6 and TNFα and components of the insulin resistance syndrome (Yudkin et al., 2000). There are several mechanisms by which IL-6 might promote atherogenesis, including its metabolic, endothelial and pro-coagulant activities (see Chapter 9). IL-6 alters insulin sensitivity, increases the release of adhesion molecules by the endothelium, increases hepatic elaboration of fibrinogen and has some pro-coagulant activities towards platelets (Yudkin et al., 2000).

Very few population-based studies have investigated the association between circulating levels of IL-6 and cardiovascular risk. The largest, conducted in approximately 28 000 healthy post-menopausal women, demonstrated a significant association with the risk of a cardiovascular event (relative risk 2.2 for highest versus lowest quartile) (Ridker et al., 2000). A smaller study did not find an association between IL-6 and risk of mortality in healthy subjects, although a significant association was apparent in women with prevalent cardiovascular disease (Volapto et al., 2001). A third, yet smaller study, reported higher circulating IL-6 levels in male patients with CHD than in controls; this difference was maintained after correction for age, smoking, hypertension, diabetes, lipids and homocysteine (Rifai et al., 1999). Increased levels of IL-6 have additionally been associated with high risk of all-cause mortality in older individuals (Harris et al., 1999; Jager et al., 1999; Gussekloo et al., 2000). The prognostic value of IL-6 in healthy individuals clearly needs to be verified, since there is only one large study which supports this. However, as with fibrinogen, the close association between IL-6 and CRP suggests that it is unlikely to emerge as an independent risk factor. Evidence for an association between plasma levels of TNFα and cardiovascular disease is similarly limited to small studies (Koukkunen et al., 2001).

A new and interesting area which is emerging in the context of cytokines and inflammation is the
identification of genetic polymorphisms in the promoter regions of cytokine genes, which can be related to elevated production of the cytokine. There is some suggestion that these polymorphisms may alter the risk of CHD, but current evidence does not support this conclusively (Herrmann et al., 1998).

7.4.9 Adhesion molecules

Adhesion molecules mediate the attachment of leukocytes to the endothelium, their transmigration into the subendothelial space, and their retention and accumulation within the artery wall (Fig. 7.6). Several families of adhesion molecules are known to exist. The key adhesion molecules, in terms of atherosclerosis, are the selectins, intercellular adhesion molecules (ICAMs) and vascular cell adhesion molecules (VCAMs). The surface expression of these molecules can be up-regulated very rapidly because they exist within an intracellular pool and translocate to the plasma membrane following cell activation. Here they engage with their complementary adhesion molecule or are recycled. At the cell surface these adhesion molecules may also be cleaved to form soluble fragments that enter the circulation. Soluble forms of ICAM-1 and VCAM-1 (termed sICAM-1 and sVCAM-1) are found in the plasma, probably as a result of shedding from the surface of activated endothelial cells (Rothlein et al., 1991).

Plasma concentrations of sICAM-1 and sVCAM-1
are reported to be higher in individuals with cardiovascular disease and PVD than in controls (Blann & McCollum, 1994; Haught et al., 1996; Morisaki et al., 1997; Caulin-Glaser et al., 1998) and appear to correlate with the extent of atherosclerosis (de Caterina et al., 1997; Peter et al., 1997; Rohde et al., 1998). However, the results of such studies are not entirely consistent, since Semaan et al. (2000) report that patients with documented cardiovascular disease have significant elevation of plasma sVCAM-1, sE-selectin, but not sICAM-1 concentrations compared with healthy subjects. In contrast, Lemos et al. (2000) reported no significant difference in the median baseline sVCAM-1 concentration in subjects who developed cardiovascular disease compared with controls during a nine-year follow-up of participants in the Physician’s Health Study. Positive correlations have been observed between sICAM-1 and triglycerides, fibrinogen, tissue-type plasminogen activator antigen and total homocysteine, whereas a negative association was observed for HDL (Rohde et al., 1999). However, while plasma sICAM-1 was significantly associated with risk of cardiovascular events in a study of healthy post-menopausal women during a three-year follow-up period (Ridker et al., 2000), in multivariate analysis, the only inflammatory marker to remain a predictor of events was hsCRP (Ridker et al., 2000). A recent meta-analysis has demonstrated that soluble adhesion molecules are unlikely to add much predictive information to that provided by established risk factors (Malik et al., 2001).

7.4.10 Heat-shock proteins

Heat-shock proteins are highly conserved protein chaperones produced by many cell types in response to environmental stress, including exposure to free radicals. Heat-shock proteins protect cells from damage, but are often themselves a target of immune responses and inflammation, and may therefore both induce and perpetuate the chronic inflammation that characterises atherosclerosis. Heat-shock protein 60/65 (Hsp-60/65) is expressed by most of the cellular constituents of the atherosclerotic plaque (Kleindienst et al., 1993), and T lymphocytes have been found to be preferentially localised to regions of high Hsp-60/65 expression. Hsp-60 is an antigen recognised by cells of the innate immune system, such as macrophages, which demonstrate a dose-dependent inflammatory response towards it.

It has been reported that increased titres of anti-Hsp-60 antibodies are associated with atherosclerosis, even after adjustment for traditional risk factors (Zhu et al., 2001). Xu et al. (2000) have reported that levels of serum Hsp-60 are significantly elevated in subjects with carotid atherosclerosis, and that these levels are correlated with common carotid artery intima/media thickness assessed by ultrasound.

7.4.11 Phospholipase A2

A2 phospholipases (PLA2) are a family of enzymes found in plasma in association with the plasma lipoproteins, LDL and HDL. These enzymes hydrolise phospholipids to generate lysophospholipids and fatty acids. Several reports link a type II, secreted form of phospholipase A2 to atherogenesis and the risk of cardiovascular disease (Ivandic et al., 1999; Kugiyama et al., 1999; Leitinger et al., 1999). The enzyme has been shown to be present in atherosclerotic lesions; PLA2-positive regions within a plaque include areas rich in extracellular matrix structures and foam cells. It is suggested that type II PLA2 may be involved in the modification of LDL in the arterial wall (Leitinger et al., 1999; Sartipy et al., 1999) and that its expression is regulated by mediators of inflammation (Cao et al., 1998).

The role of PLA2 as a risk factor for cardiovascular disease has been investigated in a small number of studies. Peripheral plasma levels of PLA2 were increased in patients with coronary spastic angina compared with control patients (Kugiyama et al., 1999), and lipoprotein-associated PLA2 levels were positively associated with risk of coronary events in a cohort of the West of Scotland Coronary Prevention Study (The WOSCOPS Study Group, 1995). In the latter study, there was no relationship between serum CRP and lipoprotein-associated PLA2. A recent study by the same authors has demonstrated that lipoprotein-associated PLA2 was able to predict cardiovascular events (fatal and non-fatal) in 580 men versus 1160 controls (Packard et al., 2000). The association remained in multivariate analysis, even though the associations of other inflammatory markers (CRP and fibrinogen) were attenuated (Packard et al., 2000). However, a prospective case-control study in 28 283 healthy middle-aged women concluded that lipoprotein-associated PLA2 was not a strong predictor of future cardiovascular risk, while the adjusted relative risk for the highest quartile of
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CRP was 4.7 (Blake et al., 2001). Thus, the value of PLA2 as a risk factor for cardiovascular disease requires further verification.

7.4.12 Paraoxonase 1

Paraoxonase-1 (PON1) is a member of a multigene family of enzymes. Although all enzymes in the family have been designated as PONs, only PON1 appears to exhibit paraoxonase activity, which is hydrolytic activity towards paraoxon and other organophosphates. PON1 is found in mammalian serum exclusively associated with HDL and is thought to impart antioxidant activity, which may protect LDL from lipid peroxidation (Durrington et al., 2001). However, although serum PON1 activity and concentration are correlated with HDL-cholesterol and apoA-I concentrations, the relationship is not strong, and it is suggested that only a subspecies of HDL particles carry PON1 (Mackness et al., 2000). It is also likely, therefore, that factors which influence serum PON1 activity will not necessarily be the same as those that regulate HDL (Durrington et al., 2001). The issue of the role of serum PON1 in inflammatory responses is complex. Some evidence suggests that serum PON1 activity is decreased during an inflammatory response (Van Lenten et al., 1995; Feingold et al., 1998) and this is speculated to both acutely and chronically exacerbate atherogenesis through effects on LDL oxidation (see Chapter 5).

7.5 Are the inflammatory risk factors equivalent?

How do markers of inflammation compare with each other in the assessment of coronary risk? Are they equally useful? There are some head-to-head comparisons, which generally suggest that they are not equivalent (Table 7.2). Some factors respond more rapidly in an inflammatory response, some are under hormonal control while others are not, and some exhibit much wider subject-to-subject variation than others. CRP has neither diurnal nor seasonal variation, is not affected by food intake, is very stable and can be measured in samples which have been stored frozen for a number of years. It is also important to note that technically, the assays for some risk factors are much better than for others and that CRP is the only inflammatory risk factor with an international standard; it may be for these reasons alone that CRP has emerged as the strongest predictor for risk, rather than because it is biologically the most important inflammatory marker.

7.6 Infectious burden, inflammation and atherosclerosis

It has sometimes been argued that one of the underlying determinants of the variation in systemic inflammation is infectious burden. The relationship between infectious burden and the risk or extent of atherosclerosis has been supported by several cross-sectional studies (see Ridker 2002). However, as with the inflammatory markers themselves, this does not necessarily imply that the relationship is causal and there may be a number of confounding factors associated independently with both infection and atherosclerosis. Prospective cohort studies evaluating exposure to infectious organisms during early life and the subsequent development of cardiovascular disease have, on the whole, reported no significant effects (see Danesh 1999). However, while these studies were large, they focused on early exposure to infection, rather than total infectious burden throughout life. Thus the actual relationship between infectious burden and atherosclerosis remains uncertain. Furthermore, given that there are many potential genetic determinants of systemic inflammation, the same infectious burden in two individuals will not necessarily have the same inflammatory consequences. This is illustrated by a recent study demonstrating that a polymorphism in the Toll-like receptor 4, which governs the inflammatory response to bacterial pathogens, results in lower levels of markers of systemic inflammation. Although subjects with a lower inflammatory response were more susceptible to bacterial infections (and would, therefore, be expected to have a greater infectious burden), they had a lower risk of carotid atherosclerosis (Kiechl et al., 2002). Caution is therefore required in interpreting the relationship between infection and atherosclerosis. It remains an intriguing possibility, however, that an inherited ability to fight infection (i.e. a pro-inflammatory phenotype) is an evolutionary adaptation, which once protected against death from infection, but in Western societies of today, where infections are less common, increases the risk of chronic vascular disease and diabetes in later life. This theory was first put forward in an excellent article by Fernandez-Real and Ricart (1999).
Table 7.2  Relative evaluation of inflammatory risk factors.

<table>
<thead>
<tr>
<th>Inflammatory marker</th>
<th>Extent of evidence</th>
<th>Limitations to interpretation and application</th>
<th>Future studies</th>
<th>Common method of assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>Large-scale prospective studies and a number of meta-analyses; improves predictive value over standard lipid screening</td>
<td>Highly sensitive assays with an international standard, but an acute phase reactant and therefore raised levels seen in other acute and chronic inflammatory conditions</td>
<td>Studies of effects of dietary factors on CRP</td>
<td>Latex-enhanced immunonephelometric assays</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Large prospective and case-control studies and several meta-analyses</td>
<td>Levels closely correlated with CRP and SAA – not likely to be additive over CRP</td>
<td>Examination of interactions between genetics and environment</td>
<td>Nephelometry, or clot-rate assay</td>
</tr>
<tr>
<td>Serum amyloid A</td>
<td>Small prospective studies</td>
<td>Not clear whether association is independent of other confounders</td>
<td></td>
<td>Nephelometry or immunoassay</td>
</tr>
<tr>
<td>Albumin</td>
<td>Some large prospective studies and a meta-analysis of eight studies</td>
<td>Inverse relationship with CVD may reflect non-inflammatory effects of a decreased plasma albumin. Non-specific; a large number of conditions may be associated with low levels</td>
<td></td>
<td>Bromocresol green colorimetric assay</td>
</tr>
<tr>
<td>Leukocyte count</td>
<td>Several prospective studies; meta-analysis of seven large prospective studies</td>
<td>A number of confounding factors, which were not all accounted for</td>
<td>More detailed studies of differential leukocyte counts and subclass analysis</td>
<td>Coulter counter</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate</td>
<td>Small prospective studies</td>
<td>Primarily reflects plasma fibrinogen concentration, therefore an indirect measure; non specific</td>
<td></td>
<td>Westergren’s technique</td>
</tr>
<tr>
<td>Circulating immune complexes</td>
<td>Small prospective studies</td>
<td>Complex, time-consuming methods; role in atherosclerosis unclear</td>
<td>More specific characterisation of the immune complexes involved in atherogenesis</td>
<td>Nephelometry or gel filtration and ELISA for immunoglobulin determination</td>
</tr>
<tr>
<td>Cytokines and growth factors</td>
<td>A few prospective studies (one large)</td>
<td>Strongly associated with CRP – prognostic value needs to be verified</td>
<td>Verification of association; further work on identification of genetic polymorphisms</td>
<td>ELISA</td>
</tr>
<tr>
<td>Soluble adhesion molecules</td>
<td>Inconsistent prospective studies; recent meta-analysis shows lack of additional predictive information over lipid screening</td>
<td>Role of circulating soluble adhesion molecules not clear</td>
<td></td>
<td>ELISA</td>
</tr>
<tr>
<td>Heat-shock proteins</td>
<td>Case-control studies; no prospective studies</td>
<td>Data not consistent</td>
<td>Requires verification</td>
<td>ELISA</td>
</tr>
<tr>
<td>PLA2</td>
<td>Some prospective studies</td>
<td>Influenced by exposure to xenobiotic toxins</td>
<td>Requires verification</td>
<td>ELISA</td>
</tr>
<tr>
<td>Paraoxonase-1</td>
<td>Case-control studies; no prospective studies. Meta-analysis supports association</td>
<td></td>
<td></td>
<td>Colorimetric assay for enzyme activity</td>
</tr>
</tbody>
</table>

CRP: C-reactive protein; ELISA: enzyme-linked immunosorbent assay; PLA2: phospholipase A2.
7.7 Pharmacological agents with anti-inflammatory effects in cardiovascular disease

7.7.1 Non-steroidal anti-inflammatory agents

Cyclo-oxygenases-1 and -2 (COX-1, COX-2) are the enzymes involved in the conversion of arachidonic acid to eicosanoids. Each enzyme is responsible for the synthesis of a distinct family of eicosanoids; for example, COX-1 catalyses the production of thromboxane A₂, a potent platelet aggregant, and vasocostriclor, whereas COX-2 is responsible for the synthesis of prostaglandin I₂, which is a thromboxane A₂ antagonist.

Aspirin (acetyl salicylic acid) is an irreversible inhibitor of platelet COX-1. Low-dose aspirin is effective in reducing the coronary event rate in patients with established atherosclerosis (Antiplatelet Trialists’ Collaboration, 1994), and this may be associated with a reduction in serum CRP (Ikonomidis et al., 1999). Low-dose aspirin also appears to confer an absolute benefit in primary prevention (Steering Committee of the Physicians’ Health Study Research Group, 1989; Collaborative Group of the Primary Prevention Project (PPP), 2001), particularly in subjects with a calculated cardiovascular event risk of more than 0.6% per annum (Sanmuganathan et al., 2001) and in those patients with a systolic blood pressure below 130 mmHg (Meade & Brennan, 2000). Classically this has been thought to be due to the platelet inhibitory action of aspirin, but it has recently been shown that inflammation plays a predominant role in the initiation and progression of lesions in atherosclerosis. In humans, it has been reported that treatment with aspirin reduces cardiovascular risk and slows carotid plaque growth in a dose-dependent fashion (Ranke et al., 1993), although this has not been a consistent finding (Delcker et al., 1995). Ridker et al. (1997) assessed the interaction between serum CRP levels and aspirin treatment in 543 apparently healthy men participating in the Physicians’ Health Study in whom MI, stroke, or venous thrombosis subsequently developed, and in 543 study participants who did not report vascular disease during a follow-up period exceeding eight years (Ridker et al., 1997). The men in the quartile with the highest CRP values had three times the risk of MI (relative risk: 2.9, p < 0.001) and two times the risk of ischaemic stroke (relative risk: 1.9, p = 0.02) of the men in the lowest quartile. The use of aspirin was associated with significant reductions in the risk of MI (55.7% reduction, p = 0.02) among men in the highest quartile but with only small, non-significant reductions among those in the lowest quartile (13.9%, p = 0.77). Continued use of aspirin in patients who have suffered a MI appears to be associated with a shift to less severe outcomes (Abdelnoor & Landmark, 1999). However, a recent report suggests that subjects with established coronary disease, who have a history of prior aspirin use, are at lower risk of non-Q wave infarction, but at higher risk for subsequent failure of standard medical therapy (Lancaster et al., 2001); the authors suggest that aspirin treatment may alter the properties of the thrombus. Nor have other non-selective non-steroidal anti-inflammatory drugs been shown to be cardio-protective (Ray et al., 2002).

Selective inhibition of COX-2 with nimesulide reduced prostaglandin I₂, biosynthesis substantially in a mouse model of atherosclerosis, but had no significant effect on thromboxane formation by platelets, which only express COX-1. By contrast, the isoform non-specific inhibitor, indomethacin, suppressed platelet function and thromboxane formation and reduced the extent of atherosclerosis, whereas nimesulide failed to increase the rate of atherogenesis. Despite their divergent effects on atherogenesis, both drugs depressed two indices of systemic inflammation, soluble intercellular adhesion molecule-1, and MCP-1 to a similar but incomplete degree.

7.7.2 Lipid lowering agents

The landmark statin trials have shown the benefits of cholesterol lowering in coronary prevention (Scandinavian Simvastatin Survival Study Group, 1994; West of Scotland Coronary Prevention Group, 1996; Collins et al., 2002). However, the statins also possess other potentially beneficial properties, including an ability to modulate inflammation (Davignon & Mabile, 2001; Sparrow et al., 2001). In animal models, statins reduced the content and activation-state of inflammatory cells within atherosclerotic lesions (Bustos et al., 1998). This may be related to their ability to inhibit macrophage proliferation and activation (Aikawa et al., 2001), and their elaboration of cytokines and growth factors such as MCP-1 (Kothe et al., 2000; Romano et al., 2000), TNFα (Solheim et al., 2001) and IL-8 (Kothe et al.,...
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Statin treatment is also associated with a rapid reduction in serum CRP levels (Strandberg et al., 2000; Albert et al., 2001; Ridker et al., 2001c), the changes in serum CRP concentrations being inversely associated with changes in HDL-cholesterol but unrelated to changes in LDL-cholesterol. Other serum markers of inflammation are reported to be unaffected by statin treatment; for example, sICAM-1, sVCAM-1, sE-selectin and sP-selectin (Sardo et al., 2001; Solheim et al., 2001). Short-term treatment with simvastatin also decreased the plasma concentrations of markers of lipid peroxidation and of stable nitric oxide metabolites, but did not alter circulating levels of IL-6 (Dekur-Smielecka et al., 2001).

(i) Fibric acid derivatives

The fibrate family of drugs is used to treat combined dyslipidaemias and has been shown to be effective in reducing coronary events (Rubins et al., 1999). They are synthetic ligands for peroxisome proliferator-activated receptor-alpha (PPARα). PPARα is expressed by the liver, skeletal muscle and heart tissue, and is involved in beta oxidation and fatty acid degradation. Fatty acids and eicosanoids appear to be the natural ligands for the PPARs, suggesting a role in the modulation of the inflammatory response. This possibility is supported by the fact that PPARα−/− knockout mice have a prolonged response to inflammatory stimuli, and PPAR activators inhibit the activation of inflammatory response genes via multiple pathways in several cell types (Staels et al. (1998); reviewed by Chinetti et al. (2000)).

(ii) Probucol

Probucol is a potent antioxidant that was originally used for its cholesterol-lowering effects (Hunninghake et al., 1980). However, its use was not found to be associated with clinical benefit in individuals with established atherosclerosis (Wallidius et al., 1994). Dietary administration of probucol (0.5%, wt/wt) efficiently reduced total plasma cholesterol levels in apolipoprotein E-deficient mice (apoE−/−) by 40%, with decreases in HDL and apoAI of 70% and 50%, respectively. Paradoxically, however, aortic atherosclerotic plaques in the probucol-treated apoE−/− mice formed more rapidly than in the untreated apoE−/− mice, and the lesions were two to four times larger and more mature regardless of sex, age and genetic background (p < 10−6). Histologically, lesions in probucol treated mice contained increased fibrous materials and cells other than foam cells, and were commonly associated with focal inflammation and aneurysmal dilatation. Probucol treatment also accelerated lesion development in apoE+/− mice fed an atherogenic diet, indicating that the adverse effect is not dependent on the complete absence of apoE. Furthermore, mice lacking apoE and apoAI have plasma lipoprotein profiles very similar to the probucol-treated apoE−/− mice, but do not have accelerated plaque development. Thus, the enhanced atherosclerosis in the probucol-treated animals is unlikely to be caused by the reduction of HDL and apoAI levels.

7.8 Dietary factors and inflammation

Several dietary constituents have been shown to modulate the inflammatory response; these include fats, antioxidants, protein and specific amino acids (reviewed by Grimble (1998)). These factors may affect the cell-mediated immune response by altering the availability of substrates required for the biosynthesis of essential molecules in the inflammatory response, affecting the composition of cell membranes and thereby intracellular signalling, or by modulating redox-sensitive pathways. Above all, given the strong relationship between body weight and CRP, it is clear that the most efficient way of reducing CRP is through weight loss (see Chapter 9).

7.8.1 Fatty acids

The key physiological roles of polyunsaturates are as components of cell membranes and as precursors of eicosanoids, which are a family of hydroxylated polyunsaturates usually derived from arachidonic acid (Fig. 7.7). Eicosanoids have important functions in regulating inflammation, immunity, cellular migration, platelet and vascular function and blood pressure. Prostaglandin E₂ (PGE₂), derived from arachidonic acid, has a number of pro-inflammatory effects and elevated levels have been observed in patients with chronic inflammatory disorders. The n-3 polyunsaturates, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), present in fish oils, competitively inhibit the conversion of arachidonic acid to eicosanoids. Furthermore, EPA is able to act
as a substrate for the synthesis of eicosanoids, which have biological properties and potencies that are distinct from those of the arachidonic acid-derived eicosanoids (Fig. 7.7). Since eicosanoids influence processes such as platelet aggregation, inflammation and immunity, it follows that ingestion of fish oil, resulting in a shift in the pattern of eicosanoid synthesis, may be anti-inflammatory. Whether these anti-inflammatory effects contribute to the decreased mortality from cardiovascular disease by fish oils reported in three landmark secondary prevention trials in men who had suffered a first MI (Burr et al., 1989; Singh et al., 1997; GISSI-Prevenzione Investigators, 1999) is not clear (see Chapter 11). However, it is notable that many of the anti-inflammatory effects of fish oil are observed only at high doses, well above those which protected individuals from cardiovascular death or sudden death in the secondary prevention trials.

(i) *Fish oil and inflammatory cytokines*

A number of studies have investigated the effect of dietary eicosapentaenoic acid. A number of studies have investigated the effect of dietary *n*-3 polyunsaturates supplementation on *ex vivo* cytokine production by human peripheral blood mononuclear cells (see Calder (2001)). Several of these studies report a reduction in cytokine production following fish oil supplementation, supporting
an anti-inflammatory effect of n-3 polyunsaturates, although a number do not. This is likely to be due to the dose employed being too low or the power of the study insufficient, or a combination of both factors (Yaqoob, 2003). In addition to their role in mediating an inflammatory response, the pro-inflammatory cytokines, TNF\(\alpha\) and IL-1 are released during the early phase of ischaemic heart disease. These cytokines decrease myocardial contractility and induce myocardial damage, and enhance the production of free radicals, which can also suppress myocardial function. It has been suggested that n-3 fatty acids may increase parasympathetic tone, leading to an increase in heart rate variability and thus protect the myocardium against ventricular arrhythmias (discussed by Das (2000)). It is pertinent to note that the most striking observation in the secondary prevention studies of fish oil supplementation was the dramatic reduction in sudden death (Burr et al., 1989; Singh et al., 1997; GISSI-Prevenzione Investigators, 1999), which has given rise to the suggestion that the principal mechanism for the protective effect of fish oil may involve an anti-arrhythmic effect (see Chapter 11).

(ii) Fish oil and soluble adhesion molecules

Studies investigating the effects of supplementation with fish oil on serum soluble adhesion molecule levels have reported equivocal findings (Abe et al., 1998; Seljeflot et al., 1998; de Caterina et al., 2000). Several studies have shown no effect on levels of sVCAM-1, while one study reported an increase (Johansen et al., 1999) and one study reported that supplementation with a moderate dose of fish oil (1.2 g EPA + DHA per day) for 12 weeks decreased plasma levels of sVCAM-1 in older subjects, but did not have any effect in young males (Miles et al., 2001). Most studies show no effect on plasma sICAM-1 levels (Abe et al., 1998; Miles et al., 2001). Two studies showed that fish oil increased plasma sE-selectin (Johansen et al., 1999; Miles et al., 2001); in one of these studies sE-selectin was increased by fish oil in young subjects, but had no effect in older subjects (Miles et al., 2001). It is pertinent to note that while there are many reported anti-inflammatory effects of fish oil, it contains n-3 polyunsaturates which are highly susceptible to oxidation, and it is possible that some of the contradictory results arise from differences in antioxidant status of subjects involved in the studies described.

(iii) Fish oil and serum inflammatory markers

De Maat et al. (1994) have reported that short-term treatment (one week) with fish oil, at a high dose of 30 g/day, has no significant effect on CRP levels in healthy young subjects. More recently, Chan et al. (2002) have demonstrated that six weeks of treatment with statin, but not fish-oil (4 g/day), reduced CRP and IL-6 concentrations in individuals with visceral obesity Madsen et al. (2003) tested the effects of two doses of fish oil (2 g/day or 6.6 g/day) against olive oil on serum CRP in healthy volunteers, but they also reported no effect. Thus there is currently no evidence to suggest that n-3 polyunsaturates are able to modulate plasma levels of CRP.

(iv) Fish oil and plaque stability

A recent study investigated the effects of fish oil in patients undergoing carotid endarterectomy, which is a surgical procedure involving the removal of plaque from the carotid artery in the neck. The patients were randomly assigned to consume control, sunflower oil or fish oil capsules until surgery, when the atherosclerotic plaque was removed. Patients in the fish oil group consumed an extra 1.4 g of the n-3 polyunsaturates, EPA and DHA per day, while those in the sunflower oil group consumed an extra 3.6 g of linoleic acid per day (Thies et al., 2003). The duration of treatment was 7 to 189 (median 42) days and did not differ significantly between the groups. The proportions of EPA and DHA were higher in carotid plaque phospholipids, cholesteryl esters and triglycerides in patients receiving fish oil compared with patients in the control group (Thies et al., 2003). Fewer plaques from patients being treated with fish oil had thin fibrous caps and signs of inflammation, and more plaques had thick fibrous caps and no signs of inflammation, compared to the other two groups; these differences were statistically significant in patients who had been treated with fish oil for more than 42 days (Thies et al., 2003). The number of macrophages in the plaques from patients receiving fish oil for more than 42 days was lower than that in the other two groups (Thies et al., 2003). These results suggest that advanced atherosclerotic plaques are dynamic and readily incorporate n-3 polyunsaturates, even when provided at relatively modest doses. Furthermore, incorporation of n-3 polyunsaturates into carotid plaques was associated with a reduced
number of macrophages and fewer signs of inflammation, suggesting that n-3 polyunsaturates induce changes that may increase the stability of atherosclerotic plaques.

7.8.2 Antioxidants

Although the anti-atherogenic effects of antioxidants are likely to be principally related to their free-radical scavenging properties (see Chapter 5), several dietary antioxidants are known to have non-antioxidant-related actions, including anti-inflammatory effects. For example, it has been shown that alpha-tocopherol decreases lipid peroxidation, platelet adhesion and aggregation, and smooth muscle cell proliferation (Konneh, 1995; Williams et al., 1997). It has also been reported to exert anti-inflammatory effects on monocytes, and to improve endothelial function (Ferns et al., 1993a; Mottram et al., 1999; Skyrme-Jones et al., 2000; Devaraj et al., 2002), although these effects are not reported consistently in the literature (McKechnie et al., 2002). Devaraj and Jialal (2000) have also reported that high dose alpha-tocopherol (vitamin E) supplementation (1200 IU/day) significantly lowered levels of CRP and monocyte IL-6 in type 2 diabetic patients with or without pre-existing macrovascular disease. Langlois et al. (2001) have reported that serum vitamin C concentrations are lower in patients with intermittent claudication who have high serum CRP levels and severe PVD. Nuclear factor-kappa B (NFκB) is a transcription factor that is critical for the regulation of several genes involved in immune and inflammatory responses (reviewed by Bowie and O’Neill (2000a)). It may be activated by several pathways (Fig. 7.8).

Bowie and O’Neill (2000b) have found that millimolar concentrations of vitamin C inhibit IL-1 and TNFα-induced activation of NFκB within endothelial cells. This inhibitory effect of vitamin C was not simply an antioxidant effect, because redox-insensitive pathways to NFκB were also blocked. Their results indicate that p38 is an intracellular target for high dose vitamin C. Goudev et al. (2000) found that a combination of antioxidants reduced circulating levels of sVCAM-1 and sICAM-1 in postmenopausal women after 12 weeks of treatment. Other antioxidants have also been shown to influence the expression of adhesion molecules in vivo and in vitro (Ferns et al., 1993b, 2000; Sen & Bagchi, 2001). These effects do not appear to be mediated by NFκB for all antioxidants in all cell types (Hoare et al., 1999; Sen & Bagchi, 2001). Apoptosis is thought to play an important role in vascular remodelling. Nardini et al. (2001) have recently reported that caffeic acid inhibits the ceramide-induced apoptotic response in the human macrophage cell line U937. In this study, vitamins E and C were ineffective in inhibiting apoptosis. Neither green nor black tea appear to have a significant effect on markers of inflammation or endothelial injury in smokers, although basal serum concentrations of beta-carotene were inversely related to IL-6 and fibrinogen (De Maat et al., 1994), and in the Physicians’ Health Study an inverse association between dietary intake of vegetables rich in carotenoids and coronary risk was demonstrated (Liu et al., 2001; see Chapter 5).

**Fig. 7.8** Summary of the evidence proposed for the oxidative stress model of NFκB activation. This model proposes that diverse NFκB activators act through a common step involving an increase in oxidative stress within the cell. Evidence is as follows: (1) hydrogen peroxide can activate NFκB directly in some cells. (2) An increase in intracellular oxidative stress has been measured in response to some stimulants. (3) Antioxidants (PDTC, NAC, α-lipoate, BHA and DFO) can inhibit pathways to NFκB. (4) Enzymes that modulate the redox status of the cell (catalase, glutathione peroxidases (GPx), SOD and 5-LOX) can, in some cases, either attenuate (−) or potentiate (+) NFκB activation. BHA: butylated hydroxyanisole; DFO: desferroxamine; GPx: glutathione peroxidase; 5-LOX: 5-lipoxygenase; NAC: N-acetylcysteine; NFκB: nuclear factor-kappa B; PDTC: pyrrolidine dithiocarbamate; SOD: superoxide dismutase. Reproduced from Bowie and O’Neill (2000a) with permission from Elsevier.
7.8.3 Alcohol and systemic inflammation

Imhof et al. (2001) have reported that among men alcohol consumption shows a U-shaped association with serum CRP concentrations; non-drinkers and heavy drinkers had higher CRP than moderate drinkers consuming 20–40 grams of alcohol per day (equating to around two to six drinks; Fig. 7.9). A similar association was found in individuals who smoked. More recently it has been reported that the effects of alcohol on CRP levels are modified by genotype at the alcohol dehydrogenase locus (Imhof et al., 2002).

7.8.4 Glycaemic load

In a study of middle-aged women, Liu et al. (2002) reported that there is a positive relationship between dietary glycaemic load, assessed by food questionnaire, and serum CRP concentrations. The association remained after adjustment for age, smoking status, alcohol intake, a history of diabetes or hypertension, and body mass index.

7.8.5 Dietary salicylates and other anti-inflammatory agents

Certain foodstuffs, for example apples, contain significant quantities of salicylate, that may contribute substantially to the intake of salicylate in the population (Ingster & Feinleib, 1997), and it has been suggested that this may be partially responsible for the decline in cardiovascular mortality in the USA. However, other investigators have argued that in a normal mixed diet, there is too little salicylate to have a significant health impact (Janssen et al., 1996). Nevertheless, some food products, for example, organic vegetable soup, have been reported to contain substantial amounts of salicylate (Baxter et al., 2001), and other dietary constituents, such as curcumin and quercetin also have anti-inflammatory properties (Ishikawa et al., 1999; Shah et al., 1999).

7.8.6 Micronutrients and the inflammatory response

Low circulating levels of certain micronutrients, including iron, zinc, selenium, copper and vitamin A, are thought to reflect the presence of an underlying inflammatory response (Galloway et al., 2000). In recent years a number of epidemiological studies have reported an association between low circulating levels of micronutrients and increased risk of cardiovascular disease, which has led to the misconception that supplementing these apparently deficient micronutrients should provide some benefit. However, if reductions in circulating micronutrient levels are an inevitable result of the presence of underlying inflammation, supplementation will not necessarily have any effect. This is indeed exemplified by the failure of some of the large micronutrient supplementation trials to affect cardiovascular disease-related outcomes (see Chapter 5).
7.9 Key points

- There is evidence that a chronic, low-grade inflammation underlies atherosclerosis, although it is not clear whether this is a cause or effect phenomenon.
- The local chronic inflammatory response is likely to result in a ‘spilling over’ of inflammatory molecules from leukocytes and endothelial cells and adipocytes into the circulation, where they may act on a number of tissues, including the liver, to induce the secretion of acute phase proteins.
- The acute phase proteins, CRP, fibrinogen and serum amyloid A, appear to be associated with risk for cardiovascular disease and, in some cases, PVD.
- A number of other surrogate markers for systemic inflammation have also been suggested, including albumin, leukocyte count, ESR, circulating immune complexes, cytokines and growth factors, soluble adhesion molecules, heat-shock proteins, phospholipase A2 and paroxonase-1. However, at present these markers remain inferior to CRP in terms of their discriminatory power to predict cardiovascular disease risk, but this may be related to the relative stability and ease of measurement of CRP and the fact that it is the only inflammatory risk factor with an international standard.
- A number of pharmacological agents with anti-inflammatory properties are currently being used in the treatment of cardiovascular disease, illustrating the potential importance of the role of inflammation in the aetiology of the disease. However, the results of clinical trials of non-steroidal anti-inflammatory drugs are inconsistent, perhaps because of opposing effects on other processes.
- There are insufficient data describing the effects of dietary components on inflammatory markers, either in healthy subjects or in cardiovascular disease patients, although there is evidence for anti-inflammatory effects of the n-3 polyunsaturates and some dietary antioxidants. This is a key area for future research.

7.10 Recommendations for future research

- Clarification of the relationships between serum amyloid A, albumin, specific leukocyte subclasses, circulating immune complexes, cytokines, soluble adhesion molecules, heat-shock proteins, phospholipase A2 and paroxonase-1 and cardiovascular and/or peripheral vascular disease.
- To date there are very few data on the influence of dietary components on inflammatory markers of cardiovascular disease. Large, well-controlled trials are therefore required for this area to progress.

7.11 Key references


Homocysteine

8.1 Introduction

Homocysteine is a sulphur-containing amino acid present in all cells, where it is involved in the generation of methyl groups required for essential cellular function (Jacobsen, 2001). Elevated plasma total homocysteine concentration can occur due to genetic defects, vitamin deficiency or renal impairment. Individuals with the rare genetic disorder homocystinuria have markedly elevated plasma homocysteine concentrations (>100 µmol/l) and have a high risk of vascular events occurring in their second and third decades (Wilcken & Wilcken, 2001). Several B vitamins are involved in homocysteine metabolism and homocysteine levels are inversely related to blood levels of folate, vitamin B₁₂ and vitamin B₆. Supplementation of individuals with homocystinuria with vitamin B₆, folic acid, vitamin B₁₂ and betaine reduces the risk of the vascular and other complications (Wilcken & Wilcken, 1997). The high incidence of coronary heart disease (CHD) and stroke among children with homocystinuria prompted the hypothesis that moderately elevated homocysteine levels (>15–20 µmol/l) may be a risk factor for CHD and stroke in the general population, and the possibility of reducing risk by supplementation with folate acid and other B vitamins (McCully, 1996; Yap et al., 2001).

This chapter examines the biochemistry and the epidemiological evidence relating homocysteine levels with risk of cardiovascular disease, and explores the possible pathophysiological mechanisms associated with elevated homocysteine levels. In addition, the chapter examines the determinants of homocysteine in the general population, the effects of vitamin supplementation on homocysteine levels and the design of large trials to assess if lowering homocysteine levels can reduce risk of cardiovascular disease.

8.2 Biochemistry

Homocysteine is a sulphur amino acid derived from methionine, following the loss of a methyl group; the formulae of these and related metabolites are shown in Fig. 8.1. Homocysteine lies at a branch point in one carbon metabolism between two metabolic cycles (remethylation and trans-sulphuration) in all cells (Fig. 8.2). In the remethylation pathway, homocysteine accepts a methyl group from methylene-tetrahydrofolate to form methionine. The remethylation reaction, catalysed by methionine synthase (MS), requires vitamin B₁₂ as a cofactor and methylene-tetrahydrofolate as substrate. Methylene-tetrahydrofolate reductase (MTHFR) plays an important role in remethylation, by supplying methyl groups as methyl-tetrahydrofolate for homocysteine remethylation. An alternate remethylation pathway utilising betaine as methyl donor is confined to the liver. Much of the methionine is activated to form S-adenosylmethionine (SAM), which is the chief donor of methyl groups for methyltransferases involved in synthesis of DNA, proteins and phospholipids. The loss of the methyl group from SAM results in the production of S-adenosylhomocysteine (SAH), which is in turn hydrolysed to form homocysteine. In the transulphuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalysed by vitamin B₆ dependent cystathionine beta-synthase (CBS). Under normal circumstances, the flow of methionine to cystathionine
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accounts for half of all methionine metabolism (Finkelstein & Martin, 1984). The remaining half of total body homocysteine is remethylated by methyl groups derived in equal amounts from methyl tetrahydrofolate or betaine. The intracellular levels of homocysteine are highly regulated and any increased production is met by export from cells. Thus blood levels of homocysteine reflect intracellular concentrations of homocysteine and the homeostatic balance of the enzymes involved in methionine metabolism to ensure a supply of methyl groups for essential reactions in all cells.

8.3 Laboratory measurement of homocysteine

In plasma, about two-thirds of homocysteine is bound to albumin, the remaining one-third forming disulphides with other thiols and only about 1% circulating in a free form (Fig. 8.1). The sum of these homocysteine forms is referred to as total homocysteine or homocyst(e)ine. The total homocysteine assays convert the multiple unstable thiols to a reduced form, which can be measured directly or after derivatisation by high performance liquid chromatography (HPLC) or gas chromatography mass-spectrometry (GC-MS) (Ueland et al., 1993; Table 8.1). The introduction of immunoassays for homocysteine determination affords a highly practical method for homocysteine determination that is now available in most routine clinical chemistry laboratories (Nexo et al., 2000).

Meticulous attention to detail in sample handling is important for homocysteine determination. Plasma concentrations of homocysteine rise rapidly unless plasma is separated from red cells immediately or kept chilled at 4°C. Homocysteine levels in unseparated whole blood stored at room temperature (UK approximately 23°C) increase by about 10% per hour and higher ambient temperatures result in a greater increase (Ueland et al., 1993). However, after prompt separation of red cells, homocysteine concentrations in plasma remain stable for several days at room temperature or 4°C and for several years at −20°C. Plasma samples can, therefore, be sent to laboratories for analysis by first class post. Ethylenediaminetetraacetic acid (EDTA) is a convenient anticoagulant (as is lithium heparin), while serum is less preferable because the inevitable separation delay leads to elevations of homocysteine. Plasma homocysteine levels can be stabilised at room temperature by collecting blood in tubes containing disodium EDTA (7.0 mg) with added sodium fluoride (17.5 mg; final sodium fluoride concentration, 60 mmol/l; Becton Dickinson UK) (Clark et al., 2003). A single blood sample, which may not be from a fasting patient, is the most widely used investigation to assess homocysteine status, and in practice a light breakfast has little influence on this (Ueland et al., 1993).

Fig. 8.1 Formulae of methionine, homocysteine and related metabolites.
Evidence relating homocysteine with risk of cardiovascular disease

8.4 Initial epidemiological evidence

The initial epidemiological evidence relating homocysteine to risk of CHD came from retrospective case-control studies, which consistently reported that cases had higher plasma homocysteine concentrations than controls (Boushey et al., 1995; Danesh & Lewington, 1998; Ueland et al., 2000). In a meta-analysis of 17 studies involving 2297 CHD cases, Boushey et al. (1995) reported that a 5 µmol/l increase in homocysteine was associated with a 70% increased risk of CHD and an 80% increased risk of stroke. Subsequently, however, prospective studies (in which blood for homocysteine determinations was collected before the onset of CHD) have reported apparently inconsistent results for risk of CHD, with some showing highly significant associations and others showing none. In 1998, Danesh and Lewington demonstrated the importance of discrepant results of different study designs which highlighted the importance of bias and reverse causality (where the occurrence of disease may alter homocysteine levels) in these studies; these authors reported that a 5 µmol/l
increase in homocysteine was associated with 30% increase risk of CHD in prospective studies (OR: 1.3; 95% CI: 1.1 to 1.5), 60% increased risk in retrospective studies with population controls (OR: 1.6; 95% CI: 1.4 to 1.7), and 90% increased risk in retrospective studies with other controls (OR: 1.9; 95% CI: 1.6 to 2.3). It is unclear to what extent the more extreme associations observed in retrospective studies compared with prospective studies is due to bias related to the effects of disease, or changes in treatment, or renal function after the onset of disease, all of which may increase the homocysteine concentrations among the cases in retrospective studies.

8.4.2 Homocysteine Studies Collaboration meta-analysis of homocysteine and risk of CHD and stroke

The Homocysteine Studies Collaboration was set up to combine individual participant data from all relevant observational studies of homocysteine and risk of CHD and stroke to produce reliable estimates of the associations of total homocysteine with CHD and stroke, with adjustment for confounding by known cardiovascular risk factors and correction for regression dilution (Homocysteine Studies Collaboration, 2002). The aim of this meta-analysis was to estimate the relative risks of CHD and stroke associated with a 25% lowering in blood homocysteine concentrations (which is equivalent to the average change in plasma homocysteine concentration achieved by folic acid supplementation), after adjusting for the effects of other cardiovascular risk factors and correction for regression dilution associated with single measurements of homocysteine (Clarke et al., 2001). The meta-analysis explored the reasons for the discrepant results of epidemiological studies of homocysteine and risk of CHD and stroke (Homocysteine Studies Collaboration, 2002). Data were obtained from 30 studies involving 5073 CHD events and 1113 stroke events. Because homocysteine levels were positively skewed, all homocysteine values were log transformed using a \( \log_2 \) homocysteine, so that a unit increase in homocysteine \( \log_2 \) is equivalent to a doubling in homocysteine levels. The odds ratios (OR) for CHD and stroke associated with a 25% lower homocysteine concentration were obtained by conditional logistic regression after adjustment for study, age and sex. The odds ratios of CHD associated with differences in homocysteine concentrations were almost twice as strong in retrospective studies compared with those in prospective studies (Fig. 8.3). The chief emphasis in this meta-analysis was on the results of prospective studies of apparently healthy populations, because the results of prospective studies are less prone to the influence of pre-existing cardiovascular disease on homocysteine levels (‘reverse causality’). Since homocysteine levels are higher in current smokers compared with non-smokers and are correlated with levels of systolic blood pressure and cholesterol levels, it is likely that such risk factors may account for some of the associations with CHD and stroke. Fig. 8.4 shows the impact of adjustment for known cardiovascular risk factors on risk of CHD and stroke in prospective studies. The substantial changes in the odds ratios and the chi-squared statistics with these adjustments suggests that a large part of the association was due to confounding. Among prospective studies, after adjustment for the effects of smoking, cholesterol and blood pressure, and correction for regression dilution, 25% lower homocysteine was associated with 11% (95% CI: 4 to 17%) lower CHD risk and 19% lower (95% CI: 5 to 31%) stroke risk. Thus, the evidence from population studies of homocysteine and CHD or stroke suggests that elevated homocysteine is a modest independent risk factor for cardiovascular disease.

8.4.3 Relevance of genetically determined differences in homocysteine levels to risk of CHD

Studies of genetic variants affecting homocysteine levels and risk of heart disease could help to provide evidence about whether the underlying associations of homocysteine with vascular disease are causally related (Brattstrom & Wilcken, 2000). Among the genetic variants associated with homocysteine levels, the C677T polymorphism for the gene encoding MTHFR is the most important (Frosst et al., 1995). Individuals who have a C to T substitution at base 677 in both copies of the gene that encodes MTHFR (TT homozygotes) have about 2.5 \( \mu \text{mol/l} \) higher homocysteine levels than those with the CC genotype (Jacques et al., 1996). Individuals with the 677TT mutation are sensitive to folate and riboflavin deficiency, and the effect on homocysteine levels may vary with differences in dietary intake of folate and riboflavin in different populations. The advantage
of studying genetic variants that influence homocysteine levels and their relationship with CHD include the observations that genetic variants reflect long-term differences in homocysteine levels, are not influenced by confounding by known risk factors for CHD and are irreversible (i.e. not susceptible to reverse causality; Davey Smith & Ebrahim, 2003). Since the genetic variants are randomly allocated one
from each parent at meiosis, the allocation of a genetic variant is referred to as Mendelian randomisation. If one could demonstrate an association between a genetic variant influencing homocysteine levels and risk of CHD, then this could provide support that the observed associations of homocysteine with CHD risk are likely to be causal. Individual studies and a previous meta-analysis had included too few cases to have adequate power to address this question reliably (Brattstrom & Wilcken, 2000). However in 2002, the MTHFR collaboration collected individual participant data from all the observational studies of MTHFR and risk of CHD to examine the relevance of 677TT genotype for MTHFR with risk of CHD (Klerk et al., 2002). Data were obtained from 40 studies involving a total of about 11 000 cases and 13 000 controls. Data on homocysteine were available in the subset of the participants; individuals with the TT polymorphism had 25% higher homocysteine levels than those with CC polymorphism. The overall results of this meta-analysis showed that individuals with TT polymorphism had 16% higher risk (95% CI: 5 to 28%) of CHD (Fig. 8.5). However, there was a significant heterogeneity between the results obtained for MTHFR and risk of CHD in European populations (OR: 1.14; 95% CI: 1.01 to 1.28) compared with North American populations (OR: 0.87; 95% CI: 0.73 to 1.05), which is explained by interaction between MTHFR 677C→T polymorphism and the higher folate status in North America. The results are consistent with the effect of this mutation on cardiovascular risk being manifest only in the presence of suboptimal intakes of folate and riboflavin (Hustad et al., 2000). Since studies of genetically-determined differences in homocysteine are free from the possibility of reverse causality, these data provide strong support for these associations being causal.

8.5 Pathophysiology of vascular disease

The exact mechanisms by which elevated homocysteine levels are believed to cause vascular disease are uncertain. It has been suggested that homocysteine causes a disruption to endothelial cell physiology, as an initial trigger that ultimately results in vascular complications associated with hyperhomocysteinemia. Endothelial cell dysfunction results in multiple biological effects, including abnormal vasoconstriction, increased platelet aggregation, monocyte adhesion and activation of coagulation, that have been discussed in Chapter 4. Several animal models have demonstrated that experimental hyperhomocysteinemia results in endothelial cell dysfunction. Harker et al. (1976) demonstrated that an infusion of homocysteine in baboons caused endothelial cell desquamation, whilst Lentz et al. (1996) showed that diet-induced hyperhomocysteinemia in monkeys caused vascular dysfunction. Celermajer et al. (1993) reported abnormal nitric oxide-mediated endothelium-dependent responses to increased blood flow in homocystinuria. Endothelial dysfunction as determined by assessment of flow-mediated vasodilation has also been reported in moderate hyperhomocysteinemia (Tawakol et al., 1997) and following experimental hyperhomocysteinemia resulting from a methionine loading. In addition, folic acid supplementation has been shown to improve endothelial function in adults with hyperhomocysteinemia (Woo et al., 1999).
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<td>2.05 (1.26–3.35)</td>
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<td>Silberberg</td>
<td>274/112</td>
<td>1.12 (0.53–2.35)</td>
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<td>Bockxmeer</td>
<td>518/140</td>
<td>1.04 (0.57–1.91)</td>
</tr>
<tr>
<td></td>
<td><strong>All studies</strong></td>
<td><strong>11 162/12 758</strong></td>
<td><strong>1.16 (1.05–1.28)</strong></td>
</tr>
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</table>

**Fig. 8.5** The odds ratios and 95% confidence intervals (CI) of CHD for MTHFR 677TT versus CC genotype by region of origin. The size of the square is inversely proportional to the variance of the log odds ratio and the horizontal lines represent the 95% CI. The combined odds ratio in the subtotals for each study design and their 95% CI are indicated by the diamond. HPFS: Health Professionals Follow-up Study; Ind: Indian Asians; Whi: whites. Reproduced from Klerk et al. (2002) with permission from the Journal of the American Medical Association.
In the presence of cupric or ferric ions, homocysteine undergoes auto-oxidation, resulting in the production of hydrogen peroxide (Starkebaum & Harlan, 1986). Supplementation with antioxidant vitamins, such as vitamins E and C, prior to assessment of flow-mediated vasodilation has been shown to attenuate the effect of hyperhomocysteinaemia on nitric oxide production (Chambers et al., 1999b). This observation suggests that the disruptive effect of homocysteine on endothelial response may be mediated by free-radical production. Recent work on CBS knock-out mice has shown that animals on a low folate or high methionine diet had abnormal vascular tone compared with control animals and the effects were proportional to the elevation of homocysteine levels. Nitric oxide prevents oxidation of low-density lipoprotein (LDL) cholesterol, inhibits smooth muscle cell proliferation, inhibits platelet aggregation and promotes vasodilation (see Chapter 5, Section 5.5 for further details). Following methionine loading, methylated arginine analogues are increased substantially in a time course that corresponds to the change in flow-mediated dilatation. These methylated arginine analogues act as a competitive inhibitor of nitric oxide synthase and limit the production of nitric oxide. The precise mechanism by which elevated homocysteine increases arginine analogues is not known. In addition to the effects on the nitric oxide-mediated pathway, elevated homocysteine levels may also cause thrombosis by increasing levels of fibrinopeptide A and prothrombin fragments 1 and 2 (Stehouwer & Jakobs, 1998) and by increasing thromboxane biosynthesis in platelets (see Chapter 6).

8.6 Determinants of homocysteine

Fig. 8.6 shows the typical skewed distribution of plasma homocysteine concentrations found in the general population, in which a small proportion have particularly high homocysteine levels. Homocysteine levels increase with increasing age by about 10% for every decade and are about 10% higher in men than in women, but the sex differences are attenuated in old age. Homocysteine levels are about 10% higher in current smokers than in non-smokers and in individuals who consume high amounts of coffee, but are lower in individuals who are moderate alcohol drinkers (Vollset et al., 2001). The mean homocysteine concentration varies according to an individual’s dietary intake of folate and other B vitamins (see Section 8.8). For practical purposes, amongst normal middle-aged adults in populations without mandatory fortification with folic acid, the plasma concentrations of plasma total homocysteine lie between 8 and 15 µmol/l (Vollset et al., 2001). Homocysteine levels are relatively stable within individuals at least for a year or so, with a within-person standard deviation of about 1 µmol/l, with a somewhat greater variation with longer intervals between measurements.

8.7 Genetic influences

Homocystinuria and severe hyperhomocysteinaemia (>100 µmol/l) are usually caused by rare inborn errors of metabolism (Wilcken & Wilcken, 2001). Cystathionine beta-synthase deficiency (see Section

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![Fig. 8.6 Distribution of homocysteine levels in the Homocysteine Studies Collaboration (2002). Reproduced with permission from the Journal of the American Medical Association.](image-url)
8.2) is the most common genetic cause of severe hyperhomocysteinaemia, with an estimated frequency of one per 300,000 living births. A thermolabile variant of MTHFR due to a C→T substitution in the gene (C677T) encoding this enzyme is a much more frequent variation affecting 5–15% of the population (Frosst et al., 1995). The C677T mutation for MTHFR causes a single amino acid substitution which results in altered binding of folate. Individuals who have the TT mutation have about 25% higher homocysteine levels than individuals with the CC variant, although the effect of this variant on homocysteine levels is attenuated in individuals who are folate replete (Jacques et al., 1996). Other recently discovered genetic variants include defects of methionine synthase (Leclerc et al., 1996) and methionine synthase reductase (Wilson et al., 1999), but these have less significant effects on homocysteine levels than MTHFR.

8.8 Dietary influences
Dietary intake of folate, vitamin B₁₂ and vitamin B₆ are the chief nutritional determinants of blood homocysteine concentrations, with folate being the predominant vitamin (Selhub et al., 1993). Observational studies show a linear relationship of increasing blood folate levels and a decreasing homocysteine concentration with increasing intake of dietary folate. The Framingham Study demonstrated that those in the top decile of intake of dietary folate had homocysteine levels that were about 5 µmol/l lower than those in the bottom decile. The more reliable evidence for the effects of individual vitamins on blood homocysteine levels comes from the clinical trials comparing the effects of such vitamins on homocysteine levels (Homocysteine Lowering Trialists’ Collaboration, 1998).

8.9 Effects of vitamin supplements on homocysteine
Supplementation with folic acid and vitamin B₁₂ are remarkably effective at reducing homocysteine levels. A meta-analysis of 12 randomised trials of folic-acid-based vitamin supplementation with biochemical endpoints involving individual patient data from 1114 participants was carried out to help determine the optimum dose of folic acid to lower homocysteine levels (Homocysteine Lowering Trialists’ Collaboration, 1998). There was substantial heterogeneity between the results of the different trials in the pre-treatment blood concentrations of homocysteine that they reported and the absolute differences that they claimed to have achieved. Both the proportional and absolute reductions in blood homocysteine levels appeared to be greater at higher pre-treatment blood homocysteine levels than at lower pre-treatment homocysteine levels. But after standardising to pre-treatment blood levels of 12 µmol/l for homocysteine and 12 nmol/l for folate (the approximate average for most Western populations), there was no longer any heterogeneity in the proportional reductions in homocysteine levels achieved in the individual trials. Under these pre-treatment circumstances, this overview demonstrated that dietary supplementation with folic acid reduced blood homocysteine levels by 25% (95% CI: 23 to 28), with apparently similar effects throughout the range 0.5 to 5 mg folic acid daily (Fig. 8.7). Vitamin B₁₂ (mean 0.5 mg daily) produced an additional 7% reduction (95% CI: 4 to 11%), whereas vitamin B₆ (mean 16.5 mg daily) had no significant effects on fasting or basal homocysteine levels. Hence, in typical Western populations (but not in the USA which has mandatory folic acid fortification of flour) the meta-analysis suggested that daily supplementation with folic acid (0.5 to 5 mg) and vitamin B₁₂ (0.5 mg) would be expected to reduce homocysteine levels by about one-quarter to one-third. In most Western populations (but again not in the USA) the average concentration of blood homocysteine is about 12 µmol/l, so supplementation would result in a reduction of about 3 or 4 µmol/l (i.e. from 12 to 8 µmol/l). In the context of mandatory folic acid fortification, the effect of additional dietary supplementation with folic acid would be expected to reduce homocysteine levels by only 10–15% in people with normal renal function. Individuals with renal disease typically have homocysteine levels that are about twice as great as those in the general population and remain responsive to folic acid with expected reductions in homocysteine levels of about 25%.

8.10 Large trials of homocysteine lowering vitamin supplements
Observational studies cannot exclude the influence of confounding factors, including dietary or other factors, which may explain the modest relative
differences observed. There is some limited evidence that lowering homocysteine levels for at least six months may be associated with a reduction in risk of surrogate markers of coronary disease. The Swiss Heart Study showed that supplementation of 205 people after a successful coronary angioplasty with folic acid, vitamin B<sub>12</sub> and vitamin B<sub>6</sub> reduced the risk of restenosis of the coronary arteries (Schnyder et al., 2001) and of death, non-fatal myocardial infarction and coronary revascularisation events by 32% (Schnyder et al., 2002). But the results of long-term clinical trials of vitamin therapy to lower blood homocysteine levels conducted in high-risk populations are required to assess whether the observed associations are causal or are reversible by treatment (Clarke & Collins, 1998). Seven on-going large-scale trials (five with prior CHD and two with prior stroke) involving 30 000 individuals should provide randomised evidence for the effects on vascular risk of homocysteine lowering therapy in populations without fortification and five trials (two with prior CHD, two with renal disease, and one with stroke) involving 20 500 individuals will be carried out in populations with fortification. In the UK, the SEARCH trial is the largest individual trial of folic acid supplementation that is currently testing the effects of folic acid (2 mg) and vitamin B<sub>12</sub> (1 mg) administered daily versus placebo on risk of cardiovascular events in 12 000 individuals who had a prior history of myocardial infarction. Two trials involving 6000 participants are assessing the effects on cardiovascular outcomes of lowering homocysteine levels in patients who have renal disease.

In addition to these trials shown in Table 8.2, the VITRO trial is assessing the effects of homocysteine lowering vitamin supplements for thromboprophylaxis in 800 participants who had a prior history of venous thrombosis. The FACIT trial is assessing the effects of folic acid supplementation on carotid artery intima-medial wall thickness in 800 healthy elderly individuals. The results of the large trials are required to establish whether the associations of homocysteine with vascular risk are reversible by treatment.

The meta-analysis of the observational studies of homocysteine and CHD (Homocysteine Studies Collaboration, 2002) suggested that a prolonged absolute difference in usual blood total homocysteine levels of 25% may be associated with about 11% less CHD and 19% less stroke. However, even if the relationship of homocysteine with cardiovascular disease is causal, this does not imply that a blood homocysteine reduction that is maintained for only a few years in a clinical trial would achieve the same result as long-term differences in usual homocysteine levels observed in epidemiological studies. Moderate
reductions in the risk of vascular diseases of about 10–15% associated with dietary supplementation with folic acid and other B vitamins would be of substantial public health importance, if these could be reliably demonstrated. But detection of reductions in the risk of vascular disease of about 10–15% associated with the use of folic acid supplements would require that large-scale clinical trials be conducted in high-risk populations and these would need to adopt regimens and procedures that will maximise the difference in intake between the treatment groups for an adequate duration. Most of the ongoing large-scale trials will be carried out in high-risk populations of patients with prior vascular disease or others at high risk of such events and have adopted similar endpoints (Table 8.2). This should enable the principal investigators in each trial to collate the individual participant data from their studies in a systematic overview or meta-analysis of the post-publication follow-up of their separate results. A systematic overview of all trials of folic-acid-based therapy would provide randomised evidence of any benefits (or hazards) of lowering homocysteine levels in about 50 000 participants (30 000 in populations without folic acid fortification and 20 000 in populations with folic acid fortification). Until the large-scale trials of homocysteine lowering therapy are completed, definitive recommendations about widespread screening of patients for elevated homocysteine levels as a risk factor for cardiovascular disease cannot be justified.

8.11 Folic acid fortification

Mandatory folic acid fortification of flour was introduced in North America for the prevention of neural tube defects in 1998 (Food and Drug Administration, 1996). The effect of folic acid fortification was to increase the population mean level of folate and decrease the mean level of total homocysteine in North America (Jacques et al., 1999; Lawrence et al., 1999). Folic acid fortification has been shown to reduce the risk of neural tube defect births by 19% in North America (Honein et al., 2001). While an expert body has recommended fortification of flour with folic acid for the prevention of neural tube defects in

<table>
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<th>Year started</th>
<th>Homocysteine-lowering regimen (daily doses in mg)</th>
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<td>Folic acid B_{12} B_{6} 2 × 2 factorial design</td>
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<td>1999</td>
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<td></td>
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the UK (Department of Health, 2000b), concerns persist about the hazards of such a policy regarding delay in the diagnosis of vitamin B₁₂ deficiency or exacerbation of either peripheral neuropathy or neuropsychiatric complications associated with vitamin B₁₂ deficiency. The results of the long-term trials assessing the effects of folic acid on cardiovascular outcomes are required before advocating the use of vitamin supplements in high-risk individuals or changing the population mean levels of folate (by fortification of flour) for the prevention of cardiovascular diseases. If clinical trials could demonstrate that lowering homocysteine levels could reduce the risk of cardiovascular disease, then introduction of fortified flour or mass fortification of other foods could represent a very effective strategy for prevention of cardiovascular diseases.

About one in ten people aged 75 years or over have subnormal levels of vitamin B₁₂ and metabolic evidence of vitamin B₁₂ deficiency (Clarke et al., 2003). The prevalence of biochemical evidence of vitamin B₁₂ and folate deficiency is greater than the prevalence of macrocytic anaemia and vitamin B₁₂-related neurological disease commonly found in clinical practice (Clarke et al., 2003). There are a number of possible explanations for this apparent paradox. The usual textbook description of the clinical correlates of vitamin B₁₂ deficiency may be less frequent than widely believed, or the typical symptoms may be attributed to ageing rather than to unrecognised B₁₂ deficiency. Furthermore, improvements in the national diet over the past half-century or so may have modified the natural history of vitamin B₁₂ deficiency and of folate deficiency towards a milder or more incomplete history. It is more likely that most persons with biochemical evidence of vitamin deficiency do not have clinically relevant symptoms or signs. Longitudinal studies are needed to ascertain the proportion of such persons with biochemical B₁₂ deficiency who may subsequently develop typical symptoms and signs of vitamin B₁₂ deficiency. The administration of folic acid to people with vitamin B₁₂ deficiency may prevent the anaemia, but not the neurological sequelae associated with vitamin B₁₂ deficiency referred to as masking of pernicious anaemia. A greater awareness of the high prevalence of vitamin B₁₂ and folate deficiency in older people could reduce the risk of deficiency-related disability in old age. More research is needed to determine the minimum effective dose of vitamin B₁₂ that can correct vitamin B₁₂ deficiency in people with intrinsic factor deficiency and the long-term effects of vitamin B₁₂ supplementation of individuals with biochemical evidence of vitamin B₁₂ deficiency, to help guide public health policy on combined fortification with folic acid and vitamin B₁₂.

8.12 Key points

- Elevated blood homocysteine is a modest independent risk factor for CHD and for stroke. A meta-analysis of the observational studies on this topic showed that among prospective studies after adjustment for other cardiovascular risk factors, a 25% lowering in homocysteine was associated with about 10% lower risk of CHD and 20% lower risk of stroke.

- About 10% of the population have a genetic variant (TT) for the gene that encodes for methylene-tetrahydrofolate reductase (MTHFR), and such individuals have about 25% higher homocysteine levels than those with the CC genotype. A meta-analysis of the observational studies of MTHFR and CHD showed that individuals with 677TT had a 16% higher risk of CHD compared with those with the 677CC genotype.

- The concordance of the risk associations with homocysteine observed in the population studies with that of the genetically determined differences in homocysteine provides support to the vascular risk associations with homocysteine being causal.

- Large trials of folic-acid-based vitamin supplements are currently underway to test whether lowering of homocysteine levels may reduce the risk of CHD and stroke.
8.13 Recommendations for future research

- Large trials (and a meta-analysis of such trials) of folic-acid-based vitamin supplements are required in people at high risk of cardiovascular disease to assess whether lowering of homocysteine levels can reduce the risk of cardiovascular disease.
- More evidence is required to determine the minimum effective doses of folic acid and of vitamin B₁₂ associated with the maximum reduction in homocysteine levels.
- Further research is needed to identify the genetic determinants of homocysteine levels when examined alone or in combination with each other and with vitamin B₁₂, vitamin B₆ and folate status.
- More research is needed on the problem of vitamin B₁₂ deficiency in older people and the relevance of this for public health policy on folic acid fortification.
- Clinical trials of homocysteine-lowering vitamin supplements are required in people with renal disease to assess the effects on risk of cardiovascular disease in this group.

8.14 Key references


9 Adipose Tissue-Derived Factors

9.1 Introduction

9.1.1 Obesity and cardiovascular disease

Obesity is the most prevalent nutritional disorder in industrialised countries and is a growing problem in the developing world (Kushner, 2002). It is associated with a significantly increased risk for cardiovascular morbidity and mortality (see Chapter 2, Section 2.4). The exact role of and mechanisms by which increased obesity promotes cardiovascular risk are poorly understood, and the extent to which this is reversible with weight loss is not yet clear. Obesity is defined by an excess of adipose tissue and often leads to adverse changes in circulating cardiovascular risk factors, which may be involved in the development of cardiovascular disease (Levenson et al., 2002; Sorisky, 2002).

9.1.2 Adipose tissue-derived signals and cardiovascular disease

There has been a long-held belief that accumulation of adipose tissue may lead to increased risk of cardiovascular disease because of associated metabolic alterations. For instance, non-esterified fatty acids (NEFA) are released from adipose tissue; with the increased adipose tissue mass of obesity, plasma NEFA concentrations are almost inevitably raised (Opie & Walfish, 1963; Flatt, 1972). Elevated plasma NEFA concentrations may relate either directly or indirectly to other risk factors for cardiovascular disease (Frayn et al., 1996; see Chapter 3). In addition, as adipocytes enlarge with fat storage, they become less metabolically active and respond less well to insulin. An impaired ability of adipose tissue to respond rapidly to insulin and other hormones could be seen as a factor causing insulin resistance, and ultimately increased cardiovascular risk, through metabolic perturbations (Frayn, 2002; see Chapter 2).

However, the past few years have seen an explosion of research on alternative, non-metabolic links between adipose tissue, insulin resistance and cardiovascular disease. Indeed, current evidence suggests that the association between obesity and cardiovascular disease can be explained, at least in part, by novel signalling molecules (adipokines) emanating from, or expressed in, adipose tissue. The diversity of these factors includes enzymes, such as lipoprotein lipase and hormone sensitive lipase, growth factors and cytokines, such as tumour necrosis factor-alpha (TNFα), interleukin-6 (IL-6) and heparin-binding epidermal growth factor-like growth factor (HB-EGF), and several other hormone-like molecules involved in metabolism (leptin, adiponectin/Acrp30, resistin and acylation stimulation protein; Fig. 9.1; Mohamed-Ali et al., 1998). It has been proposed that the secretion of molecules such as leptin, adiponectin and IL-6 by adipose tissue, combined with the actions of adipose tissue-expressed TNFα in obesity, could underlie the association of insulin resistance with endothelial dysfunction and coagulopathy, leading to coronary heart disease (CHD). Whilst firm evidence from intervention or epidemiological studies for links between adipose tissue-derived factors and cardiovascular disease is still lacking in many instances, this is such an important emerging area of research that it was considered appropriate to include a discussion of this subject in this report.

While elevated total body fat is associated with increased morbidity and mortality rates, the risk factor profile for CHD is more adverse when this excess fat
is mainly stored on the upper body (within the abdominal cavity around the viscera) than in the gluteal regions or on the lower limbs. Thus, obese subjects with excess intra-abdominal fat deposition suffer greater metabolic consequences than do similarly overweight subjects with a predominantly subcutaneous distribution of adipose tissue (see Chapter 2). There is some evidence that the visceral and subcutaneous adipose tissue depots differ in their expression and production of adipokines (Arner, 2001; Vidal, 2001).

Two forms of adipose tissue have been identified: brown (BAT) and white (WAT). In humans, significant amounts of BAT are found only in neonates, primarily in the thoracic cavity surrounding the great vessels (James & Trayhurn, 1981). However, a small amount of brown fat is also present within human white fat depots and there is conversion between these two types of tissue (Cinti, 2002). For example, in humans with pheochromocytoma, a tumour that secretes catecholamines, large brown fat depots develop (Lean et al., 1986). While the relative contribution that brown fat cells make to total energy balance of adult humans is debatable, these cells do exist and may represent an important target for physiological/dietary/pharmacological intervention (Champigny et al., 1991).

In the remainder of this chapter the products of the WAT, both expressed and secreted, will be discussed along with evidence for their involvement in insulin resistance, endothelial dysfunction, hypertension and other cardiovascular risk factors, which may form the molecular basis for the association between obesity and heart disease. Evidence will also be presented, where available, for intervention strategies involving dietary (i.e., low fat, high fibre diets) and lifestyle (i.e., physical activity) measures that have been demonstrated to be effective in improving the obesity-associated cardiovascular risk profile in the regulation of these adipose-derived factors and for the potential importance of variability in genes expressed in adipose tissue.

9.2 Leptin

Leptin is a 16 kDa hormone produced largely by subcutaneous adipose tissue and to a lesser extent in gastric epithelium, placenta and skeletal muscle (Friedman, 2002). The cloning and sequencing of this molecule catapulted adipose tissue research into a new era, opening up a novel area of endocrinology in the 1990s (Zhang et al., 1994). Leptin receptors are present in most tissues. It has a variety of roles depending on the cell from which it is derived, as well
as on its target tissue. It is a mediator of energy expenditure, and interacts with other hormonal mediators and regulators of energy status and metabolism such as insulin, glucagon, the insulin-like growth factors, growth hormone and glucocorticoids, acting as an endocrine, paracrine as well as an autocrine factor. It is a permissive factor for puberty, signalling to the hypothalamus when sufficient energy has been stored to embark on the energy-expensive reproductive cycle. It also signals metabolic status and modulation between the fetus and the maternal metabolism (Margetic et al., 2002).

The major function of this hormone appears to be in the adaptation to reduced energy intake and body fat stores rather than as a restraint to limit energy intake and obesity (Crowley et al., 2002; Havel, 2002). Leptin helps to maintain stability of body weight and energy storage over long periods of time by regulating hepatic insulin action, peripheral glucose utilisation, food intake and thermogenesis (Leibel, 2002). It also appears to interact with acute signals of satiety emanating from the gastrointestinal tract, such as cholecystokinin (Attele et al., 2002). Evidence suggests that most human obesity is a leptin-resistant state, caused either by decreased leptin transport into the central nervous system (CNS) or reduced signalling distal to the leptin receptor. A relative deficiency in leptin is biologically significant, while excess leptin appears physiologically less relevant (Havel, 2002).

9.2.1 Leptin and cardiovascular risk

Leptin may be involved in the development of various cardiovascular risk factors. It has been associated with impaired fibrinolysis (Soderberg et al., 1999) and been shown to be an independent predictor of coronary events, with levels significantly higher in men who subsequently experienced a coronary event compared to those who remained disease-free (Wallace et al., 2001). Leptin levels also correlate with inflammatory risk factors for heart disease, such as cytokines, fibrinogen and C-reactive protein (CRP) (Van Dielen et al., 2001; see Chapter 7 for further details of these factors).

A direct effect of leptin on blood pressure has also been reported, perhaps through sympathetic activation (Hall et al., 2001). In rats, a chronic increase in circulating leptin caused a sustained increase in arterial pressure (Shek et al., 1998). A direct effect of leptin on the pathogenesis of hypertension independent of obesity has been reported in transgenic mice over-expressing the leptin gene. Plasma leptin concentrations in these ‘skinny’ mice are comparable to those found in obese subjects. However, they are devoid of adipose tissue, have significantly reduced food intake and body weight and elevated blood pressure (Ogawa et al., 2002). Also in humans, plasma leptin correlates with blood pressure, and patients with essential hypertension have been shown to be hyperleptinaemic (Adamczak et al., 2000).

Leptin receptors are also expressed on vascular endothelial cells and mediate angiogenesis (Park et al., 2001). Furthermore, genetically obese (ob/ob) mice that are leptin deficient show impaired endothelial function that is reversed on leptin replacement (Winters et al., 2000). Evidence from animal studies suggests that leptin also affects platelet function and haemostasis. Both ob/ob (leptin deficient) and diabetic (db/db) (leptin resistant) animals exhibit delayed thrombotic occlusion and form unstable thrombi that frequently embolise. Leptin administration prior to injury shortens the time to occlusion and stabilises the thrombi in leptin deficient (ob/ob), but not leptin resistant (db/db), mice (Konstantinides et al., 2001).

Recent in vitro data suggest that leptin stimulates adenosine-monophosphate-activated protein kinase (AMPK), an enzyme that modulates fatty acid and carbohydrate metabolism (Winder & Hardie, 1999). In response to a fall in intracellular adenosine-triphosphate/adenosine-monophosphate (ATP/AMP) ratio, AMPK becomes activated by phosphorylation of its alpha-subunit and the activated AMPK phosphorylates a number of enzymes involved in biosynthetic pathways, causing their inactivation and reducing further ATP utilisation (Winder & Hardie, 1999). In adipocytes, AMPK is known to inhibit both lipolysis and lipogenesis by regulating enzymes engaged in lipid metabolism (Sullivan et al., 1994). Leptin, through its effect on AMPK, down-regulates the activity of the key regulatory enzyme controlling fatty acid synthesis, acetyl CoA carboxylase, in liver, skeletal muscle, and pancreas. This leads to partitioning of fatty acids into mitochondrial beta-oxidation rather than triglyceride storage. Thus, the activation of AMPK by leptin would increase muscle fatty acid oxidation and insulin sensitivity, and leptin deficiency or resistance could contribute to fat accumulation in macrophages and skeletal muscle cells...
(Atkinson et al., 2002; Minokoshi et al., 2002). Indirect in vivo evidence for this comes from studies in patients with lipoatrophy where leptin replacement significantly improves insulin resistance and hyperlipidaemia (Petersen et al., 2002).

### 9.3 Acylation stimulating protein (ASP)

Acylation stimulating protein (ASP) is a small, basic serum protein capable of stimulating triglyceride synthesis in fibroblasts and adipocytes in vitro (Cianflone et al., 2003). Sequence analysis of ASP has shown that it is identical to C3adesArg, the inactive fragment of the complement anaphylatoxin peptide, C3a, and it is generated by mature adipocytes secreting the three complement proteins: complement protein C3, factor B and factor D (adipsin) (Kildsgaard et al., 1999).

ASP acts mainly as an adipocyte autocrine factor and plays a central role in the metabolism of adipose tissue. Adipocytes may express a specific C3adesArg (ASP)-receptor that is distinct from the cloned C3a-receptor (Martin et al., 1997). It increases the efficiency of triglyceride synthesis by stimulating fatty acid incorporation in adipocytes. ASP also enhances translocation of glucose transporters (GLUT 1, 3 and 4) from the cytoplasm to the plasma membrane to stimulate adipocyte glucose uptake (Tao et al., 1997) and activates diacylglycerol acyltransferase (DGAT), which is a key enzyme in triglyceride synthesis (Yasruel et al., 1991). Furthermore, while increasing lipogenesis, ASP also inhibits the activity of hormone sensitive lipase (HSL) and HSL-mediated lipolysis by stimulating free fatty acid re-esterification (Van Harmelen et al., 1999). These effects of ASP are insulin independent.

Evidence that ASP deficiency may have a major impact on energy homeostasis and insulin action comes mainly from rodent studies. ASP-deficient mice, that are genetically deficient in C3, are hyperphagic (i.e. have an abnormally increased appetite), yet have significantly reduced fat mass and are resistant to weight gain even when placed on a high fat diet (Murray et al., 2000). ASP/C3 knockout mice, when compared with wild-type animals, are more insulin sensitive, with reduced fasting insulin levels and improved glucose tolerance, perhaps due to their increased lean mass. These animals demonstrate delayed postprandial lipid clearance. Exogenous ASP administration increases triglyceride and NEFA clearance in normal and obese mice, suggesting a role in postprandial lipid disposition (Murray et al., 1999). However, Wetsel et al. (1999) found no significant differences in the triglyceride, cholesterol, or free fatty acid concentrations in the plasma of fasted normal and ASP/C3 knockout mice; furthermore, they also failed to show any impairment in ability of these mice to clear triglycerides and NEFA from the circulation when challenged with an oral fat load when compared with their wild-type littermates. Genetic deficiency of ASP(C3ades-Arg) does not cause elevated apolipoprotein B (apoB) levels in mice (Wetsel et al., 1999). In humans, ASP deficiency, or more specifically adipsin deficiency, is rare. In these individuals there are no data on insulin action or lipid metabolism. However, indirect evidence that ASP may be involved in regulating human lipid metabolism comes from a report that genes that affect total cholesterol, low-density lipoprotein (LDL), and triglycerides also influence circulating ASP levels (Martin et al., 2004).

In humans, circulating ASP levels correlated with percentage body fat, while C3 was shown to be inversely related to insulin sensitivity as assessed by a euglycaemic clamp or by fasting insulin concentrations, independently of body adiposity (Weyer & Pratley, 1999). Insulin action and insulinaemia are closely related to the fasting complement C3, but not ASP concentration (Weyer et al., 2000a). A study comparing obese subjects with and without type 2 diabetes with lean non-diabetic volunteers also reported higher ASP concentration in the obese groups. Although plasma ASP concentration did not change significantly after a fat challenge, it correlated negatively with insulin sensitivity and positively with the magnitude of postprandial lipaemia in non-diabetic, but not in the diabetic, men. This suggests that ASP is associated with whole-body glucose and lipid metabolism in non-diabetic individuals, whereas in diabetes there may be an impairment in the regulatory role of ASP in lipid and glucose metabolism (Koistinen et al., 2001).

#### 9.3.1 Acylation stimulating protein and cardiovascular disease

Levels of ASP and its precursors C3 and adipsin, are all raised in obesity, as well as in type 2 and type 1 diabetes (Pomeroy et al., 1997; Maslowska et al., 1999; Koistinen et al., 2001). ASP or adipsin has been
shown to be positively associated with various cardiovascular risk factors including insulin, glycated haemoglobin (HbA1c), leptin, CRP, plasminogen activator inhibitor-1 (PAI-1) and tissue plasminogen activator (tPA) (Mavri et al., 1999; Ebeling et al., 2001; Ylitalo et al., 2001). C3 was higher in patients with high blood pressure (Mantov & Raev, 1996). It also predicts myocardial infarction (Muscari et al., 2001; Ylitalo et al., 1999). Adiponectin levels were elevated, especially in those with hyperapolipoprotein B (Cianflone et al., 1997).

### 9.4 Adiponectin

Adiponectin, a 30 kDa adipocyte protein, structurally related to collagen and TNFα, was identified in the mid-1990s (Scherer et al., 1995; Maeda et al., 1996). It has multiple names: complement-related protein 30 (ACRP30), adipose most abundant gene transcript (apM1), adiponectin, adipoQ, and gelatin-binding protein (gdp28) (Tsao et al., 2002). In this chapter, for the purpose of simplicity, it is referred to as adiponectin (Arita et al., 1999).

Circulating adiponectin concentrations have been shown to be decreased in obese individuals, and this reduction has been proposed to have a role in the pathogenesis of atherosclerosis and cardiovascular disease associated with obesity and other components of the metabolic syndrome (Arita et al., 1999; Funahashi et al., 1999). Adiponectin levels were higher in women than in men (Spranger et al., 2003) and lower in patients with coronary artery disease. In men, lower serum adiponectin levels (6.4 ± 2.4 versus 7.9 ± 3.0 mg/l) were associated with high-normal blood pressure (systolic: 133 ± 4, diastolic: 79 ± 5 mmHg), faster heart rate and smaller LDL, even after adjustment for body mass index (BMI), compared to those with optimal blood pressure (systolic: 110 ± 7, diastolic: 65 ± 6 mmHg) (Kazumi et al., 2002). Systemic levels of adiponectin and its gene expression in adipose tissue were reduced in patients with type 2 diabetes (Statnick et al., 2000). Its levels were negatively correlated with fasting insulin concentrations and positively correlated with insulin sensitivity, as assessed by glucose disposal during euglycaemic and hyperinsulinaemic clamps. The relationship between adiponectin and insulin action was independent of body adiposity (Weyer et al., 2001). Furthermore, a decline in circulating adiponectin levels coincides with the onset of insulin resistance and the development of type 2 diabetes in both animal models of obesity and in humans (Hotta et al., 2001; Lindsay et al., 2002; Spranger et al., 2003).

It has recently been shown that adiponectin circulates in serum as a low molecular weight (LMW) hexamer and a larger multimeric, high molecular weight (HMW) complex of 12–18 subunits (Pajvani et al., 2003). Interestingly in male mice adiponectin circulates mainly in the LMW form, whereas females have a more balanced distribution of the two forms. Levels of the two complexes also responded differentially to insulin and glucose treatment, with selective loss of the HMW form. Furthermore, the ratio between these two oligomeric forms, (HMW/(HMW + LMW)), rather than the absolute amounts, was critical in determining insulin sensitivity. Pajvani et al. showed that db/db mice display decreased (HMW/ (HMW + LMW)) values compared with wild type littermates, as do type 2 diabetic patients compared with insulin-sensitive individuals, despite similar total adiponectin levels (Pajvani et al., 2004; Tonelli et al., 2004). In mice and in humans, thiazolidinedione therapy improved this ratio. In a number of type 2 diabetic cohorts it was also a better indicator of improvements in insulin sensitivity than total serum adiponectin levels. Intravenous injection of HMW adiponectin, but not the hexameric adiponectin, dose-dependently decreased serum glucose. These observations suggest that the HMW adiponectin complex may be the active form of this protein.

#### 9.4.1 Actions of adiponectin

Although the site and mechanism of adiponectin’s actions are not completely clear, data show that it has insulin sensitising and anti-inflammatory properties. Recently, two receptors (AdipoR1 and AdipoR2) have been cloned and shown to bind and mediate several of the functions of adiponectin (Yamauchi et al., 2003a). AdipoR1 is expressed ubiquitously, but is most abundant in skeletal muscle, while AdipoR2 is found mainly in hepatocytes. Adiponectin appears to modulate endothelial function, has an inhibitory effect on vascular smooth muscle cell proliferation and decreases the synthesis of adhesion molecules (Ouchi et al., 1999). Adiponectin may also be involved...
in the modulation of inflammation (Ukkola & Santaniemi, 2002). It is accumulated preferentially in the injured vascular wall than in intact vessels, and has been shown to suppress macrophage-to-fatty cell transformation in vitro. Increased plasma adiponectin reduces atherosclerosis in vivo in apoE-deficient mice. Adiponectin migrates to foam cells in the fatty streak lesions and suppresses vascular cell adhesion molecule-1 (VCAM-1) and class A scavenger receptor messenger RNA (mRNA) levels, and tends to reduce levels of TNFα (Okamoto et al., 2002). More recently, transgenic mice overexpressing the globular domain of adiponectin crossed with ob/ob or apoE-deficient mice showed an amelioration of atherosclerosis, which was associated with decreased expression of class A scavenger receptor and TNFα (Yamauchi et al., 2003b). In these animals there was also amelioration in diabetes and insulin resistance. This was associated with increased expression of molecules involved in fatty acid oxidation such as acyl-CoA oxidase, and molecules involved in energy dissipation such as uncoupling proteins 2 and 3 (UCP2 and 3) and increased fatty acid oxidation in skeletal muscle of these mice (Yamauchi et al., 2003b). Administration of the globular region of adiponectin to mice induced weight loss in animals consuming a high fat, high sucrose diet without decreasing food intake, an effect that was associated with reductions in circulating fatty acids and increased fatty acid oxidation in muscle (Fruebis et al., 2001). In vitro and in vivo evidence in rodents suggests that this is through induction of AMPK and inactivation of acetyl CoA carboxylase (Tomas et al., 2002; Yamauchi et al., 2002). Recombinant adiponectin has been shown to reduce serum glucose in normal mice and in mouse models of diabetes, without stimulating insulin secretion (Berg et al., 2001). Adiponectin also enhanced insulin suppression of glucose production by isolated hepatocytes, suggesting it may lower glucose by acting directly on the liver. In addition, adiponectin improved glucose tolerance in insulin-resistant db/db mice and reduced insulin resistance associated with low adiponectin levels in mice with either lipodystrophy or obesity-induced insulin resistance, although complete reversal of insulin resistance in lipodystrophic animals required co-administration of leptin (Combs et al., 2001; Berg et al., 2002). The improvements in insulin sensitivity were associated with decreased triglyceride content of muscle and liver and increased fatty acid oxidation in muscle, and accompanied by increased expression of genes for proteins involved in fatty acid transport and utilization (Berg et al., 2002). Thus adiponectin appears to reduce hepatic glucose production and increase muscle glucose utilisation, perhaps by increasing fat oxidation and thereby reducing circulating NEFA levels and intramyocellular lipid accumulation (Berg et al., 2002).

9.5 Adipose tissue-derived cytokines

TNFα and IL-6 are pro-inflammatory cytokines with potent effects in host defence (Das, 1991). TNFα is a low molecular weight (17 kDa) peptide with a broad range of biological and immunological effects, including antiviral action, growth regulation and immunomodulation (Rosenblum & Donato, 1989). IL-6 is 22–28 kDa and synthesised as a 212 amino acid (aa) precursor protein, with a 28 aa signal peptide and a 184 aa mature segment (Akira et al., 1993).

Adipose tissue is a significant source of endogenous TNFα, with its expression in fat tissue being elevated in both rodent and human obesity (Bullo et al., 2002). Evidence for significant circulating levels of this cytokine is variable and it is thought to operate mainly via autocrine/paracrine mechanisms in both adipose tissue and skeletal muscle (Hotamisligil & Spiegelman, 1994). No regional differences have been reported in the expression or production of TNFα (Dusserre et al., 2000; Arner, 2001). IL-6, on the other hand, is expressed in adipose tissue; significant amounts of this cytokine are released, largely by the visceral adipose tissue, into the systemic circulation and its plasma levels increase with obesity (Mohamed-Ali et al., 1997; Fried et al., 1998).

9.5.1 Cytokine receptors

Two structurally distinct TNF receptors, TNFRI and TNFRII, have been identified (Hotamisligil, 1999). TNFRI is thought to mediate most of the functions of TNFα, while the actions of RII are as yet unclear, and may be mainly cell specific. Both these receptors are expressed in human adipose tissue. Soluble forms are present in the circulation and produced at least in part by adipose tissue, with levels increasing with measures of adiposity (Mohamed-Ali et al., 1999). While the nature of their physiological function is still unclear they are thought, at least in vitro, to inhibit the ligand binding cell surface
receptor, thereby acting as antagonists (Hasegawa et al., 2001).

The biological activities of IL-6 are initiated by binding of the ligand to a single receptor. The IL-6 receptor (IL-6R) comprises two chains, a ligand binding, predominantly extracytoplasmic chain (IL-6R; gp80) and the signal transducing gp130 chain (Hirano, 1998). The gp130 chain by itself has little or no IL-6 binding property, but it plays a part in signal transduction (Hirano et al., 1997). The binding of IL-6 to IL-6R is predominantly an extracellular process. This complex, IL-6/IL-6R, can be formed with either soluble or membrane-bound IL-6R (Kishimoto et al., 1992). The binding of IL-6 to IL-6R in the presence of gp130 leads to the formation of high affinity binding sites, gp130 dimerisation and signal transduction (Hirano, 1998). Unlike in the case of TNFα, where the soluble receptors may function as inhibitors for the ligand, both recombinant and naturally produced circulating, soluble IL-6R (sIL-6R) enable cells that express gp130 but not IL-6R to respond to IL-6 (Hirano, 1998). The signal transducing gp130 is abundantly expressed in most cell types, while IL-6R is expressed in a variety of cells in extremely low quantity (Kishimoto et al., 1992).

9.5.2 Role of TNFα in adipose tissue
TNFα has distinct effects on adipose tissue. It induces insulin resistance by down-regulating the tyrosine kinase activity of the insulin receptor and decreasing the expression of GLUT-4 glucose transporters (Hotamisligil, 2000). TNFα also decreases adipose tissue leptin production (Bruun et al., 2002) and increases PAI-1 content in adipocytes (He et al., 2000). It reduces lipoprotein lipase activity in white adipocytes and stimulates hepatic lipolysis (Sethi & Hotamisligil, 1999). TNFα treatment of several in vitro models of adipogenesis clearly showed that it is a potent inhibitor of adipocyte differentiation. This antiadipogenic property is accompanied by suppression of developmental and metabolic markers of fat cell differentiation, such as peroxisome proliferator-activated receptor-gamma2 (PPAR-γ2), lipoprotein lipase, glycerol-3-phosphate dehydrogenase and GLUT-4. Moreover, TNFα promotes lipolysis in mature adipocytes and, subsequently, a reversion of the adipocyte phenotype (Hube & Hauner, 1999). Taken together, the results of a variety of studies suggest that TNFα may act as an important autocrine or paracrine regulator of fat cell function that serves to limit adipose tissue expansion, probably by inducing insulin resistance which may in turn cause metabolic disturbances. However, this concept is mainly based on animal data and is so far only partially supported by studies in humans. A clearer understanding of the molecular mechanisms of TNFα production and action in human adipose tissue may help to find new approaches for the treatment of insulin resistance and its associated complications.

9.5.3 Acute and chronic release of IL-6
Many different cells, including macrophages, endothelial cells, smooth and skeletal muscle cells, and adipocytes, produce IL-6. However, the characteristics and regulation of IL-6 production differ depending on cellular origin. During infection or in response to infectious stimuli such as lipopolysaccharide (LPS), IL-6 is released from immune cells (e.g. monocytes and macrophages). Skeletal muscle cells, on the other hand, produce physiologically significant quantities of IL-6 in response to exercise (Pedersen et al., 2001). Both post-exercise and during infection the magnitude of the cytokine response is far greater but of a shorter duration, lasting hours or perhaps days, than that seen in metabolic diseases such as obesity. In obesity there is a chronic, low-level elevation in the circulating IL-6, probably of adipocyte origin (Fig. 9.2). The metabolic consequences of acute, as opposed to chronic, elevations in circulating IL-6 levels are quite different.

9.5.4 Effects of acute elevation in IL-6
In humans, acute elevation in IL-6 levels, as seen after intravenous administration, is associated with increased plasma glucose clearance rate and hyperinsulinaemia, indicating that IL-6 stimulates glucose uptake in vivo (Stouthard et al., 1995). It also increases basal and insulin-stimulated glucose uptake by 3T3.L1 and 3T3.F442A adipocytes in vitro (Stouthard et al., 1996). Infusion of IL-6 was shown to cause elevated serum triglycerides and NEFA concentrations (Stouthard et al., 1996). This hypertriglyceridaemia was caused by stimulation of hepatic triglyceride secretion and was independent of endogenous catecholamines (Metzger et al., 2001). IL-6 has been shown to stimulate insulin release from a hamster islet cell line (Shimizu et al., 2000). IL-6 stimulates
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1.1 Glucose and Fatty Acid Oxidation

Glucose and fatty acid oxidation, and induces the release of glucagon and cortisol (Tsigos et al., 1997). IL-6 release from contracting skeletal muscle increases when muscle glycogen availability is reduced and increases glucose uptake. Also during exercise, IL-6 may have immunomodulatory effects, where it inhibits the expression of TNFα while inducing various anti-inflammatory cytokines such as interleukin-1 receptor antagonist (IL-1Ra) and interleukin-10 (IL-10) (Pedersen et al., 2003). IL-6 infusion also increases plasma cortisol, and that indirectly causes a reduction in lymphocyte numbers. Thus, collectively the data suggest that acute increases in IL-6 are beneficial, by increasing lipolysis, glucose availability and being anti-inflammatory.

9.5.5 Effects of chronic elevation of IL-6

Chronic elevations in circulating IL-6 concentrations are seen with increasing age, in type 2 diabetes, CHD and obesity, conditions often associated with increases in adipose tissue mass (Goodpaster, 2002). In these conditions up to a third of the systemic IL-6 is adipose tissue-derived, with a significant proportion of this production being constitutive (Mohamed-Ali et al., 1997; Flower et al., 2003). That these levels may be adequate to induce the acute phase response may be concluded from elevated CRP also seen in obesity (McLaughlin et al., 2002). IL-6 has also been shown to inhibit the expression and secretion of the adipose tissue insulin sensitizer, adiponectin (Bruun et al., 2003; Fasshauer et al., 2003). IL-6, produced by adipose tissue, may also affect adipogenesis in a paracrine/autocrine fashion, and play a role in the pathogenesis of obesity. Indirect evidence for a possible role for IL-6 in adipogenesis comes from studies on the senescence accelerated mouse-P6 (SAMP6) (Kajkenova et al., 1997). These animals have been shown to have a higher percentage body fat, and ex vivo bone marrow cultures from these mice exhibited an increase in the number of colony-forming unit adipocytes, as well as an increase in the number of fully differentiated marrow adipocytes. Long-term bone marrow cultures from SAMP6 produced more IL-6. These data suggest a switch in the differentiation programme and support the existence of a reciprocal relationship between osteoblastogenesis and adipogenesis. This may explain the association of elevated systemic IL-6, decreased bone formation and the resulting osteopaenia with increased adiposity seen with advancing age in both animals and humans.

The data on the effect of IL-6 on lipolysis are also conflicting. In a study looking at the effect of food intake, sympathetic activation and lipolysis, it was reported that while TNFα correlated with lipolysis, IL-6 did not (Orban et al., 1999). Also, in vitro studies have shown that in 3T3-L1 and 3T3-F442A adipocytes, chronic exposure to IL-6 inhibits beta-adrenergically stimulated lipolysis (D. K. Clarke & V. Mohamed-Ali, unpublished observations). However, previous reports have shown that IL-6 reduces
lipoprotein lipase activity in adipose tissue and increases lipolysis (Mattacks & Pond, 1999). Therefore, while there is apparently a physiological difference between acute and chronic elevations in IL-6, the available data are not entirely clear and require further investigation.

9.5.6 IL-6 and CHD

IL-6 may play a key role in several mechanisms that contribute to the development of CHD (Fig. 9.3; Yudkin et al., 2000). IL-6 is a powerful inducer of the hepatic acute phase response, and several acute phase proteins, such as CRP and fibrinogen, are potent cardiovascular risk factors (see Chapter 7). Elevated concentrations of CRP are found in patients with acute coronary syndromes, and predict future risk in healthy subjects. Elevated levels of fibrinogen, with autocrine and paracrine activation of monocytes by IL-6 in the vessel wall contribute to the deposition of fibrin. The acute phase response is associated with increased blood viscosity, platelet number and activity. IL-6 decreases lipoprotein lipase activity and monomeric lipoprotein lipase (inactive form) levels in plasma (Wallberg-Jonsson et al., 1996). Lipoprotein lipase has been shown to mediate the uptake of lipoproteins into cells, with the monomeric form inhibiting the uptake mediated by the dimeric lipoprotein lipase (Krapp et al., 1995). Therefore, the IL-6 mediated reduction in monomeric lipoprotein lipase may increase macrophage uptake of lipids. In fatty streaks and in the atheromatous ‘cap’ and ‘shoulder’ regions, macrophage foam cells and smooth muscle cells express IL-6, suggesting a role for this cytokine along with interleukin-1 (IL-1) and TNFα, in the progression of atherosclerosis.

Circulating IL-6 stimulates the hypothalamo-pituitary-adrenal (HPA) axis, activation of which is associated with central obesity, hypertension and insulin resistance. IL-6 receptors are present in the hypothalamus, which supports a direct central role for this cytokine. IL-6 stimulates both thermogenesis and satiety, through a range of central effects, including prostaglandin synthesis and corticotrophin releasing hormone (CRH) release (Mastorakos et al., 1993). Thus IL-6 may be involved in the pathogenesis of CHD through a combination of autocrine, paracrine and endocrine mechanisms (see Chapter 7, Section 7.4.8).

Although obesity increases circulating levels of IL-6 which contribute to some of the maladaptive consequences of obesity, the net effects of chronically increased circulating cytokine concentrations remain to be clarified.
9.6 Angiotensinogen

Angiotensinogen is a precursor of angiotensin II. Angiotensinogen II has potent vasoconstrictor effects and the renin–angiotensin system has important modulatory activities in the atherogenic process (Brasier et al., 2002). Angiotensinogen is expressed abundantly in liver and in a number of peripheral tissues, including adipose tissue (Engeli et al., 2000). Rat adipocytes synthesize and secrete this protein, and the levels of angiotensinogen mRNA are comparable to those in liver (Hainault et al., 2002). In humans, angiotensinogen is expressed in adipose tissue as well as in primary and cultured adipocytes (Jones et al., 1997; Engeli et al., 1999; Schling et al., 1999). Angiotensinogen gene expression is highly differentiation-dependent in cultured murine adipocytes (Ailhaud et al., 2000). Adipose tissue expresses all the necessary enzymes to convert angiotensinogen into angiotensin II. Angiotensinogen II may also influence adipocyte differentiation by interactions with adipocyte angiotensin receptors (Zorad et al., 1995), inducing adipose cells to produce prostacyclin (Safonova et al., 1997).

9.7 Resistin

Resistin is the latest addition to the adipose tissue secretory products (Steppan et al., 2001a). The murine resistin mRNA encodes a 114-amino acid polypeptide containing a 20-amino acid signal sequence. The secreted protein, resistin, is a 94-amino acid disulphide-linked dimer (Steppan et al., 2001b). The administration of anti-resistin antibody to mice with diet-induced obesity, insulin resistance and hyperglycaemia, partially corrected their blood glucose levels and improved their sensitivity to exogenous insulin (Steppan et al., 2001a). These data suggest that the raised circulating resistin levels might contribute to the hyperglycaemia and insulin resistance seen in this model (Vidal-Puig & O’Rahilly, 2001). Acute administration of recombinant resistin to normal mice led to a modest impairment of glucose tolerance. Reduced adipocyte resistin expression was associated with increased insulin sensitivity in a mouse model of insulin resistance. Resistin expression in 3T3-L1 adipocytes was significantly up-regulated by high glucose concentrations and suppressed by insulin (Haugen et al., 2001). Furthermore, troglitazone, a thiazolidinedione anti-hyperglycaemic agent, as well as TNFα, suppress resistin expression (Shojima et al., 2002). While these data provide some evidence for a role for resistin in insulin resistance, the resistin receptor and downstream signalling pathway are as yet unknown.

However, expression of the gene in human adipose tissue is much less than that observed in mouse adipose tissue and is derived mainly from non-fat cells (Fain et al., 2003). The mouse and human proteins appear to differ greatly, and data do not, as yet, support an important role for resistin in human insulin resistance (Nagaev & Smith, 2001; Janke et al., 2002).

9.8 Peroxisome proliferator-activated receptors (PPARs)

Peroxisome proliferators (PPs) have been shown to regulate cell proliferation, differentiation and metabolism via activation of the peroxisome proliferator-activated receptor (PPAR). PPARs are members of the nuclear hormone receptor family. Three subtypes of PPAR have been described, α, δ (also called β) and γ, encoded by different genes. There is relatively specific expression of the PPAR mRNA in adipose tissue, making it a candidate gene for the regulation of lipid storage and adipose tissue metabolism. PPARα promotes the conversion of pre-adipocytes to adipocytes, adipogenesis, and in adipocytes it enhances insulin sensitivity by controlling the expression of genes in glucose and lipid metabolism. There are three mRNA isoforms of the PPARγ gene: γ1, γ2 and γ3, with the γ2 isoform being most abundant in adipose tissue.

Members of another family of transcription factors C/EBP (CCAAT/enhancer binding protein), also play a regulatory role in energy metabolism. The α, β and δ forms are co-expressed in significant levels in the liver and adipose tissue (Cowherd et al., 1999). PPARγ, along with C/EBPα, is central to the mechanism of adipocyte differentiation, and both are essential for adipose conversion to occur (Fig. 9.4). Over-expression of either PPARγ, C/EBPα, or the immediate controller of their expression, C/EBPβ, leads to differentiation (Fajas et al., 1998). Further evidence for PPARγ involvement comes from incubation of pre-adipocytes with a PPARγ ligand, such as thiazolidinediones, which is sufficient to cause adipocyte differentiation (Takamura et al., 2001).

Activation of the PPARγ gene also has a powerful, site-specific effect on adipose metabolism and lipid deposition, and greatly affects the postprandial
handling of triglyceride-rich lipoproteins. These depot-specific effects may be mediated by differential regulation of key metabolic genes, including 11β-HSD-1, and UCP-1. PPARγ also controls the expression of key enzymes of lipid metabolism (lipoprotein lipase, fatty acid binding and transfer proteins, hormone-sensitive lipase), as well as secretory and expressed proteins (i.e. leptin and TNFα) thus possibly influencing insulin sensitivity (Spiegelman, 1998; Olefsky, 2000).

In Zucker diabetic fatty (ZDF) rats, treatment with PPARγ agonists dose-dependently lowered plasma glucose and enhanced insulin sensitivity. Furthermore, in mice that over-express the human apolipoprotein A-I gene these agonists elevated high-density lipoprotein (HDL) cholesterol and lowered plasma triglycerides, thus improving primary cardiovascular risk factors (Etgen et al., 2002). Overall, results suggest that activation of PPARγ improves multiple factors of the insulin resistance syndrome and its associated cardiovascular risk.

9.9 Other adipose factors associated with cardiovascular disease

Obesity is associated with both arterial and venous thrombosis, and may therefore represent abnormalities in coagulation and haemostasis (De Pergola & Pannacciulli, 2002). Several studies have shown that obese patients have higher plasma concentrations of all prothrombotic factors (fibrinogen, von Willebrand factor and factor VII) than in non-obese controls, with a positive association with central fat. Similarly, plasma concentrations of PAI-1 have been shown to be higher in obese patients than in non-obese controls and to be directly correlated with visceral fat (Juhan-Vague et al., 2000; Pannacciulli et al., 2002). The issue of whether adipose tissue contributes directly to plasma PAI-1, its products stimulating other cells to produce PAI-1, or whether it primarily contributes indirectly has not yet been resolved (Yudkin et al., 1999b). Obesity is also characterised by higher plasma concentrations of antithrombotic factors, such as tissue-type plasminogen activator (tPA) and protein C, than those found in non-obese subjects; the increase in these factors may represent a protective response to counteract the increase in prothrombotic factors.

Another molecule of interest is heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF). Produced locally by vascular macrophages and smooth muscle cells, this has been suggested to induce the migration and proliferation of vascular smooth muscle cells (Kayanoki et al., 1999). HB-EGF mRNA is abundantly expressed in human adipose tissue and increases in the fat tissues of obese mice (Matsumoto et al., 2002). Plasma HB-EGF levels also increase in parallel with fat accumulation in humans, and subjects with coronary artery disease have higher plasma HB-EGF levels. Thus fat accumulation may induce adipose tissue production of HB-EGF and contribute to the higher incidence of vascular disease in obesity (Matsumoto et al., 2002; Shah & Catt, 2003).

9.10 Nutritional regulation of adipocyte factors

Nutritional status, that is, feeding, fasting, weight loss and weight gain, regulate the production of
several of the adipocyte hormones. The following section outlines the evidence that is available for the effect of nutrition on these factors.

9.10.1 Nutritional regulation of leptin production
Fasting leptin levels generally reflect body fat mass, and therefore weight gain is associated with an increase, and weight loss a decrease, in systemic leptin levels. Insulin and glucose are the major mediators of leptin production by adipose tissue. Infusions of both glucose and insulin increase circulating leptin concentrations. Also fasting and feeding regulate circulating leptin concentrations independently of changes in body adiposity. However, in isolated adipocytes, insulin-stimulated glucose metabolism, rather than insulin, appears to mediate the effects of insulin on the leptin promoter activity (Lee et al., 2001; Moreno-Aliaga et al., 2001). Thus the effects of glucose and insulin on leptin may be secondary to those in response to changes in aerobic glucose metabolism (Havel, 2000).

Diets with identical energy contents but different dietary composition can also have different effects on leptin concentrations. Disproportionate decreases have been reported in response to fasting or restriction of energy intake to a much larger extent than would be expected for small reductions of adiposity (Agus et al., 2000). Thus dietary composition induces leptin-mediated metabolic adaptations to energy restriction. This may be a mechanism to ensure that increased energy intake is triggered well before body energy stores are compromised.

Leptin levels in serum fluctuate, with a nocturnal peak shortly after midnight and a mid-morning trough. This effect may be insulin-mediated, as starvation abolishes nocturnal rhythmicity and results in a fall in systemic leptin levels (Wagner et al., 2000).

There is some evidence that changes in dietary macronutrient intake modulate leptin concentrations and its diurnal pattern. High fat meals, which induce smaller insulin and glucose responses, suppress both the amplitude of the nocturnal peak and circulating leptin levels, with the largest differences observed 4–6 hours after each meal (Havel et al., 1999). This could explain, to some extent, the recognised effects of high fat diets to promote increased food intake, weight gain and obesity.

Fructose consumption induces insulin resistance, impaired glucose tolerance, hyperinsulinaemia, hypertriglyceridaemia and hypertension in animal models. However, the more limited studies in humans have produced conflicting results (Elliott et al., 2002) (see Chapter 2, Section 2.7). In animals, diets very high in fructose or sucrose, particularly in association with induction of hypertriglyceridaemia, also had a detrimental effect (Daly et al., 1997). Fructose, like fat, produces a smaller postprandial insulin response than glucose, and therefore fructose consumption also reduces circulating leptin concentrations (Mooradian et al., 2000). Thus diets that are high in dietary fructose could increase the likelihood of weight gain and its associated metabolic sequelae.

Dietary fatty acid composition is also an important determinant of circulating leptin level in diet-induced obesity (Peyron-Caso et al., 2002). Intake of polyunsaturates influences adipose tissue expression of leptin, and of several lipogenic enzymes and transcription factors. Specifically, feeding of fish oil containing n-3 polyunsaturates has been associated with a reduction in adipose hypertrophy, perhaps through a leptin-mediated process. Leptin stimulates triglyceride depletion in WAT without increasing free fatty acid release, favouring fatty acids versus glucose as a fuel source. Thus by elevating circulating leptin, polyunsaturates may prevent leptin resistance and weight gain (Hynes & Jones, 2001).

9.10.2 Regulation of acylation stimulating protein
Weight loss or fasting often correlates with a fall, and weight gain a rise, in levels of ASP and its precursors (Pomeroy et al., 1997; Koistinen et al., 2001). Body fat, insulin action, gender and glucose tolerance status are the main determinants of C3 levels (Weyer et al., 2000b). In studies looking at the effects of oral hypoglycaemic agents, both glibenclamide and pioglitazone decreased glucose and glycated haemoglobin (HbA1c), as well as C3, but there was an increase in BMI (Ebeling et al., 1999, 2001). As for leptin, there is the suggestion that obesity involves ASP resistance at the adipose tissue levels, which then results in increased hepatic fatty acid flux, stimulating increased very-low-density lipoprotein (VLDL) and LDL production (Cianflone et al., 2003). Evidence to support this comes from an in vitro study using cells from subjects with hyperapolipoprotein B, in whom plasma ASP levels were raised, and these cells showed reduced response to ASP compared with cells from subjects with normal circulating ASP (Zhang et al., 1998).
An oral fat load in humans does not change systemic circulating ASP; however, postprandial ASP release from subcutaneous adipose tissue can be measured in venous plasma, with peak release 4–5 hours after meals (Saleh et al., 1998). Insulin and chylomicrons potently increase ASP secretion in isolated adipocytes (Maslowska et al., 1997), suggesting that changes in ASP production in response to fasting and food ingestion could be mediated by insulin. Retinoic acid also increases C3 and ASP production from adipocytes in vitro (Scantlebury et al., 2001). However, additional work is needed to understand the nutritional regulation of ASP production and its underlying mechanisms.

9.10.3 Nutritional regulation of cytokines

While very low energy diets that result in weight loss and decreased body fat mass, with improved insulin sensitivity, are not always associated with significant changes in serum or adipose tissue TNFα levels, they do induce significant decreases in IL-6 levels in both adipose tissue and serum (Bastard et al., 2000). Gallistl et al. (2001) found that in obese children a three-week programme of energy restriction (3.8–5.0 MJ daily; approximately half the estimated average requirement) combined with physical activity was associated with a significant decrease in body weight, blood pressure and heart rate, as well as in systemic levels of IL-6 and leptin. Thus, restriction of energy intake and increase in physical activity were associated with more favourable serum concentrations of IL-6 (Gallistl et al., 2001).

Beneficial effect of n-3 fatty acids in cardiovascular disease has been shown, but the mechanism is unclear (Das, 2000). n-3 fatty acids inhibit synthesis of cytokines (TNFα, IL-1 and IL-2) which are released during the early stages of CHD, and may also improve leptin secretion (see Section 9.10.1). Selective nutritional intervention based on specific fatty acids, such as n-3 and n-6 lipids and vitamin E, on the secretion of other serum cytokines, such as IL-6, have been reported (Venkatraman & Chu, 1999); however, whether this secretion is from adipose tissue has not been investigated.

In summary, circulating IL-6 concentrations reflect, at least in part, adipose tissue production. The reduced production and serum concentrations of this cytokine after weight loss may play a role in the improved cardiovascular risk profile. Evidence for TNFα is less clear, perhaps reflecting its mainly autocrine/paracrine mode of action.

9.10.4 Regulation of adiponectin

In marked contrast to the increased levels of many other adipocyte-derived hormones, circulating adiponectin concentrations are reduced in obesity (Arita et al., 1999; Funahashi et al., 1999). Weight loss increases circulating adiponectin concentrations in non-diabetic and diabetic humans (Yang et al., 2001). Similar to leptin, circulating adiponectin concentrations are higher in women than in men, and there is a significant gender difference in the adiposity-independent response of circulating adiponectin during acute energy restriction (Havel, 2002).

Although there is little information available on the mechanisms regulating adiponectin production, several studies have reported that PPARγ agonists, such as thiazolidinediones, increase both adiponectin gene expression and circulating adiponectin levels in humans (Hirose et al., 2002). Adiponectin expression is increased with markers of adipocyte differentiation and its secretion is enhanced by the calcium ionophore, ionomycin, and inhibited by cyclic adenosine-monophosphate (cAMP) analogues. Beta-adrenergic agonists, activators of adenylate cyclase, TNFα and glucocorticoids are also reported to inhibit adiponectin gene expression and secretion (Havel, 2002). Interestingly, subjects with severe insulin resistant diabetes due to dominant negative mutations that inactivate PPARγ are not obese, but have very low circulating adiponectin levels (Combs et al., 2002). There was an inverse correlation between adiponectin and leptin in vivo in women (Matsubara et al., 2002).

Additional work is needed to understand the physiological and nutritional mechanisms involved in regulating the production of this adipocyte hormone, depot-specific differences in its production, its paradoxical reduction in obesity and its potentially protective effect in cardiovascular disease.

9.10.5 Regulation of angiotensinogen

The level of angiotensinogen expression and its plasma levels are increased in obesity. Fasting reduces angiotensinogen release from adipocytes and re-feeding increases mRNA levels above fed levels (Frederich et al., 1992). These effects of fasting and re-feeding on
angiotensinogen regulation have been adipose tissue specific, with liver angiotensinogen mRNA and serum angiotensinogen concentrations being unaffected. Furthermore, systolic blood pressure was modulated in a manner parallel to adipocyte angiotensinogen expression (Frederich et al., 1992). Its mRNA was increased in adipose tissue of obese mice (ob/ob and db/db). In cultured adipocytes, angiotensinogen mRNA was upregulated by insulin and downregulated by beta-adrenergic agonists (Jones et al., 1997). This may explain the hypotensive effects of energy restriction and the hypertension associated with obesity. Angiotensinogen in adipocytes mainly regulates the regional blood flow to adipose tissue rather than lipolytic activity and substrate oxidation (Townsend, 2001). Thus by affecting both substrate availability indirectly and pre-adipocyte differentiation, angiotensinogen could potentially regulate adipose size in response to nutritional signals.

9.10.6 Adipogenic transcription factors and diet

Changes of adiposity in response to vitamin A status have been shown to correlate with changes of PPARγ2 expression. Vitamin A-deficient diets cause a marked increase in fat mass, and to a smaller degree in body weight (Ribot et al., 2001). Hypertrophy of WAT depots has been correlated with enhanced PPARγ2 expression. In contrast, hypertrophy of BAT has correlated with a decrease of PPARγ2 expression that may contribute to the reduced thermogenic potential of BAT under conditions of vitamin A restriction (Ribot et al., 2001). Treatment with trans-retinoic acid (tRA) has been shown to trigger a reduction of adiposity and body weight that correlates with a down-regulation of PPARγ2 expression in all adipose tissues. The effects of tRA were more pronounced in epididymal WAT, where C/EBPα and ADD1/SREBP1c (adipocyte determination and differentiation-dependent factor-1/sterol regulatory element binding protein-1) levels were also reduced (Ribot et al., 2001). The response to tRA was impaired in the epididymal WAT and BAT of animals fed the vitamin A-deficient diet. The results emphasise the importance of retinoids as physiological regulators of adipose tissue development and function in intact animals. The transcription factors PPARγ and SREBP-1 mRNAs were reduced after incubation with n-3 polyunsaturates, whereas the expression of C/EBPα was unchanged (Hontecillas et al., 2002).

9.11 Physical activity and adipokines

Physical activity has effects on energy balance, sympathoadrenal drive, and hormonal and metabolic homeostasis. A low level of physical activity contributes to low total energy expenditure and promotes weight gain (Blair et al., 1989); evidence from studies in twins and from epidemiological data suggests that it is the strongest environmental determinant of body and adipose tissue mass (Young & Steinhardt, 1995). Several of the adipokines are influenced by both the amount and type of physical activity.

Several studies have reported effects of exercise on leptin (Kraemer et al., 2002). Acute short-duration exercise, regardless of intensity, gender or body weight, does not appear to significantly alter leptin levels, despite changes in regulating hormones or metabolites such as insulin, glucose and catecholamines (Weltman et al., 2000; Fisher et al., 2001). Results of studies of acute but longer-duration exercise are variable, mostly depending on the length of sampling after cessation of exercise. Sixty minutes of cycle ergometry or treadmill exercise did not change leptin production from subcutaneous adipose tissue or its systemic levels for up to 4 hours of recovery (Torjman et al., 1999; Racette et al., 2001). However, when monitoring was prolonged for more than 48 hours after exercise, leptin levels were shown to decline (Essig et al., 2000). Large changes in energy expenditure, such as seen in marathon running or glycogen-depleting exercise, have also been shown to produce significant reductions in circulating leptin levels (Tuominen et al., 1997; Leal-Cerro et al., 1998). This reduction in leptin is overcome by feeding. Both exercise training and weight training produce reductions in leptin levels, independent of changes in body composition, insulin or glucocorticoids, but only in patients with type 2 diabetes and not in normal subjects (Halle et al., 1999b; Ishii et al., 2001; Kanaley et al., 2001).

Very few reports are available of the effects of exercise on ASP or on adiponectin. ASP increases acutely during a timed run and returns to baseline shortly afterwards (Smith et al., 1990). Also, in a large population-based study there was an inverse relationship between C3 and physical activity (Muscari et al., 2000). In subjects undergoing weight training for six months, insulin action improved significantly without any change in body mass or fat mass. In these subjects, plasma adiponectin did not
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change significantly. However, in subjects in a weight loss programme there was a significant increase in adiponectin after weight loss accompanied by enhanced insulin action. Thus adiponectin does not appear to contribute to the exercise-related improvements in insulin sensitivity (Hulver et al., 2002).

IL-6 expression increases acutely after exercise in both skeletal muscle and adipose tissue (Febbraio & Pedersen, 2002). This increase in the expression of IL-6 is significantly blunted by the ingestion of carbohydrate (Keller et al., 2003).

In a study of spontaneously hypertensive rats fed a fructose-rich diet during 16 weeks of exercise training, animals that received angiotensin-converting enzyme inhibitors and were exercised showed elevated TNFα expression in the epidydymal fat pad, but this was negatively correlated with fat pad weight. Leptin, conversely, was positively correlated with fat pad weight. So, TNFα expression is tissue-specific and its up-regulation by exercise may have contributed to reduction in fat cell volume via induction of apoptosis or increased lipolysis (Kawamura et al., 2002).

9.12 Genetic determinants of adipokines

In several adipocyte genes, mutations and polymorphisms have been reported which may modify human obesity and cardiovascular risk.

9.12.1 Leptin gene mutations

Mutations in the leptin gene and its receptor have been reported, and are associated with obesity and hyperphagia in both mice and humans (O’Rahilly, 2002). In these subjects, leptin administration induces weight loss, predominantly as body fat, and decreases hyperphagia. Heterozygous relatives of patients with absolute leptin deficiency have a high incidence of obesity, relatively low leptin levels for their BMI, and a greater percentage body fat than would be predicted by their BMI compared with matched controls or their homozygous wild-type relatives (O’Rahilly, 2002). However, a large proportion of obesity cannot be explained by these mutations. Polymorphisms within the leptin gene have been reported with association to obesity and cardiovascular risk factors. A tetranucleotide repeat polymorphism in the 3′-flanking region of the leptin gene is associated with hypertension independent of obesity and insulin resistance in Japanese subjects (Shintani et al., 2002).

9.12.2 C3 polymorphisms

C3 polymorphisms have been reported in humans, termed C3F and C3S, depending on fast or slow electrophoretic mobility (Welch et al., 1990). This polymorphism is a cytosine-to-guanine shift at 364 bp on exon 3, and leads to an arginine instead of glycine at position 102 (Botto et al., 1990). The C3S variant was more common than C3F, especially amongst South Asians and African Americans. While there was little evidence to suggest that the polymorphisms are associated with altered circulating levels of C3 or ASP, there are data showing increased association of C3F with atherosclerosis, hypertension, hypercholesterolaemia and partial lipodystrophy (Kristensen, 1978; Botto et al., 1990).

9.12.3 Adiponectin gene

A genome-wide scan examining the loci influencing six traits associated with obesity and insulin resistance reported a quantitative trait locus on chromosome 3q27, where the adiponectin gene is located (Comuzzie et al., 2001). In another study two single nucleotide polymorphisms (SNPs: 45T →G and 276G →T) in the adiponectin gene were examined. Homozygotes for the haplotype defined by the combined polymorphisms had significantly lower plasma levels of adiponectin. These subjects were more centrally obese, and had higher levels of blood pressure, plasma glucose and insulin, as well as high total and low high-density lipoprotein (HDL) cholesterol (Menzaghi et al., 2002).

9.12.4 IL-6 genotype

Several functional variants in the promoter region of the IL-6 gene have been reported with the most common being −174G →C (Fishman et al., 1998). In vitro studies have shown that in HeLa cells the −174G construct gave similar expression as the −174C allele in unstimulated cells, but upon stimulation the increase in promoter activity was greater for the G than the C construct (Terry et al., 2000). Although demonstrating functionality, extrapolation of this effect to the in vivo situation is difficult. Several studies have now shown conflicting results regarding the association of
this variant with plasma levels of IL-6. The initial study suggested that the −174C allele was associated with lower mean plasma levels of IL-6 and was significantly more common in patients with peripheral vascular disease (Flex et al., 2002). However, in other studies the −174C allele was associated with higher IL-6 levels in subjects with aneurysmal disease, in patients undergoing coronary artery bypass grafting, in newborns but not in adults, and was more common amongst smokers (Brull et al., 2001; Jenny et al., 2002). These data imply that there is a genetically determined difference in the degree of the IL-6 response to a stressful or inflammatory situation that is influenced by this promoter variant. However, whether this or the other described IL-6 polymorphisms alter adipose tissue IL-6 release or function is not known.

9.12.5 Angiotensin gene polymorphism (AGT)

The AGT M235T polymorphism has been shown to be associated with body fatness in women, but not in men. Homozygotes for the T allele had higher body fat mass than the MM homozygotes or the heterozygotes. In the more obese women, the carriers of the T allele showed a higher resting diastolic and systolic blood pressure, and diastolic blood pressure following exercise than non-carriers, whereas no such difference was found in the lean women (Rankinen et al., 1999).

9.12.6 PPARγ polymorphisms

In humans, a relatively common Pro12Ala substitution in PPARγ-specific exon B, resulting in a PPARγ2 form with reduced transcriptional activity in vitro, has been described (Yen et al., 1997; Deeb et al., 1998). In vivo, the Pro12Ala substitution has been associated in some, but not all, studies with lower BMI and improved insulin sensitivity (Beamer et al., 1998; Deeb et al., 1998; Mori et al., 1998; Ek et al., 1999; Koch et al., 1999; Mancini et al., 1999; Ringel et al., 1999; Valve et al., 1999; Jacob et al., 2000; Vaccaro et al., 2000).

An association of this polymorphism with reduced risk of diabetes has also been demonstrated (Altshuler et al., 2000). In carriers of the Ala allele, as compared to non-carriers, a more efficient suppression of lipolysis in adipose tissue and lower circulating NEFA concentrations have been observed during a hyperinsulinaemic-euglycaemic clamp (Stumvoll et al., 2001). However, a recent study did not show any relationship between the Pro12Ala polymorphism and fasting NEFA concentrations in the general population (Vaccaro et al., 2002).

In summary, there is evidence that suggests that alterations in the adipocyte genes are associated with modification of cardiovascular and obesity risk. Furthermore, a recent report of the analysis of gene–diet interactions suggests that the allelic variants of adipose genes (leptin, TNFα, PPARγ2) might strongly affect diet-related obesity and cardiovascular risk (Nieters et al., 2002). Thus dietary habits would also be of relevance when investigating obesity genes (Luan et al., 2001).

9.13 Conclusion

Obesity is associated with cardiac and vascular disease through well-known mediators such as hypertension, type 2 diabetes and dyslipidaemia, as well as the less well-characterised mediators such as chronic inflammation and hypercoagulation (see Chapter 2, Section 2.4). However, the molecular basis for these associations is less clearly defined. Prolonged periods of excess energy intake lead to increased adipose tissue mass in man. Although there is a wealth of information from animal studies on the regulation of energy homeostasis and related unfavourable factors, knowledge of the regulation of energy balance in human subjects is still insufficient.

It is apparent that a large number of proteins produced by adipose tissue, both intracellular and secreted, function along with central and other peripheral tissues, in the co-ordination of energy homeostasis and fuel metabolism. In obesity, peripheral tissue resistant to leptin, combined with adiponectin deficiency may lead to decreased fat oxidation in skeletal muscle, macrophages and liver, and increased fat deposition within these tissues. Obesity associated increase in ASP increases fat storage through increased triglyceride synthesis and decreased intracellular lipolysis. There is also increased expression of TNFα in fat and muscle, which directly causes insulin resistance. Increased visceral expression of IL-6 increases secretion of hepatic acute phase proteins such as CRP and fibrinogen. There is evidence for the synthesis and release of these factors being involved in endothelial dysfunction, as well as altering the activities of the sympathetic nervous system and hypothalamo–pituitary–adrenal axis, thus
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9.14 Key points

- The adipose tissue secretes a diverse number of factors (adipokines) that include enzymes, growth factors, cytokines and several other hormones involved in fatty acid and glucose metabolism.
- These adipokines are implicated in chronic inflammation, insulin resistance, dyslipidaemia, hypertension and endothelial dysfunction, perhaps contributing directly to increased cardiovascular risk.
- Weight loss or fasting often correlates with a fall, and weight gain and excess energy intake a rise, in levels of several of the adipokines (leptin, IL-6, ASP), reflecting changes in adipose tissue mass.
- However, adiponectin is unique as weight loss is associated with an increase, and obesity with a reduction, in circulating levels.
- In humans, detailed macronutrient and micronutrient effects on adipokines are sparse.
- Several of the adipokines also appear to be influenced by both acute and longer duration exercise. However, these data are mainly based on animal studies.

9.15 Recommendations for future research

- Interactions between genotypic variations of these molecules and nutritional regulation remain to be elucidated.
- The effect of exercise on adipokine expression and secretion needs to be determined.

9.16 Key references


10 Fetal and Maternal Nutrition

10.1 Low birthweight and adult cardiovascular disease

The concept that longevity is determined by events in early life is not new. In 1934, Kermack et al. showed that death rates from all causes in the UK and Sweden fell between 1751 and 1930 with each successive year-of-birth cohort. They rejected one possible explanation, that ‘a more healthy race of children was born in each successive decade’, and concluded that it was the result of better childhood living conditions brought about by social reforms. In 1977, Forsdahl discovered a geographical correlation within Norway between coronary heart disease (CHD) mortality in 1964–67 and infant mortality rates 70 years earlier (1896–1925); he suggested that growing up in poverty caused ‘permanent damage’ perhaps due to a ‘nutritional deficit’, which resulted in ‘life-long vulnerability’ to an affluent adult lifestyle and high fat intakes (Forsdahl, 1977). Studies in the UK a decade later shifted the focus back to pre-natal rather than childhood events. Blood pressure was found to be inversely related to birthweight in young men and women in the 1946 national birth cohort (Wadsworth et al., 1985). Differences between UK local authority areas in neonatal mortality (a marker for low birthweight) in 1921–25 predicted variation in death rates from stroke and CHD in 1968–78 (Barker & Osmond, 1986). Barker went on to propose that the roots of cardiovascular disease lay in undernutrition during fetal life and infancy, and in the effects of poverty on mothers.

The discovery in the UK county of Hertfordshire, of ledgers recording the birthweight of men and women born during 1911–30 made it possible to show that lower birthweight and weight at one year were associated with an increased risk of death in adult life from CHD and stroke (Barker et al., 1989; Osmond et al., 1993). There was an approximate doubling of cardiovascular disease mortality from those weighing less than 5.5 lb (2.5 kg) at birth to those weighing 9.0–9.5 lb (4.1–4.3 kg) (Fig. 10.1). Since then, epidemiological studies in the UK, Europe, USA and India have confirmed a link between low birthweight and adult cardiovascular disease (Barker et al., 1993c; Osmond et al., 1993; Frankel et al., 1996a; Martyn et al., 1996; Stein et al., 1996; Forsen et al., 1997; Rich-Edwards et al., 1997; Leon et al., 1998; Eriksson et al., 1999, 2000, 2001; Forsen et al., 1999).

The effects are linear and graded across the whole range of birthweight, with no evidence of threshold effects (Fig. 10.1) and are independent of adult socioeconomic status (Frankel et al., 1996b; Rich-Edwards et al., 1997; Leon et al., 1998). Studies using birth records with gestational age data indicate that it is restricted fetal growth rather than pre-term delivery, which carries the risk of cardiovascular disease (Barker et al., 1993c; Leon et al., 1998), although the risk for premature babies has been inadequately studied (Irving et al., 2000; Whincup & Cook, 2000). The majority of studies are limited to birthweight as a measure of fetal growth, but there is some evidence that body proportions at birth show stronger associations with cardiovascular disease. For example, low ponderal index at birth (weight/length3) predicted CHD better than birthweight alone in Finland (Forsen et al., 1997; Eriksson et al., 1999), and a low birthweight/head circumference ratio predicted stroke mortality in Sheffield, UK (Martyn et al., 1996).
10.2 Size at birth and cardiovascular disease risk factors

Subsequent work has shown that lower birthweight and other measures of small size at birth are also associated with higher levels of several cardiovascular disease risk factors.

10.2.1 Insulin resistance syndrome

Blood pressure, type 2 diabetes, insulin resistance, and combinations of these (the insulin resistance syndrome (IRS); see Chapter 2, Section 2.3) are consistently related to low birthweight in a large number of studies in different populations (Hales et al., 1991; Barker et al., 1993a; Valdez et al., 1994; Lithell, 1996; Yarborough et al., 1998; Rich-Edwards et al., 1999; Byberg et al., 2000; Eriksson et al., 2002b; Levitt et al., 2000; Mi et al., 2000). Studies relating to blood pressure, diabetes and insulin resistance have been summarised in systematic reviews (Huxley et al., 2000; Newsome et al., 2003); there are similar associations in children (Huxley et al., 2000; Whincup et al., 2002; Newsome et al., 2003). The associations are stronger for systolic than diastolic blood pressure, and (in adults) stronger for disease outcomes, either hypertension (Fig. 10.2) or cardiovascular disease, than for blood pressure measurements. This could be because of anti-hypertensive treatment effects on blood pressure (which also differ according to birthweight) (Lackland et al., 2002), or because the birthweight effect is small until a threshold is passed, after which it is amplified. Associations with type 2 diabetes appear to be mediated mainly through insulin resistance (Phillips, 1996), rather than reduced insulin secretion (Phillips et al., 1994), although several studies have shown raised insulin precursors, suggesting disordered insulin secretion (Hales et al., 1991; Byberg et al., 2000).

10.2.2 Lipids and clotting factors

Although plasma lipids and clotting factors show some associations with size at birth, these are weaker and much less consistent. A systematic review by Owen et al. (2003) concluded that total cholesterol shows only a weak inverse association with birthweight. High density lipoprotein (HDL) cholesterol concentrations are positively, and triglyceride concentrations inversely, related to birthweight in some (Fall et al., 1995b; Bavdekar et al., 2000) but not most studies (Fall et al., 1992; Frankel et al., 1996a; Lithell, 1996; Byberg et al., 2000; Eriksson et al., 2002b; Levitt et al., 2000). Postprandial lipid concentrations were unrelated to birthweight in Hertfordshire (Byrne et al., 1997) as were lipoprotein(a) (Lp(a))

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**Fig. 10.1** Standardised mortality ratios for cardiovascular disease in men and women below the age of 65 years born in Hertfordshire, UK, according to birthweight. 2.2 pounds (lb) = 1 kg. Reproduced from Osmond et al. (1993) with permission from BMJ Publishing Group.
concentrations. Fibrinogen and factor VII concentrations are not related to birthweight (Barker et al., 1992; Martyn et al., 1995b). However, total- and LDL-cholesterol, apolipoprotein B (apoB) and fibrinogen concentrations were associated with smaller abdominal circumference at birth, recorded in obstetric records in Sheffield (Barker et al., 1993b; Martyn et al., 1995b). Plasminogen activator inhibitor-1 (PAI-1) was increased in low birthweight men in the one published study which has measured it (Byberg et al., 2000; for further information about PAI-1 see Chapter 6).

10.2.3 Measurements of cardiovascular structure and function

Martyn et al. (1998) showed that arterial intima-media thickness and the risk of carotid stenosis, examined using ultrasound, were increased in lower birthweight men and women. The intima–media thickness findings were not confirmed in a later study (Gale et al., 2002). In a follow-up of the Newcastle 1000 Families Study, carotid intima–media thickness was increased in lower birthweight men and in women of lower socioeconomic class at birth (Lamont et al., 2000). In the only study to examine peripheral vascular disease (PVD), ankle brachial pulse index was not significantly related to birthweight (Martyn et al., 1998). Pulse wave velocity, a measure of poor arterial compliance, was increased in old age in UK men and women with small head and abdominal circumferences at birth (Martyn et al., 1995a), but showed no association with size at birth in another UK study of young adults (Montgomery et al., 2000) or in a study in India (Kumaran et al., 2000). Left ventricular mass was unrelated to birthweight in three studies (Vijayakumar et al., 1995; Zureik et al., 1996; Kumaran et al., 2000). Flow-mediated dilatation in large arteries, a measure of endothelial function (see Chapter 4, Section 4.2), was reduced in young adults (Leeson et al., 1997) and children (Goodfellow et al., 1998) of lower birthweight. Studies in infants, children and young adults of lower birthweight have also shown evidence of impaired microvascular function (Serne et al., 2000).

10.2.4 Obesity

Fetal growth restriction has been shown in experimental animals to result in increased adult fat mass (Vickers et al., 2000). There is no evidence that lower birthweight is associated with higher total body fat in adult life in normal human populations. If anything, people of lower birthweight tend to become ‘thinner’ adults as measured by body mass index (BMI) (Sorensen et al., 1997; Byberg et al., 2000; Table 10.1; Fig. 10.3). However, this may reflect reduced lean mass rather than reduced adiposity (Phillips, 1995;
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Table 10.1 Birthweight and adult obesity: Uppsala, Sweden (men, n = 1268).

<table>
<thead>
<tr>
<th>Birthweight (kg)</th>
<th>&lt;3.25</th>
<th>−3.75</th>
<th>−4.25</th>
<th>≥4.25</th>
<th>p</th>
<th>p*</th>
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<tr>
<td>50 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triceps skinfold (mm) (TR)</td>
<td>10.3</td>
<td>10.9</td>
<td>11.2</td>
<td>11.3</td>
<td>0.02</td>
<td>0.2</td>
</tr>
<tr>
<td>Subscapular skinfold (mm) (SS)</td>
<td>17.4</td>
<td>16.6</td>
<td>16.7</td>
<td>16.6</td>
<td>0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>SS/TR</td>
<td>1.78</td>
<td>1.60</td>
<td>1.57</td>
<td>1.54</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>70 years</td>
<td></td>
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<tr>
<td>BMI (kg/m²)</td>
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<td>26.4</td>
<td>26.6</td>
<td>26.8</td>
<td>0.007</td>
<td>–</td>
</tr>
<tr>
<td>Waist (cm)</td>
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<td>94.9</td>
<td>95.5</td>
<td>96.2</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>Hip (cm)</td>
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<td>100.4</td>
<td>101.4</td>
<td>101.7</td>
<td>&lt;0.001</td>
<td>0.02</td>
</tr>
<tr>
<td>Waist/hip</td>
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<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>0.9</td>
<td>0.03</td>
</tr>
</tbody>
</table>

p values adjusted for age; p* for age and body mass index (BMI).
TR: triceps; SS: subscapular; SS/TR: subscapular/triceps ratio.
Source: Adapted from Byberg et al. (2000) with permission from Springer-Verlag.

Kahn et al., 2000; Weyer et al., 2000b; Gale et al., 2001. In the only study to use specific methods to measure body composition (dual energy absorptiometry; DEXA), lower birthweight was associated with a markedly lower lean body mass at age 70 years, but was only weakly related to fat mass (Gale et al., 2001, Fig. 10.4). Using anthropometric measurements in young men, Kahn et al. (2000) showed that the birthweight–BMI association was attenuated by 68% after adjustment for thigh muscle + bone area, but only by 30% after adjustment for subcutaneous fat area. These findings suggest that low birthweight may be associated with an increased adult fat/lean mass ratio, but this requires further study. Turning to functional measures: leptin concentrations were increased in low birthweight men and women in one study (Phillips et al., 1999; for more information about leptin, see Chapter 9). Two studies have reported increased metabolic rate in low birthweight adults (Weyer et al., 2000b; Eriksson et al., 2002a).

There is some evidence that small size at birth is associated with later central obesity (indicated by increased waist circumference, waist/hip ratio and subscapular/triceps skinfold ratio). The subscapular/triceps ratio is consistently higher in adults and children of lower birthweight (Table 10.1; Malina et al., 1996; Bavdekar et al., 2000; Byberg et al., 2000; Okosun et al., 2000). Although waist circumference and waist/hip ratio are inversely related to birthweight in some studies (Law et al., 1992; Han et al., 1995; Barker et al., 1997; Serne et al., 2000) this is weaker and less consistent (Valdez et al., 1994). Where present, the association may reflect larger hip circumference (and, therefore, frame size and muscle mass) in higher birthweight individuals, rather than abdominal obesity in people of low birthweight (Table 10.1).

In summary, high blood pressure, glucose intolerance and insulin resistance, all features of the insulin resistance syndrome (see Chapter 2, Section 2.3.2),
are consistently associated with low birthweight or other measures of reduced fetal growth. The same is true for other features of the insulin resistance syndrome; for example, truncal obesity, reduced endothelial function, and PAI-1 concentrations, though these were measured in fewer studies. However, low birthweight does not consistently predict abdominal obesity or the lipid abnormalities found in the insulin resistance syndrome (hypertriglyceridaemia and low HDL-cholesterol concentrations). Cholesterol and fibrinogen concentrations are not related to low birthweight, but may be related to low abdominal circumference at birth.

10.3 Post-natal growth and adult obesity

Cardiovascular disease and its risk factors also show associations with patterns of growth in infancy and childhood. In Hertfordshire, men with lower weight at the age of 1 year, and lower weight gain between birth and one year, had higher cardiovascular disease mortality (Barker et al., 1989), left ventricular mass (Vijayakumar et al., 1995), serum fibrinogen and cholesterol concentrations (Barker et al., 1992; Fall et al., 1992), and a higher prevalence of type 2 diabetes (Hales et al., 1991). These effects were strong in men, and notably absent in women (Fall et al., 1995b). There are few adult cohorts with infant data, but inverse associations between weight at 1 year and both cardiovascular disease mortality and type 2 diabetes have been confirmed in men born in Finland (Eriksson et al., 2001).

In contrast, childhood and adolescent weight or BMI gain are associated with an increased risk of adult disease. Upward crossing of centiles, or an increase in standard deviation (SD) score, for weight or BMI during childhood was associated with higher blood pressure in young adults in the UK (Law et al., 2002), an increased risk of CHD and type 2 diabetes in Finnish men and women (Eriksson et al., 1999, 2001; Forsen et al., 1999, 2000; Fig. 10.5), and an increased risk of impaired glucose tolerance and type 2 diabetes in young Indian adults (Bhargava et al., 2004). Weight gain in childhood may interact with size at birth; in Finland, an increase in BMI from birth to 7 years was associated with an increased risk of adult CHD only in those who were small or thin at birth (Fig. 10.6).

An early adiposity rebound, the point in childhood at which BMI starts to rise having fallen since infancy, is known to be associated with an increased risk of later obesity (Rolland-Cachera et al., 1984). The Finnish data confirmed this (Fig. 10.7) and also showed that an early adiposity rebound is a risk factor for adult diabetes. The cumulative incidence of type 2 diabetes was 8.6% in men and women whose adiposity rebound occurred before the age of 5 years compared with 1.8% in those with an adiposity rebound after 7 years (Eriksson et al., 2003). What determines the age of adiposity rebound is unknown, but in Finland lower weight at one year predicted an
earlier rebound (Eriksson et al., 2003) (Fig. 10.7). Similar findings have been shown in a birth cohort of Indian men and women with detailed measurements of growth throughout childhood (Bhargava et al., 2004).

The apparent protective effect of high infant weight in Hertfordshire and Finland, suggests that infant weight gain should be protected, and that ‘catch-up’ growth should even be promoted in low birthweight babies. However, this is uncertain. A recent follow-up study of (mainly) pre-term babies showed higher concentrations of 32–33 split pro-insulin (evidence of insulin resistance) in children who received a high-nutrient formula during infancy and had a high weight gain in the first post-natal month (Singhal et al., 2003). The authors proposed that compensatory weight gain in early life may be harmful in terms of risk for adult chronic disease (see Section 10.6). More data are needed, taking into account both short- and long-term outcomes, before firm recommendations can be made about optimal weight gain in infancy. Weight and BMI gain during later childhood and adolescence are, however, clearly associated with a greater risk of adult disease.
It is upward crossing of centiles, or an increasing SD score, rather than absolute weight or BMI at any point, which is crucial, and indeed most children on this high-risk upward trajectory are not obese as children (Fig. 10.5). To identify them would require serial (longitudinal) measurements during childhood, and suitable reference standards against which to compare them (see Chapter 2, Section 2.4.10).

Adult obesity is clearly a risk factor for cardiovascular disease (see Chapter 2, Section 2.4.4). Its relevance to this chapter is that it adds to, and in some studies interacts with, the effects of low birthweight. The most adverse cardiovascular disease risk profile is consistently found in men and women who were small at birth but became obese adults (Fig. 10.8). The effects of adult BMI on CHD, high blood pressure, type 2 diabetes, and insulin resistance are greater in individuals of low birthweight (Hales et al., 1991; Frankel et al., 1996b; Bavdekar et al., 2000). Similar additive and/or interactive effects have been described between size at birth and other aspects of adult lifestyle; for example, between ponderal index at birth and adult socioeconomic status on CHD (Barker et al., 2001), and between weight in infancy and the effects of smoking on fibrinogen concentrations (Barker et al., 1992).

The role of height growth is not yet clear. Short adult stature is a well-known risk factor for cardiovascular disease (Paffenbarger et al., 1966; Marmot et al., 1978; Waaler, 1984; Notkola et al., 1985; Walker et al., 1989; Barker et al., 1990; Palmer et al., 1990; Yarnell et al., 1992; Hebert et al., 1993; Krahn et al., 1994; Leon et al., 1995; Rich-Edwards et al., 1995). However, increased height growth in childhood was associated with later CHD in women (Forsen et al., 1999), and with high blood pressure, diabetes and insulin resistance in adults and children of both sexes (Leon et al., 1996; Forsen et al., 1999, 2000; Bavdekar et al., 2000; Eriksson et al., 2002b). This apparent discrepancy may indicate that an advanced tempo of growth, which would produce tall height in childhood but early maturation and a reduced adult height, is associated with later risk (Bavdekar et al., 2000), though this has not been established. Effects may vary between populations; a study in Jamaica showed that stunted children had higher blood pressures (Gaskin et al., 2000).

**10.4 Variation with sex**

In general, relationships between small size at birth and adult cardiovascular disease and risk factors (especially the insulin resistance syndrome variables) are similar in both sexes. However, in Finland, CHD was most strongly associated with low ponderal index at birth in men and with short birth length in women (Forsen et al., 1997, 1999). The strong associations of cardiovascular disease mortality with low weight at 1 year in the Hertfordshire men were not seen in women (Osmond et al., 1993; Fall et al., 1995c). Similarly, apoB, fibrinogen and factor VII concentrations, which were inversely related to small abdominal circumference at birth (Sheffield) and low weight at 1 year (Hertfordshire) in men, showed no associations with either size at birth or infant weight in women (Fall et al., 1995c).

**10.5 Variation with ethnicity**

There are few data linking size at birth with cardiovascular disease and its risk factors among ethnic minorities living in the UK, many of which have lower birthweights than the white Caucasian population. Whincup et al. (2002) showed higher insulin
resistance in British South Asian children of lower birthweight. Studies from India, China, Japan, Jamaica and among US Hispanics and black South Africans have shown associations between smaller size at birth and higher blood pressure, glucose intolerance and insulin resistance (Valdez et al., 1994; Stein et al., 1996; Bavdekar et al., 2000; Levitt et al., 2000; Mi et al., 2000; Suzuki et al., 2000; Miura et al., 2001). Higher subscapular/triceps ratios have been shown in Indian children (Bavdekar et al., 2000) and in white, black and Hispanic US children of lower birthweight (Oksun et al., 2000).

There is evidence of variation between populations, however, which may be attributed to ethnic differences. Type 2 diabetes was associated with low birthweight in young Indian adults (Fall, 2001) but with a high ponderal index at birth in older men and women (Fall et al., 1998). A study of blood pressure in children in seven countries showed differences in associations with birth measurements (Law et al., 2000). In China and Central and South America, higher blood pressure was associated with ‘proportionate’ smallness at birth (reductions in birthweight, length, and head and chest circumferences), while in Sweden it was associated with ‘asymmetrical’ smallness at birth (low ponderal index). In Nigeria, blood pressure was not related to size at birth. In Jamaica, higher insulin resistance was associated with shorter length at birth rather than lower birthweight (Forrester et al., 1996; Bennett et al., 2002).

It is difficult to interpret these findings, and to determine whether they represent consistent ethnic differences. Asymmetrical smallness at birth is classically attributed to undernutrition in late pregnancy when skeletal growth is more or less complete and head growth is actively protected, but soft tissues (especially muscle and abdominal viscera) are ‘sacrificed’. Birthweight is reduced more than birth length, resulting in ‘thinness’ (i.e. a low ponderal index). Symmetrical smallness on the other hand, is attributed to impaired growth from early pregnancy, resulting in low birthweight, small head, short length and a normal ponderal index. There are ethnic differences in size, shape and body composition at birth. For example, studies of South Asian babies have shown a marked reduction in mid-arm circumference and abdominal girth, but relative fat-sparing compared with white Caucasian babies (Yajnik et al., 2002, 2003). The ponderal index at birth correlates less strongly with direct measures of body fat in Indian neonates compared with UK white Caucasian newborns (Leary et al., 2003). More data are required on fetal growth patterns in different ethnic groups, and their associations with later disease.

10.6 The ‘Fetal Origins of Adult Disease (FOAD)’ hypothesis

Barker (1998) has proposed that the associations between small size at birth and cardiovascular disease reflect permanent effects of fetal undernutrition (Fig. 10.9). The fetus is dependent on the transfer of nutrients from the mother and adapts to an inadequate nutrient supply in a number of ways: prioritisation of brain growth at the expense of other tissues, such as the abdominal viscera; reduced secretion of, and sensitivity to, the fetal growth hormones insulin and insulin-like growth factor-1 (IGF-I); and up-regulation of the hypothalamo–pituitary–adrenal (HPA) axis. The FOAD hypothesis proposes that, although occurring in response to a transient phenomenon (fetal undernutrition), these changes become permanent or ‘programmed’ because they occur during critical periods of early development.

The mechanisms by which this could occur at a cellular and tissue level have been reviewed (Lucas, 1991; Waterland & Garza, 1999). Programmed changes may include reduced insulin sensitivity (Phillips, 1996), low muscle mass (Phillips, 1995), reduced pancreatic beta cell mass (Hales & Barker, 1992) and nephron numbers (Mackenzie & Brenner, 1995), altered liver (Hales et al., 1996) and arterial structure (Martyn & Greenwald, 1997), increased left ventricular mass (Vijayakumar et al., 1995), and up-regulation of the HPA axis (Phillips et al., 1998) and sympathetic nervous system (Phillips & Barker, 1997; Fig. 10.9). The FOAD hypothesis suggests that these changes not only lead directly to adult cardiovascular disease, but also render the individual more susceptible to the effects of environmental stressors, such as obesity arising in later life.

The hypothesis is supported by examples in experimental animals of permanent structural, metabolic, endocrine and behavioural effects resulting from transient nutritional insults in utero, including undernutrition of the mother (Barker, 1998). In rats, maternal protein restriction in pregnancy leads to higher blood pressure (Langley & Jackson, 1994), impaired glucose tolerance (Ozanne & Hales, 1999), insulin resistance (Vickers et al., 2000; Ozanne et al.,
2001), and altered hepatic architecture and function (Hales et al., 1996) in the adult offspring. Animal experiments allow more sophisticated study of the mechanisms of programming at tissue and cellular level. For example, Hales, Ozanne and co-workers have shown that insulin resistance and impaired glucose tolerance in adult offspring of protein-deprived rats result from reduced gene expression for PI3 kinase, an enzyme within the insulin-signalling pathway (Ozanne & Hales, 1999; Ozanne et al., 2001).

Associations of cardiovascular disease and its risk factors with different body proportions at birth may reflect undernutrition during critical periods in gestation for the development of tissues and organ systems (Barker, 1998). It has been suggested that male and female fetuses have different growth priorities, and thus adapt differently to undernutrition, reflected in sex differences in associations with neonatal body proportions (Barker, 1998; Forsen et al., 1999). Since size at birth is a strong determinant of infant growth, the associations with low infant weight may reflect pre-natal events (Barker, 1998; Barker et al., 1989). Alternatively, since the hyper-plastic development of many tissues continues in early infancy, it may indicate similar programming effects of the infant environment to those suggested for fetal nutrition (Fall et al., 1992; Eriksson et al., 2001).

There are a number of possible explanations of why weight and height gain in childhood, on a background of fetal restriction, might be associated with disease. Low birthweight babies tend to catch up (compensatory growth), and the rapidity of post-natal growth may simply indicate the severity of growth retardation (in relation to growth potential) at birth (Leon et al., 1996). Alternatively, the process of catch-up may be disadvantageous in itself (Lucas et al., 1999). In animals, compensatory growth can lead to adverse short- and long-term effects, operating through a variety of mechanisms (Metcalfe & Monaghan, 2001). One may be excess demand on other tissues that are not capable of compensatory hyperplasia, such as the pancreas (Hales & Barker, 1992; Wilkin, 2001). Another may be through altered body composition. McCance (1962) observed excessive fat gain in pigs if they were placed on a high

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**Fig. 10.9** The fetal origins hypothesis. IGF: insulin-like growth factor.
plane of nutrition after a period of early post-natal undernutrition; he suggested that good nutrition at this stage emphasised the development of tissues like fat that maintain the capacity for growth throughout life, but could not recover tissues (such as muscle) that develop earlier and lose the capacity for cell division. Another possibility is that the hormones driving catch-up growth have adverse cardiovascular and metabolic effects (Lever & Harrap, 1992). Low birthweight children who have caught up in weight and height have higher insulin-like growth factor-I (IGF-I) concentrations, which in turn correlate with blood pressure (Fall et al., 1995c).

10.6.1 Genes versus environment

The fetal origins hypothesis has also been called the ‘thrifty phenotype’ hypothesis, a name coined by Hales and Barker (1992) after Neel’s ‘thrifty genotype’ (Neel, 1962, 1998). ‘Thrifty’ can mean ‘saving’ (storing resources in times of plenty in preparation for leaner times) or ‘economy’ (making judicious use of meagre resources). Neel (1962) suggested that diabetes is caused by ‘thrifty’ genes which were selected for in mankind’s distant past when the supply of food was precarious; he was deliberately vague about the nature of these genes, but suggested that they conveyed a ‘fast insulin trigger’ and thus the ability to store food rapidly as fat (savings). He suggested that these genes became diabetogenic in a modern setting of plentiful nutrition. The thrifty phenotype hypothesis suggests that the undernourished fetus develops insulin resistance and other metabolic changes as a strategy for immediate survival, to down-regulate and prioritise growth (economy), for which it pays a price later in life, generally after the reproductive period. Both thrifty genes and the thrifty phenotype could become detrimental on exposure to plentiful nutrition. Variations in the concept of the thrifty genotype are the strongest alternative to the FOAD hypothesis as an explanation for the associations between low birthweight and cardiovascular risk.

Correlations between parent and offspring birthweights and between birthweights of half-siblings related through either the mother or the father show stronger maternal than paternal effects, which suggests that the ‘maternal environment’ is a more powerful influence on fetal growth than genes (Morton, 1955; Klebanoff et al., 1998). Nevertheless, there are clearly significant genetic effects on size at birth (Godfrey et al., 1997; Frayling & Hattersley, 2001). Hattersley and Tooke (1999) proposed that, since insulin is a major growth hormone in fetal life, genes associated with either insulin resistance or reduced insulin secretion would lead to reduced fetal growth as well as an increased risk of adult diabetes (the ‘fetal insulin hypothesis’, Fig. 10.10). That this is biologically feasible is shown by the fact that birthweight is reduced in a number of genetic syndromes causing impaired insulin secretion or insulin resistance (Frayling & Hattersley, 2001). All of these are too rare to explain the observed birthweight–disease associations, but more commonly occurring polymorphisms, linked both to small size at birth and

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**Fig. 10.10** Genes or environment? The fetal origins hypothesis (left) and the fetal insulin hypothesis (right). Reproduced from Frayling and Hattersley (2001) with permission from Oxford University Press.
adult diabetes, have recently been described. Dunger et al. (1998) reported an association between a polymorphism of the insulin variable number of tandem repeats (INS-VNTR) locus and low birthweight. A mitochondrial DNA variant associated with adult insulin resistance, has been linked to low ponderal index at birth (Casteels et al., 1999). The associations between these two genetic markers and size at birth differed (in opposite directions) according to the degree of catch-up growth in infancy. Recently, a polymorphism of the promoter region of the IGF-I gene has been associated with lower birthweight, reduced insulin secretion, type 2 diabetes and CHD (Vaessen et al., 2002). The robustness of these associations remains to be tested. The INS-VNTR polymorphism and the mitochondrial DNA variant were unrelated to size at birth in other studies (Poulton et al., 1998; Ong et al., 1999).

Twin studies have classically been used to distinguish between genetic and environmental effects but may be misleading in the case of fetal growth (Phillips et al., 2001). Twins are smaller at birth than singletons, but do not experience greater cardiovascular morbidity or mortality. Studies linking cardiovascular disease risk to the difference in birthweight between twin pairs have shown inconsistent results. For example, although a study using the Danish twin registry showed that the smaller of monozygous twin pairs was more likely to become diabetic (Poulton et al., 1997), this has not been confirmed in other twin populations (Baird et al., 2001). However, several features of the biology of fetal growth in twins limit the conclusions that can be drawn from these studies (Phillips et al., 2001). The mechanisms underlying the growth restriction of twins probably differ from those limiting growth in singleton fetuses. Higher disease concordance rates for monozygous than dizygous twins may reflect their shared intra-uterine environment as well as shared genes. Twin–twin interactions (for example, the diffusion of steroid hormones from one twin to another) may reduce within-pair differences in programming effects.

Recent reports of associations between low offspring birthweight and an increased risk of cardiovascular disease and insulin resistance in parents (both mothers and fathers) could be evidence of common genetic factors (Davey Smith et al., 1997, 2000; Smith et al., 2001; Lawlor et al., 2002). However, intergenerational effects may also have environmental explanations. Assortive mating, whereby men and women select partners of similar size and cultural background, and shared socioeconomic conditions, along with related aspects of health and lifestyle, such as poor nutrition, smoking and stress, could lead to both low birthweight and later disease in either parent. It was possible to account for some, but not all, of these factors in these studies. Associations shown between low offspring birthweight and type 2 diabetes in fathers but not mothers is more powerful evidence of genetic effects (Lindsay et al., 2000; Hypponen et al., 2003). The lack of effect in mothers argues against these resulting from a shared environment. Maternal gene effects may have been masked by gestational diabetes, or the effect could act entirely through paternal genes (paternal imprinting). Fathers of low birthweight babies did not, however, have increased insulin resistance or diabetes in two other recent studies from India (Vajnik et al., 2001a, b).

The time trends in CHD and type 2 diabetes in Western countries and in different socioeconomic groups during the twentieth century, and the recent rise in developing countries, suggest a susceptibility to environmental changes, which could equally well have a genetic basis (thrifty genotype) or arise from fetal programming (thrifty phenotype). However, these would make different predictions for the future. The former would predict continuing high levels of disease unless people reduce their lifestyle risk factors and become less obese. The thrifty phenotype hypothesis would predict a downturn in disease as better nutrition of girls and mothers leads to improved fetal nutrition. CHD has been falling in the USA and Europe for 30 years despite increasing adult obesity, and only modest reductions in classical lifestyle-related risk factors (see Chapter 1, Sections 1.3.2 and 1.4.7). The incidence of stroke has also fallen since the early 1950s in the UK (Gale & Martyn, 1997; see Chapter 1, Section 1.3.2). In contrast, type 2 diabetes is increasing in all populations worldwide (King et al., 1998; see Chapter 2, Section 2.5.1). However, the increase has been less marked in developed than developing countries, and a fall in incidence has been reported in one population, the Nauruan islanders (Dowse et al., 1991).

The ‘genes versus environment’ debate is currently stimulating a great deal of hypothesis-testing research in this field. With increasing understanding of epigenetic effects and gene–environment interactions, it is no longer possible to think of diseases as being
either ‘genetic’ or ‘environmental’ (Morris, 1997). It was recently shown that the Pro12Pro allele of the peroxisome proliferator-activated receptor gamma (PPARγ) gene is associated with increased insulin resistance, but only in men and women of low birthweight (Eriksson et al., 2002c). Effects of vitamin D receptor genotype on adult bone mass vary according to birthweight (Dennison et al., 2001). In contrast, weight at 1 year, and the beta-fibrinogen G/A and factor VII R353Q polymorphisms had independent effects on fibrinogen and factor VII concentrations (Henry et al., 1997). It is clearly possible to permanently alter gene expression by manipulation of intra-uterine nutrition (Ozanne et al., 2001). Such epigenetic effects may underlie the ‘thrifty phenotype’, and it is notable that many of the genes associated with the control of fetal growth (controlling growth factor levels, processes of cell proliferation, apoptosis and placental function, and determining fetal demand) are ‘imprinted’ i.e. modulated by epigenetic processes (Gluckman & Pinal, 2003; Reik et al., 2003). In rats, a maternal low protein diet during the pre-implantation period leads to hypertension and altered organ/body weight ratios in the offspring in adult life (Kwong et al., 2000). These effects are associated with altered expression of imprinted genes known to control fetal growth (Kwong et al., 2003). Epigenetic effects can persist across generations. Feeding ‘agouti’ mice with a methyl supplemented diet during pregnancy leads to permanent and heritable effects on offspring coat colour, which is regulated by well-characterised genes (Wolff et al., 1998). At this time, it seems likely that environmental effects, genes and interactions between the two contribute to the observed associations linking birthweight to adult disease.

10.7 Clinical importance of the effects of poor fetal growth

It has been argued that intra-uterine undernutrition is of little clinical importance because statistically birthweight explains little of the variation in adult disease (Joseph & Kramer, 1997; Boyko, 2000; Lamont et al., 2000; Huxley et al., 2002). Effects are most marked at the extremes of birthweight, where there are relatively fewer individuals. Based on the Hertfordshire data, Joseph and Kramer (1997) estimated that 26% of CHD deaths would be averted if all babies weighed 9–9.5 lb (4.1–4.3 kg) at birth, 9% if babies were born one birthweight category (approximately 1 pound or 0.5 kg) heavier, and 2% if more realistic increases in birthweight (100–200 g) were achieved. Boyko (2000) calculated that the type 2 diabetes population attributable risk for low birthweight ranged from 0.02 to 0.25 in four studies, compared with population attributable risks for adult BMI of 0.43 (women) and 0.25 (men) based on US National Health and Nutrition Examination Survey (NHANES) data.

These calculations are potentially misleading. Crude birth measurements and imprecise estimates of gestational age at birth would lead to underestimation of birthweight effects. Birthweight is also an insensitive marker of the dynamic process of fetal growth (Harding, 2001) (Fig. 10.11), and does not capture the effects of fetal undernutrition on body composition or the development of specific tissues. Babies born to mothers exposed to the Dutch famine of 1944–45 during early gestation had normal birthweights, and yet an increased risk of adult obesity and dyslipidaemia (see Section 10.8.1). The large

![Fig. 10.11 Possible fetal growth trajectories. Reproduced from Harding (2001) with permission from Oxford University Press.](image-url)
numbers of men and women required to show the effect of birthweight on cardiovascular disease (Fig. 10.1) contrast with the small numbers of rats required, for example, to show an effect of nutritionally-induced defects in gene expression in the insulin signalling pathway on adult glucose intolerance (Ozanne et al., 2001). The true impact of intrauterine undernutrition will not be known until there are better tissue-level markers of programming than birthweight.

Another reason why calculations based on birthweight alone may underestimate intra-uterine effects is that, as described above, effects of size at birth are conditioned by childhood growth and adult obesity. Data from Finland show that combinations of birth, infant and childhood measurements predict large differences in the risk of CHD, hypertension and diabetes (Barker, 1999; Eriksson et al., 1999, 2001; Table 10.2).

10.8 The role of nutrition

Fetal growth depends on the uptake of nutrients, which occurs at the end of a complex materno-fetal supply line (Harding, 2001; Fig. 10.12). This includes intake, i.e. the mother’s appetite, diet and absorption. Even the pre-implantation embryo is sensitive to its nutritional environment (Robinson et al., 1999), which can alter subsequent fetal growth (Kwong et al., 2000). The nutrient content of uterine fluid is influenced by maternal diet (Porter et al., 2003). Maternal diet during the peri-conceptual period influences the size of the placenta; maternal undernutrition at this stage can paradoxically increase placental size (Godfrey, 2002). In later pregnancy, the range and quantity of nutrients arriving at the placenta depend on maternal intermediary metabolism: her endocrine status, her partitioning of nutrients between storage, utilisation or circulation, and her cardiovascular adaptations to pregnancy, such as plasma volume expansion, which influence uterine blood flow. These are influenced by maternal nutrition in ways that are poorly understood. Similarly, little is known about the influence of maternal nutrition on the specific and non-specific placental transport systems which carry nutrients from maternal to fetal circulations, or the processes which control the division of nutrients between the mother, the placenta itself and the fetus (Godfrey, 2002). The link between maternal food intake and fetal nutrition is certainly indirect.

This helps to explain why the impact of maternal diet on fetal growth remains unclear. Fetal growth can be readily restricted in experimental animals by

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**Table 10.2** Hazard ratios for death from coronary heart disease (number of deaths) among 3641 men born in Helsinki, Finland, according to their ponderal index at birth and body mass index at age 11 years, adjusted for length of gestation.

<table>
<thead>
<tr>
<th>Ponderal index (kg/m³)</th>
<th>Body mass index* (kg/m²)</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>≤15.5</td>
</tr>
<tr>
<td>≤25</td>
<td>2.7 (21)</td>
</tr>
<tr>
<td>–27</td>
<td>1.5 (14)</td>
</tr>
<tr>
<td>–29</td>
<td>2.2 (17)</td>
</tr>
<tr>
<td>&gt;29</td>
<td>1.0 (4)</td>
</tr>
</tbody>
</table>

*Cut-off points are approximately quartiles.

Source: Reproduced with permission of the BMJ Publishing Group from Eriksson et al. (1999).
Cardiovascular Disease

reducing maternal intakes of energy and protein during pregnancy (Harding, 2001). Energy and protein deficiency in the mother is also clearly associated with intra-uterine growth retardation in humans. Size at birth rises in a linear fashion with maternal BMI (World Health Organization, 1995), and during acute famine birthweight falls by several hundred grams (Susser & Stein, 1994). Greater maternal weight gain in pregnancy is also associated with higher birthweight (Kramer, 1987). However, randomised trials of energy and/or protein supplements in well-nourished populations have shown only small effects on birthweight (Kramer, 2001). There is some evidence that supplementing mothers with micronutrients, or improving the quality of the diet with micronutrient-dense foods, leads to an increase in fetal growth, but this has not been sufficiently tested in trials (Fall et al., 2003). There are paradoxical relationships too: attempts to increase birthweight by giving mothers high-density protein supplements have consistently reduced fetal growth (Rush, 1989).

In an observational study in Southampton, UK, it was found that mothers with high carbohydrate intakes in early pregnancy and low dairy or meat protein intakes in late pregnancy had babies with low placental and birthweights (Godfrey, 2002; Godfrey et al., 1996). Such effects could be population-specific. Due to the obvious difficulties, very few trials, of either micro- or macronutrients, have started pre-conceptually. This needs to be evaluated, because recent studies of the embryo have shown that, while its nutrient requirements are quantitatively small, they are specific both qualitatively and temporally. Minor changes can cause major alterations in subsequent growth and size at birth (Robinson et al., 1999). At present, however, we know of no way to reliably increase fetal growth, by dietary means, in well-nourished human populations.

Fetal growth is related to maternal height and birthweight. This probably has both genetic and environmental components, and suggests that undernutrition of the mother during her own fetal life and childhood growth may limit the growth of her fetus. Effects of the mother’s current nutritional status may be influenced by her own past nutrition and that of earlier generations. Nevertheless, birthweights do increase in populations moving from poor to better nutritional circumstances (Dhawan, 1995).

High levels of maternal physical activity have been associated with reduced fetal growth in undernourished populations (Rao et al., 2003). In well-nourished US women, however, moderate exercise has only a small effect on birthweight and may even result in higher birthweight, possibly because of improved placental blood flow (Clapp, 2000). Strenuous exercise on the other hand, appears to reduce birthweight in all populations (Clapp, 2000).

10.8.1 Maternal nutritional status and cardiovascular disease risk in the offspring

If maternal nutritional status in pregnancy has an important effect on the programming of adult disease, correlations would be expected between measures of maternal nutrition and cardiovascular disease in the offspring. The problem is that there are limited data to test this, especially in populations old enough to measure disease outcomes. What data exist are limited to maternal measurements recorded in clinical obstetric notes (usually only weight and height), old dietary surveys, and famine studies.

(i) Maternal anthropometry

Several studies have shown that low maternal weight gain, BMI or skinfolds in pregnancy are associated with higher offspring blood pressure (Godfrey et al., 1994; Clark et al., 1998; Adair et al., 2001; Fig. 10.13).

![Fig. 10.13](image-url)

**Fig. 10.13** Children’s systolic pressure adjusted for sex and current weight according to the mother’s triceps skinfold thickness at 15 weeks of gestation. Source: Godfrey et al. (1994)
This is not consistent in all studies (Laor et al., 1997; Mi et al., 2000). Low maternal weight or BMI is also associated with evidence of increased adult insulin resistance in the offspring (Mi et al., 2000; Eriksson et al., 2002b; Table 10.3). A study in India showed that CHD was associated with low maternal weight (Stein et al., 1996). Conversely, in Finland, mortality from CHD was increased in men and women whose mothers were short and had a high BMI (Forsen et al., 1997), and in India, type 2 diabetes was strongly associated with higher maternal weight and pelvic diameters (Fall et al., 1998). It is possible that these effects of large maternal size or obesity reflect gestational diabetes, although this is speculative.

(ii) Famine studies

In 1944–45 part of The Netherlands suffered a period of famine when rations fell to less than 3.3 MJ (800 kcal) a day (the Dutch hunger winter). The population was previously well-nourished and food supplies were restored quickly after 5 months. Pregnant mothers experienced extreme undernutrition for sharply delineated periods of gestation. Birthweights were normal among women exposed to famine in early gestation, but reduced by 350 g in those exposed in late gestation (Susser & Stein, 1994). An early follow-up study showed increased obesity in young men whose mothers experienced famine in early gestation (Ravelli et al., 1976). Cardiovascular disease risk factors were recently measured in 700 men and women born before, during and after the famine. Late gestation exposure to famine was associated with glucose intolerance, increased measures of insulin resistance, and a small (non-significant) increase in type 2 diabetes, compared with subjects conceived after the famine (Ravelli et al., 1998).

Early gestation exposure was associated with higher LDL/HDL-cholesterol concentrations (Roseboom et al., 2000a) and (in women) higher BMI and waist circumference. There were no associations between famine exposure and fibrinogen concentrations (Roseboom et al., 2000b) or blood pressure (Roseboom et al., 1999), although a higher percentage of maternal energy intake as protein (assessed using official ration data) was associated with lower blood pressure (Roseboom et al., 2000a). Famine exposure was associated with increased infant mortality, but there were no effects on adult mortality (so far assessed only up to the age of 50 years) (Roseboom et al., 2000b).

Stanner et al. (1997) studied men and women exposed to famine during the 1941–42 siege of Leningrad. Those who experienced famine during infancy (infancy exposed) and those whose mothers also experienced famine during pregnancy (intra-uterine + infancy exposed) were compared with men and women born outside the siege area (unexposed). The Leningrad famine lasted longer and was less acute in onset and termination than the Dutch famine, and birthweights were not recorded. Those exposed to famine in utero and/or in infancy showed increased subscapular/triceps skinfold ratios, diastolic blood pressure, ischaemic changes on electrocardiogram (ECG), and PAI-1 activity and antigen, but lower factor VII concentrations than unexposed subjects. Unlike the Dutch study, there were no effects on glucose and insulin variables. There were

<table>
<thead>
<tr>
<th>Component</th>
<th>≤19.2</th>
<th>−20.5</th>
<th>−22.3</th>
<th>&gt;27.3</th>
<th>All</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125</td>
<td>122</td>
<td>125</td>
<td>124</td>
<td>124</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>2-hour glucose (mmol/l)</td>
<td>7.4</td>
<td>7.0</td>
<td>7.1</td>
<td>5.6</td>
<td>6.7</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting insulin (pmol/l)</td>
<td>49</td>
<td>50</td>
<td>41</td>
<td>40</td>
<td>45</td>
<td>0.07</td>
</tr>
<tr>
<td>2-hour insulin (pmol/l)</td>
<td>399</td>
<td>299</td>
<td>252</td>
<td>181</td>
<td>273</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.56</td>
<td>1.37</td>
<td>1.10</td>
<td>1.07</td>
<td>1.26</td>
<td>0.06</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.14</td>
<td>2.70</td>
<td>2.80</td>
<td>2.76</td>
<td>2.85</td>
<td>0.01</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.35</td>
<td>1.42</td>
<td>1.35</td>
<td>1.41</td>
<td>1.38</td>
<td>&gt;0.2</td>
</tr>
</tbody>
</table>

All values adjusted for sex and BMI. HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Source: Adapted from Mi et al. (2000) with permission of the American College of Physicians.
no differences in risk factors between those exposed in utero and during infancy, except for higher von Willebrand factor concentrations in the in utero exposure group. Men and women exposed in infancy were more likely to complain of angina than those in the in utero exposed group (Stanner et al., 1997).

(iii) Follow-up of dietary surveys and trials

Three studies suggest that the balance of maternal protein and carbohydrate intakes during pregnancy is related to blood pressure in the offspring. During 1948–54, pregnant women attending Aberdeen Maternity Hospital recorded seven-day food diaries in late gestation. The offspring were traced and studied for cardiovascular disease risk factors at the age of 40 years (Campbell et al., 1996; Shiell et al., 2000). Blood pressure was not directly related to maternal intakes of energy, protein, fat, carbohydrate, calcium, or a range of vitamins. At low maternal protein intakes (<50 g/day), a higher percentage energy intake from protein was associated with lower blood pressure. The reverse was true at high protein intakes (Campbell et al., 1996). In an attempt to replicate these findings, Shiell et al. (2001) studied young men and women born in Motherwell, Scotland 1952–76, where mothers were advised to eat a high-meat, low carbohydrate diet to prevent pre-eclampsia. Dietary intakes of ten specific foods were recorded by trained clerical staff. Mean protein intakes were higher than in Aberdeen (88 g versus 73 g). High intakes of meat and fish were associated with higher blood pressure. There was a weak inverse association between blood pressure and carbohydrate-rich food intakes. The highest blood pressures were found in men and women whose mothers had high meat/fish intakes but low intakes of green vegetables (Table 10.4). In both studies, blood pressure was inversely related to birthweight, independently of maternal dietary effects. In a study in the Philippines, maternal intakes of protein, fat and total energy were measured at 30 weeks’ gestation by 24-hour dietary recall, and the children followed up in adolescence (Adair et al., 2001). In boys, a higher percentage of maternal energy derived from protein, and in girls a higher percentage derived from fat, were associated with lower blood pressures. Mean protein intakes were not reported, but are likely to have been low in this population.

These studies have many limitations, namely crude dietary measures and large losses to follow-up. It is difficult to put the findings together. The diet survey studies show some consistency, and suggest that the balance rather than absolute intakes of protein and carbohydrate may influence blood pressure in the offspring, and that both low and high protein intakes may have adverse effects. They also indicate that there may be maternal diet effects which are not mediated through reductions in birthweight, and conversely, that birthweight is a marker for factors unrelated to maternal diet, as measured. So far, there is no strong conclusive evidence that normal variations in dietary intakes during pregnancy have important effects on cardiovascular disease risk in the offspring, but this has not been sufficiently studied. A number of prospective studies of maternal diet in pregnancy are due to report outcomes in the children in the next few years, including the Princess Anne Study (Godfrey et al., 1996), the Southampton Women’s Survey (which has pre-conceptual dietary data) and the Pune Maternal Nutrition Study, India (Rao et al., 2001).

Table 10.4  Mean systolic blood pressure of men and women, adjusted for gender, body mass index, alcohol consumption and cuff size according to their mother’s consumption of meat, fish and green vegetables in late pregnancy.

<table>
<thead>
<tr>
<th>Green vegetables (portions/week)</th>
<th>Meat and fish (portions/week)</th>
<th>Regression coefficient (mmHg/portion/week)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤11</td>
<td>−15</td>
</tr>
<tr>
<td>&lt;7</td>
<td>120 (131)</td>
<td>120 (93)</td>
</tr>
<tr>
<td>≥7</td>
<td>118 (58)</td>
<td>120 (94)</td>
</tr>
<tr>
<td>All</td>
<td>119 (189)</td>
<td>120 (187)</td>
</tr>
</tbody>
</table>

Number of subjects given in parentheses. *Overall SD = 11.5 mmHg.
Source: Reprinted from Shiell et al. (2001), with permission from Lippincott, Williams and Wilkins.
In the only follow-up study so far of a randomised controlled trial of a nutritional intervention in pregnancy, Belizan et al. (1997) followed up children born during a trial of maternal calcium supplementation to prevent pregnancy-induced hypertension in Argentina; calcium supplementation was associated with lower blood pressure, with a larger effect in children with a BMI above the median.

### 10.9 Maternal diabetes and fetal macrosomia

Although the main focus of this chapter is fetal undernutrition and low birthweight, recent data show that maternal diabetes, which results in fetal ‘over-nutrition’ and macrosomia, may also have adverse long-term effects on the offspring. Studies of the Pima Indians first showed that offspring of diabetic mothers have an increased risk of obesity and type 2 diabetes compared with offspring of non-diabetic mothers or women who became diabetic later (Dabelea et al., 2000a; Fig. 10.14). The difference in risk holds true for siblings born before and after the onset of maternal diabetes, and is not seen among offspring of diabetic fathers (Dabelea et al., 2000b). Among the Pima Indians, who have a high incidence of gestational diabetes, this effect produces a U-shaped relationship between birthweight and adult diabetes, with an increased risk of diabetes at both ends of the birthweight distribution. The increased risk at high birthweights is attributable to maternal diabetes (McCance et al., 1994). Similar data from a large cohort of nurses in the USA (Rich-Edwards et al., 1999) show that this high birthweight effect is also seen in non-Pima populations. These data suggest that as long as the mother is not diabetic, there is no increased risk of type 2 diabetes even at extremely high birthweights. With increasing levels of obesity worldwide, maternal diabetes is increasing (King, 1998). Effects of fetal ‘overnutrition’ may therefore contribute to the epidemic of type 2 diabetes (see Chapter 2, Section 2.5). This has important implications for interventions to improve maternal nutrition in developing countries; interventions which simply increase maternal body fat may have adverse consequences if they lead to impaired maternal glucose tolerance.

![Graph](image-url)

**Fig. 10.14** Prevalence of (a) type 2 diabetes and (b) obesity in offspring of non-diabetic, pre-diabetic and diabetic women (Pima Indians). Reproduced from Dabelea et al. (2001a).
10.10 Key points

- Fetal growth restriction resulting in low birthweight, and low weight gain during infancy, are associated with an increased risk of adult cardiovascular disease, hypertension, type 2 diabetes and the insulin resistance syndrome.

- The fetal origins of adult disease (FOAD) hypothesis proposes that these associations reflect permanent metabolic and structural changes resulting from undernutrition during critical periods of early development. An alternative explanation is that both reduced fetal growth and cardiovascular disease risk factors have common genetic origins.

- Poor fetal and infant growth may contribute to the high rates of cardiovascular disease seen among ethnic minorities in the UK.

- Cardiovascular disease risk is also increased in men and women who had an early adiposity rebound, those who crossed centiles for weight and BMI upwards during childhood, and those who are obese in adult life. The adverse effects of childhood and adult obesity on cardiovascular disease and its risk factors are exaggerated in people of low birthweight, and relatively small in those of high birthweight.

- Fetal growth is related to maternal size and body composition. Poor maternal diet is a major cause of low birthweight globally, but its impact on fetal growth in ‘well-nourished’ Western populations has been inadequately studied and remains unclear.

- In experimental animals, hypertension and insulin resistance can be consistently programmed in the offspring by restricting the mother’s diet in pregnancy. There are currently insufficient data to determine whether maternal nutritional status and diet Programme cardiovascular disease risk in humans.

- In addition to low birthweight, fetal ‘macrosomia’ due to maternal gestational diabetes leads to an increased risk of obesity and type 2 diabetes. Maternal obesity is a strong risk factor for gestational diabetes.

- Mothers should be encouraged to attain a healthy BMI (avoiding excessive thinness as well as obesity) and to adopt a varied and balanced diet before and during pregnancy. However, there are currently insufficient data to set exact BMI or weight gain targets or to make specific dietary recommendations to pregnant mothers, with the aim of increasing fetal growth or reducing adult cardiovascular disease outcomes. Moderate physical activity during pregnancy can be recommended.

- There is insufficient evidence to make recommendations about catch-up growth during infancy in low birthweight babies. Accelerated weight and BMI gain in childhood is a clear risk factor. Population-wide reduction in childhood BMI should be a public health priority. Current data suggest that the greatest benefit in terms of individual risk-reduction would be in people who had a low birthweight.
10.11 Recommendations for future research

- More research is needed into the determinants of fetal growth and the physiology of the materno-fetal supply line, from maternal food intake to placental function. Studies into the effects of maternal diet should consider micronutrient intakes and status, the balance of macronutrients, and possible paradoxical effects. They should incorporate serial measurements of fetal growth, measurements of neonatal body composition (not just birthweight) and follow-up measurements of cardiovascular risk outcomes in the children. Pre-conceptual nutritional status should be evaluated. It would be helpful to study cardiovascular outcomes in people born during randomised controlled trials of maternal dietary supplementation.

- Epidemiological research should include studies of the genes known to influence fetal growth and those associated with cardiovascular disease, and examine gene–environment interactions.

- The setting up of a multi-ethnic birth cohort should be considered.

- Research is needed into the long-term effects of infant ‘catch-up’ growth, aetiology of obesity and the determinants of the age of adiposity rebound. New methods need to be developed to detect, prevent and treat obesity in children and adults. It needs to be established whether targeting efforts to prevent obesity to a ‘high risk’ low birthweight group would be an effective strategy.

- Further research is needed into the long-term effects on the offspring of maternal gestational diabetes.

10.12 Key references


11 Diet and Cardiovascular Disease: Where Are We Now?

11.1 Introduction

Since the UK recommendations for cardiovascular disease were published in 1994 (Department of Health, 1994), knowledge of the pathophysiology and risk factors for heart disease has progressed, providing the impetus to re-examine current dietary guidelines. Furthermore, less restrictive dietary fat recommendations have been published in The Netherlands and the USA (see Chapter 13, Section 13.3). The Dutch recommendations reflect recognition that reduction in dietary saturated fatty acids can be achieved by either low fat diets or by substitution of saturates with unsaturated fatty acids.

The primary emphasis of current UK guidelines is on reduction in total fat and saturates, and there is concern that there is insufficient emphasis being placed on weight management and promotion of physical activity. Furthermore, experts have questioned the strategy to reduce the intake of total fat because favourable effects on low-density lipoprotein (LDL) cholesterol may be offset by adverse effects on high-density lipoprotein (HDL) cholesterol (see Section 11.7). In addition, knowledge is growing about the ability of other aspects of diet to modulate blood lipid levels and other risk factors.

The purpose of this chapter is to consider current UK dietary recommendations for the prevention of cardiovascular disease, published in 1994, in the context of the emerging knowledge considered in this report. In particular, this chapter considers whether evidence for impact of dietary components on 'emerging' risk factors is sufficiently strong to provide a basis for revision of current public health messages for prevention of cardiovascular disease. Because of recent developments in the evidence relating to dietary fat, especially fat quality (fatty acid profile), particular attention is paid to this dietary component, and this supplements the information provided in Chapter 3.

11.2 Structure of the chapter

The tables within this chapter present an integration of the information provided in earlier chapters. These tables reflect the available evidence from a population perspective; they should not be assumed to provide advice for individuals.

Accompanying text is also provided. First to be considered is diet in the context of the conditions known to increase risk of cardiovascular disease, namely obesity (body fatness; Section 11.3), hypertension (Section 11.4) and type 2 diabetes (Section 11.5). These sections are followed by a discussion of the influence of diet on insulin sensitivity (Section 11.6).

The next section (11.7) is devoted to the influence of dietary fat and fat quality (fatty acid profile). Section 11.8 concentrates on the effect of other dietary factors on blood lipids, and is followed by a section addressing the influence of diet on some of the emerging risk factors discussed in detail in earlier chapters, e.g. endothelial dysfunction, oxidative stress and inflammation-related factors (Section 11.9).

Information is also provided on maternal and fetal nutrition. Low birthweight and low weight in infancy have recently emerged as risk factors for adult disease (Chapter 10). The mechanisms underlying these associations are unknown and there is considerable debate as to whether they have an environmental basis (possibly linked to maternal nutrition) or are genetic in origin.
In order to consider the relevance of the evidence to public health messages for prevention of cardiovascular disease, the Task Force has taken account of a number of important considerations, namely whether:

(1) There is evidence not only from observational epidemiology and secondary prevention trials but also detailed efficacy studies where ‘recommended’ intake levels can be defined.

(2) Effects are observed in ‘healthy’ (this may be high risk healthy) as well as diagnosed diseased populations (coronary heart disease (CHD) and non-CHD; hyperlipidaemic and non-hyperlipidaemic; diabetic and non-diabetic).

(3) Effects are observed at intake levels that are feasible and can/could be achieved through food. For example, the intake levels of long chain n-3 polyunsaturates, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) of 2–4 g/day used in some studies described in earlier chapters are 10–20 times normal intake levels and could not currently be achieved through food.

Other considerations are the advancement of new knowledge and technologies regarding gene–nutrient interactions and the growing interest in the concept of ‘optimum’ diets. Currently, personalised dietary advice to reduce risk of disease, based on an individual’s genetic profile is the focus of much debate (see Buttriss (2003) and Williams (2003)). However, before bringing individuality to public health recommendations (see Chapter 13) numerous research questions on the role of specific nutrients in the population’s diet need to be addressed.

From the foregoing chapters, it will readily be seen that the current state of knowledge regarding impact of diet on emerging risk factors remains incomplete as yet. Not many of the risk factors or diet components covered in earlier chapters meet the criteria required to provide revised public health messages. However, in many cases the available data provide useful indicators of those areas where data are promising and where further research would most valuably be placed.

### 11.3 Diet and body fatness: amount and distribution

The level of obesity in Britain has tripled since the 1980s and more than half the adult population is now overweight (see Fig. 11.1 and Chapter 2, Section 2.4). Among women there is a clear social gradient, with lower socioeconomic groups having a far higher prevalence (Fig. 11.2). Increases in body weight during childhood are also apparent (see Chapter 2, Section 2.4.10 and Chapter 13, Section 13.6.2). Such is the concern about these trends that the National Audit Office (2001) has considered obesity. More

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**Fig. 11.1** Prevalence of overweight (BMI 25–30 kg/m²) and obesity (BMI > 30 kg/m²) in adults aged over 16 years in England. BMI: body mass index. Source for 1986–87 Gregory et al. (1990), for 1991–93 Bennett et al. (1995) and for 1994–2002 Department of Health (2002).
recently, the Parliamentary Health Select Committee has conducted an inquiry about obesity, focusing on health implications, trends, causes of the rise in recent decades, what can be done, whether the institutional structures are in place to deliver an improvement, and recommendations for national and local strategy (www.parliament.uk/commons/selcom/hlthhome.htm).

The number of British adults dieting to lose weight has doubled during the period 1986/7 to 2000/1 (Gregory et al., 1990; Henderson et al., 2002) to 24% of women and 10% of men, but there is little evidence that these efforts are resulting in long-term weight loss. For long-term sustainable weight loss, the ideal approach is to increase physical activity while modifying eating behaviour to achieve a nutritionally balanced intake (see Table 11.1 and Chapter 12). Efforts are hampered by the misconception among the public that scientific opinion is constantly changing about the best approach for weight loss, resulting in apathy and scepticism regarding dietary advice. Consequently, individuals frequently attempt to lose weight using non-scientific prescriptions that often purport to promote something unique for stimulating weight loss, jumping from one ‘celebrity diet’ to the next.

11.3.1 Low fat diets

A recent systematic review of randomised trials (Pirozzo et al., 2003) has compared the efficacy of low fat diets, as a means of achieving sustained weight loss, with other dietary restrictions. In six trials (from 3–18 months duration with follow-up of 6–18 months) there was no significant difference between low fat and other weight reducing diets in terms of sustained weight loss. Furthermore, the overall weight loss at the 12–18 month follow-up was very small (2–4 kg). Further comments about the effectiveness of different types of diets on weight loss can be found in Section 11.7, which focuses on dietary fat.

There is strong evidence that the carbohydrate content of a diet, under ad libitum conditions, plays an important part in body weight regulation. In particular, low carbohydrate, high fat diets promote overeating and weight gain (Saris, 2003). So far there is little evidence that the type of carbohydrate (low glycaemic versus high glycaemic) is of importance in relation to energy balance and weight control, and a high carbohydrate intake may be crucial in preventing weight gain in individuals at risk of obesity. However, the impact of carbohydrate source and class, as well as the form in which the carbohydrate is consumed (i.e. solid or liquid), requires further consideration (Saris, 2003).

11.3.2 Glycaemic index

Glycaemic index (GI) is a measure of the rate at which sugar is absorbed into the bloodstream after eating a specific food. It is determined by comparing the blood glucose response to ingestion of 50 g of available carbohydrate from a test food with that of a reference food (either glucose or white bread). The question of whether the GI of foods has a role to play in appetite and body weight regulation, and thereby obesity, remains highly controversial (Astrup, 2002a). On the one hand, the public has been warned (largely by the media) of the negative health effects of high GI foods such as potatoes, white bread and rice, recommending instead that these are replaced by low GI carbohydrates, such as whole-grain foods (Pawlak et al., 2002). On the other hand, others have cautioned against premature public health advice, given the limitations of the available data (Raben, 2002). Raben’s systematic review of 31 short-term studies (<1 day) that measured appetite sensations following low GI versus high GI foods, most of which were statistically under-powered, found that results were inconsistent. In 20 longer term (up to...
Table 11.1  Body fatness, hypertension, diabetes: synthesis of the available information on diet and cardiovascular disease.

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body fatness: amount and distribution</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>Consistent benefit is seen. Increasing physical activity changes body composition, decreasing body fat and increasing muscle mass. Intervention studies that increase physical activity improve features of the insulin resistance syndrome, decrease vascular problems, development of diabetes &amp; death rate (see Table 11.2).</td>
<td>Weight loss is associated with a fall in some adipokines, e.g. leptin and IL-6, and an increase in adiponectin. This may reduce the risk of cardiovascular disease.</td>
<td>The prevalence of obesity in England has more than trebled since 1980: in 2002, 22% of men and 23% of women were clinically obese. A further 43% of men and 34% of women were overweight (BMI 25–30). Obesity is also rising in children. These trends are reflected worldwide. Both increased physical activity and reduced energy intake can reduce body weight. A combination of the two approaches is preferable.</td>
</tr>
<tr>
<td>Energy intake</td>
<td>Consistent benefit seen with decreased fat intake: meta-analyses of randomised trials show that a 10% fall in dietary fat results in 3–4 kg weight loss in normal/overweight subjects and 5–6 kg in obese subjects; sufficient to reduce type 2 diabetes risk by 50%. NB: The effects seen in studies of well motivated, compliant subjects are not typically replicated in larger, long-term community-based trials. After 12–18 months of follow-up, a recent systematic review of 6 RCTs found a reduction in weight of just 2–4 kg (see Section 11.7.1). The effect on heart disease risk factors of varying the macronutrient composition of the diet has been much debated. In short-term studies, a low fat/high carbohydrate diet may have adverse effects on HDL and triglycerides (see Section 11.7), although LDL-cholesterol falls. These adverse effects may be relatively short lived (CARMEN study, Chapter 1, Section 1.5.2) and can be countered by physical activity. (Also see Section 11.7 and Table 11.3.)</td>
<td>A systematic review has concluded that weight loss whilst on a low carbohydrate diet results from a decrease in energy intake rather than an effect of the CHD reduction per se. There is a lack of data on possible adverse effects despite the popularity of such diets (see Section 11.3.1). Animal studies have suggested a link between low fat/high carbohydrate intakes and elements of the insulin resistance syndrome; this has yet to be clearly demonstrated in humans.</td>
<td>Concern has been expressed about the suitability of low carbohydrate/high protein diets for people with cardiovascular disease, type 2 diabetes, dyslipidaemia or hypertension. In the short-term, a low fat/high carbohydrate diet may have adverse effects on blood lipids, but this has not been demonstrated in a longer (6 month) study. Until further information is available, for those who are overweight or obese, low fat diets are advisable, but an increase in physical activity is also needed to counteract the potential negative effects on blood lipid profile.</td>
</tr>
</tbody>
</table>

continued
### Table 11.1 (continued)

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat quality</strong></td>
<td></td>
<td>There is some evidence that dietary fat quality affects the tendency to fat storage. Unsaturated fatty acids are more rapidly oxidised in the postprandial period than are saturates (Jones et al., 1985) and so might be less readily deposited in adipose tissue. In a cross-sectional study, reported intake of polyunsaturates was not related to adiposity, whereas intakes of saturates and monounsaturates correlated positively with adiposity, as did total fat intake (Doucet et al., 1998). These studies give some support to the idea that polyunsaturates may be less 'fattening' than saturates.</td>
<td>Insufficient evidence on which to base recommendations. (Also see Table 11.3 and Section 11.7.)</td>
</tr>
<tr>
<td><strong>Carbohydrate quality</strong></td>
<td>A review of over 70 human intervention studies has revealed no clear pattern between GI and body weight regulation (Raben, 2002).</td>
<td></td>
<td>The ideal study has yet to be conducted: effect of ad libitum low versus high GI foods.</td>
</tr>
<tr>
<td><strong>Hypertension</strong></td>
<td></td>
<td>In the Intersalt study, BMI and high alcohol intake (&gt;500 ml alcohol per week in men, and &gt;300 ml in women) had independent effects on blood pressure (Intersalt Cooperative Research Group, 1988). In other studies, associations with diabetes and smoking have been reported.</td>
<td>In England, 41% of men and 33% of women have hypertension or are in receipt of treatment. Genetic factors may account for 20–40% of variations in blood pressure in the general population. Each 20 mmHg difference in systolic blood pressure has been shown to be associated with a two-fold difference in death rates from CHD, stroke and other vascular causes (see Chapter 1, Section 1.2.3). Therapy can typically lead to a 10 mmHg fall, equating to a 40% reduction in risk of stroke death and a 30% reduction in heart disease deaths in middle-age (effect in older people almost as great; see Chapter 1). Achieving a healthy weight is an important factor in reducing blood pressure.</td>
</tr>
<tr>
<td><strong>Body weight</strong></td>
<td>Most RCTs have shown that even a modest weight loss of 3–9% is associated with a significant reduction in systolic and diastolic blood pressure of about 3 mmHg in overweight people (Hermansen, 2000). In a meta-analysis, each 1 kg weight loss in obese hypertensive patients was associated with a reduction in systolic and diastolic pressures of 1.2 and 1.0 mmHg, respectively (Staessen et al., 1988). Upper body obesity is especially associated with hypertension (Hermansen, 2000).</td>
<td></td>
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</tr>
</tbody>
</table>

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200 Cardiovascular Disease
Alcohol

In a meta-analysis of 15 RCTs (2234 subjects, heavy drinkers), alcohol reduction was associated with a significant reduction in mean systolic and diastolic blood pressures, −3.31 mmHg and −2.04 mmHg, respectively (Xin et al., 2001).

Salt and sodium

Intervention studies have adopted either dietary manipulation or dietary advice. The DASH sodium study demonstrated the ability of a reduction in sodium intake to reduce systolic blood pressure, but the greatest effect was seen when sodium reduction was combined with other dietary interventions (see Additional comments column). Dietary advice to reduce salt intake has proved effective in some studies, although the size of the effect tends to diminish with time (Trials of Hypertension Prevention Collaborative Research Group, 1997; Hooper et al., 2002); such an approach may be more effective in high-risk groups (Whelton et al., 1998).

A number of meta-analyses of RCTs have been conducted (Scientific Advisory Committee on Nutrition, 2003). Three of these suggest that decreases in blood pressure in response to sodium reduction are insufficient to justify population-wide dietary advice on salt restriction (Midgley et al., 1996; Ebrahim & Smith, 1998; Graudal et al., 1998), but these included trials of short duration for example. A recent meta-analysis which only included studies of at least 4 weeks duration (17 trials in hypertensives and 11 in normotensives) found a beneficial effect of salt reduction (He & MacGregor, 2002).

These findings are supported by data from a large number of cross-sectional and prospective studies. In the MRFIT study (over 11 000 middle-aged men), alcohol intake was significantly and positively associated with both systolic and diastolic blood pressure (Stamler et al., 1997).

In a prospective study, a strong positive association was seen between alcohol consumption and risk of mortality from stroke, with men drinking 35 or more units having double the risk of non-drinkers (Hart et al., 1999).

Animal studies (rodents and chimpanzees) have shown that a high salt intake increases blood pressure (Scientific Advisory Committee on Nutrition, 2003).

Cross-sectional studies (such as the Intersalt study) have demonstrated a relationship between urinary sodium excretion and both systolic and diastolic blood pressures (Intersalt Cooperative Research Group, 1988). Consistent effects have been shown in various study populations, including different ethnic groups, different age groups of adults, hypertensives and non-hypertensives.

SACN concluded that although data on 24-hour urinary sodium suggest that a high salt intake has adverse effects on cardiovascular disease mortality, there are insufficient reliable data on morbidity and premature mortality outcomes to reach clear conclusions (Scientific Advisory Committee on Nutrition, 2003). The greatest reductions in blood pressure are observed when a diet rich in fruits, vegetables and low fat dairy products, and reduced in saturates and total fat, is combined with a low salt intake.

Moderation in alcohol consumption is an important factor in reducing blood pressure.

continued
**Table 11.1 (continued)**

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Other nutrients and dietary patterns</strong></td>
<td>Diets rich in fruits and vegetables have been shown to reduce blood pressure, <em>e.g.</em> an increase to ~5 servings of fruit &amp; vegetables a day reduced blood pressure to a clinically significant extent in healthy middle-aged men and women. Supportive evidence is available from the DASH study (see above) and various other RCTs. Meta-analyses have shown a reduction in systolic but not diastolic pressure with calcium supplementation in both normotensive and hypertensive subjects (Hermansen, 2000). In a meta-analysis of 33 RCTs (Whelton <em>et al.</em>, 1997), potassium supplementation was associated with a significant reduction in systolic and diastolic pressures, −3.1 and −2.0 mmHg, respectively. RCT data for magnesium is conflicting. A recent 3-month randomised trial demonstrated a reduction in mean blood pressure of 18/11 mmHg from soy milk providing 80 mg of the isoflavone genistein per day in subjects with mild hypertension (Rivas 2002). The blood pressure lowering effect correlated with urinary genistein excretion. This finding needs to be confirmed by other studies.</td>
<td>The role of fat intake as a risk factor for stroke remains uncertain, although use of cholesterol lowering drugs reduces risk (Gariballa, 2000).</td>
<td>There is consistent evidence that a diet rich in fruit &amp; vegetables has a small but clinically significant effect, which has been calculated to correlate at the population level with a 17% reduction in incidence of hypertension, 6% in CHD risk and 15% in stroke risk. With the exception of potassium, mechanistic data for other nutrients (<em>e.g.</em> calcium and magnesium) are lacking.</td>
</tr>
<tr>
<td><strong>Maternal and/or fetal nutrition</strong></td>
<td>An RCT of maternal calcium supplementation in pregnancy showed lower blood pressure in the offspring as children.</td>
<td>There is a large body of epidemiological evidence that low birthweight is associated with increased blood pressure in childhood and adult life, and higher rates of adult hypertension. The effects of low birthweight are exacerbated by rapid gain in BMI in childhood.</td>
<td>The environmental (<em>e.g.</em> maternal nutrition) and genetic factors underlying these associations are not known. Further research is needed to clarify the mechanisms and to identify interventions to improve fetal growth and prevent excessive weight gain during childhood.</td>
</tr>
</tbody>
</table>
Type 2 diabetes

- **Low energy diet/ increased physical activity**
  
  Controlled trials in high risk subjects (without type 2 diabetes) have clearly shown benefits of diet (low fat/low energy) and physical activity at achievable levels, and shown this to be more effective than drug treatment (see Section 11.7.1). Type 2 diabetes is associated with a 2–4 fold greater risk of cardiovascular disease death compared with non-diabetics (see Chapter 2, Section 2.5).

  There is now strong evidence that low fat, low energy diets in combination with physical activity reduce risk of type 2 diabetes.

- **Maintenance of healthy body weight**
  
  RCTs provide convincing evidence that diabetes risk is reduced in adults who maintain a normal BMI and are physically active throughout adult life (Jehangir *et al*., 2004).

  These findings are supported by cohort and other studies.

- **Glycaemic control**
  
  An RCT in subjects with type 2 diabetes on a very high fibre diet (25 g soluble fibre + 25 g insoluble fibre) led to a decrease in blood glucose and insulin compared with an isocaloric diet with moderate fibre (Chandalia *et al*., 2000; see Section 11.5).

  In a crossover study, a low GI diet improved glycaemic control (as well as decreasing cholesterol and normalising fibrinolytic activity) compared with a high GI diet (identical with respect to macronutrient composition and fibre content). Epidemiological evidence supports an association between whole-grain/fibre intake and decreased risk of developing diabetes (30% reduction in a meta-analysis of seven studies).

  Epidemiological evidence also supportive of an association between low GI and reduced risk of diabetes.

  Good evidence exists that a high carbohydrate/high fibre diet improves glycaemic control in diabetes.

  The GI concept may be a useful adjunct to therapy, but long-term studies are required before it can be recommended as a primary strategy.

- **Vitamin D status**
  
  There is provisional evidence that polymorphisms of the genes involved in the metabolism of vitamin D may predispose to type 2 diabetes (Boucher *et al*., 1995).

- **Maternal and/or fetal nutrition**
  
  No RCTs.

  There is a large body of epidemiological evidence that low birthweight is associated with an increased risk of type 2 diabetes. The effects of low birthweight are exacerbated by rapid gain in BMI in childhood. Gestational diabetes in the mother is also a risk factor for obesity and type 2 diabetes in the offspring (see Chapter 10).

  Further research is needed to clarify the mechanisms and to identify interventions to improve fetal growth and prevent excessive gain in weight during childhood. Measures to prevent gestational diabetes, such as a avoidance of maternal obesity, can be recommended. Good diabetes control during pregnancy is likely to be important.

BMI: body mass index; DASH: Dietary Approaches to Stop Hypertension; GI: glycaemic index; IL-6: interleukin-6; MRFIT: Multiple Risk Factor Intervention Trial; RCT: randomised controlled trial; SACN: Scientific Advisory Committee on Nutrition.
6 months) intervention studies looking at weight loss, a 1.5 kg loss was achieved on a low GI diet compared with 1.6 kg on a high GI diet, indicating there is currently no evidence that low GI diets are superior to high GI diets with regard to long-term body weight control. However, Pawlak et al. (2002) cite evidence supporting potential beneficial effects on risk factors for cardiovascular disease and diabetes, e.g. lowering of plasma triglycerides, increase in plasma HDL-cholesterol or reduction in postprandial hyperinsulinaemia (see Section 11.6.3).

A concern of jumping ahead of the evidence is that GI classifications do not sit well with those currently used, e.g. high GI foods include recommended starchy foods, such as potatoes and rice, and also bananas. Among low GI foods are pasta, pulses and apples (Raben, 2002).

### 11.3.3 High protein, low carbohydrate diets

Recommendations for increased consumption of protein are among the most common approaches of popular or fad diets, and the popularity of diet books promoting high protein intakes with emphasis on some form of carbohydrate restriction is of concern to health professionals (Stein, 2000; St Jeor et al., 2001). Fad diets often work in the short term because they are low-energy diets in disguise, i.e. energy intake as a result of following the diet is lower than the person’s requirements, though they are not necessarily appropriate for long-term use because of their micronutrient content. However, successful long-term weight loss depends on the consumption over a long period of time of less energy than is expended, in the context of a nutritionally balanced diet.

The attributed ‘success’ of high-protein diets is palatability coupled with the amelioration of hunger. Numerous studies have examined the effect of dietary protein on energy intake (see Eisenstein et al. (2002)). In short-term studies, dietary protein modulates energy intake via the sensation of satiety and increases total energy expenditure by increasing the thermic effect of feeding, although this latter effect is quantitatively small (0.09 kg/month) and confirmatory studies are needed. Notably, when energy intake was fixed these effects did not contribute to weight and fat loss. There is a paucity of evidence for longer-term effects and the stability of such effects. Eisenstein et al. (2002) reviewed seven fixed-energy-intake studies and found that weight loss and fat loss did not differ between control and higher-protein diets.

A systematic review (Bravata et al., 2003) concluded that weight loss whilst on these diets was primarily the result of a decrease in energy intake and increased diet duration, rather than the result of reduced carbohydrate per se. Furthermore, Bravata et al. (2003) revealed that such diets have been popularised in the absence of detailed information on their possible adverse effects in particular groups, such as those with cardiovascular disease, type 2 diabetes, dyslipidaemia or hypertension. In particular, concerns about abnormal metabolism of insulin, impaired liver and kidney function, and salt and water depletion have been highlighted. Furthermore, there is a paucity of data about the effects of carbohydrate intakes below 20 g/day, about use for periods of over 90 days, about use by people over 50 years and about the suitability of the diet for people with diabetes trying to lose weight.

### 11.4 Diet and hypertension

According to the 1998 Health Survey for England (Department of Health, 1999a), 41% of men and 33% of women have hypertension or are in receipt of treatment for the condition. The development of hypertension (an independent risk factor for cardiovascular death; see Chapter 1, Sections 1.2.3, 1.4.3 and Chapter 2, Section 2.6) is dependent on the interaction of dietary factors, alcohol consumption, obesity and its association with metabolic syndrome, physical inactivity and genetic predisposition (Scientific Advisory Committee on Nutrition, 2003). The relative risk of cardiovascular disease increases as blood pressure rises even within what is considered the normal range of blood pressures, indicating that a large number of people are at risk. A number of dietary factors have been reported to influence blood pressure and hence potentially influence the risk of hypertension (see Table 11.6), in particular sodium (see Chapter 13, Section 13.4.3), but also potassium, magnesium and calcium (Scientific Advisory Committee on Nutrition, 2003). Inverse associations with blood pressure have also been shown for particular dietary patterns, e.g. diets rich in fruits and vegetables (John et al., 2002). For example, consuming approximately five portions of fruit and vegetables per day (self-reported) for a six-month period led to a significant fall in blood pressure compared with the
control group, a difference of 4.0/1.5 mmHg (John et al., 2002). From the Dietary Approaches to Stop Hypertension (DASH) studies, it would seem that the influence of at least some of these factors may be additive (Appel et al., 1997; Sacks et al., 2001). The initial DASH study showed that, compared to the typical US diet, a diet rich in fruit and vegetables (a source of potassium), low in fat, and incorporating low-fat dairy products (a source of calcium) could lower blood pressure, even without changes in body weight or salt intake. In the follow-up DASH study (Sacks et al., 2001), a similar diet was employed, but this time salt intake was also modified (3 g/day versus 6 g versus 9 g). The effects on blood pressure of the combined approach (DASH diet plus sodium restriction) were additive.

With regard to the association between sodium intake and blood pressure, in its recent review the Scientific Advisory Committee on Nutrition (SACN) concluded that although studies that have prospectively collected 24-hour urine (a good marker of sodium intake) suggest that a high salt intake has adverse effects on cardiovascular disease mortality, there are insufficient reliable data on morbidity and premature mortality outcomes to reach clear conclusions (Scientific Advisory Committee on Nutrition, 2003). The SACN concluded that reducing the average salt intake in Britain would confer significant public health benefits by contributing to a reduction in cardiovascular disease burden. However, they also concluded that the greatest reductions in blood pressure are observed when a diet rich in fruits, vegetables and low fat dairy products, and reduced in saturates and total fat, is combined with a low salt intake. The recommendations they made for salt intake can be found in Chapter 13, Section 13.4.3 (see Table 11.1 for further information).

### 11.5 Diet and diabetes

There are about 1.4 million people in the UK with diabetes mellitus (mainly type 2) and Diabetes UK (www.diabetes.org.uk accessed 2003) estimates that a further million have the condition but are yet to be diagnosed. The prevalence is 2.4% among Caucasians within the British population, but in some black and ethnic minority groups the prevalence of type 2 diabetes can be three to five times higher (see Chapter 1, Section 1.3.3 and Chapter 2, Section 2.5.1). It is particularly high in South Asian populations (six times more common), and among people of African or African-Caribbean (three times) or Middle Eastern or Chinese descent, compared with the white population (Department of Health, 2002). The incidence is increasing in all age groups, particularly among black and ethnic minority groups; it is now seen in children and young people. Risk of type 2 diabetes is particularly high among those who are overweight or obese, and among those with a family history of the disease. Prevalence rises steeply with age: one in 20 people over the age of 65 years has diabetes, and this rises to one in five over the age of 85 years. It is also more prevalent in lower socioeconomic groups, but in the case of women this may be a consequence of the higher rates of overweight.

The current dietary advice for the management of diabetes is essentially little different to that for the general population, stressing the need to attain or maintain a healthy weight, to cut down on fat (especially saturates), to eat more fruit and vegetables, to use less salt and, in addition, to eat regular meals based on starchy carbohydrates to help to control blood glucose levels (www.diabetes.org.uk). The scientific underpinning for current advice has been comprehensively summarised by Franz et al. (2002). The biggest change in the last 20 years is the advice on fat and carbohydrate. Whereas, in the past, fat was not particularly restricted, a low fat diet, especially with respect to saturates, is now advocated. Rather than restricting carbohydrate, this is now stressed as a primary focus and, with regard to glycaemic effects of carbohydrates, the total amount present in meals and snacks is considered to be more important than source or type (Franz et al., 2002).

A particular role for high fibre diets in terms of glycaemic control has been shown in a randomised controlled trial (25 g soluble fibre plus 25 g insoluble fibre) (Chandalia et al., 2000) and this is supported by other studies (Jarvi et al., 1999; Mann, 2001). The study of Chandalia et al. (2000) also found a reduction in LDL-cholesterol; however, large amounts of dietary fibre were required (50 g/day compared with a typical high intake of 24 g/day), and it is not clear whether the palatability and gastrointestinal side effects would be acceptable to most people (see Table 11.1). Furthermore, there is supportive epidemiological evidence from prospective trials that high fibre diets may reduce the risk of developing diabetes by as much as 30% (Liu, 2003). Nevertheless, although the use of low GI foods may reduce...
postprandial hyperglycaemia and hence contribute to glycaemic control (Brand-Miller et al., 2003), there is insufficient evidence of long-term benefit to support the use of a low GI diet as a primary strategy in food/meal planning (Franz et al., 2002).

The guidelines also stress the importance of regular physical activity and maintenance of healthy body weight (see Table 11.1 for a summary of additional aspects). Such guidelines have been developed to help in the management of established diabetes, but considerable interest is now focused on the potential of diet to influence insulin sensitivity in individuals yet to develop diabetes (Scott, 2003; see Section 11.6). This has led, for example, to renewed consideration of the macronutrient composition of energy-reduced diets (see Section 11.6.3).

The effects on glycaemia and lipaemia reported for specific sources of soluble fibre, psyllium and beta-glucan, are mixed and require further study (Franz et al., 2002).

11.6 Diet and insulin sensitivity

11.6.1 Fat quality and insulin sensitivity

Although a few studies have attempted to address the issue of insulin resistance with respect to fat quantity and/or quality, these are either largely observational in nature or are relatively under-powered controlled intervention trials. A number of observational studies have focused on associations between dietary, plasma and tissue fatty acid compositions in relation to insulin action or risk of metabolic syndrome and diabetes. In the US Nurses’ Health Study, intakes of dietary saturated or monounsaturated fatty acids were neutral, but intake of polyunsaturated fatty acids was positively associated, and that of trans fatty acids was negatively related to increased risk of type 2 diabetes (Salmerón et al., 2001). Other prospective studies have shown that risk of type 2 diabetes is greatest in subjects with relatively high proportions of saturates and low proportions of unsaturated fatty acids in blood lipids at baseline (Vessby et al., 1994; Laaksonen et al., 2002). In serum phospholipids, saturates were negatively and polyunsaturates were positively associated with measured insulin sensitivity (Pelikanova et al., 2001). Higher proportions of oleic and linoleic acids in plasma phospholipids were subsequently shown to be associated with lower fasting blood glucose and greater glucose effectiveness (Louheranta et al., 2002). In general, these studies support the hypothesis that unsaturated fatty acids are protective and saturated fatty acids are harmful with respect to risk of type 2 diabetes (see Table 11.2). However, observational studies such as these are limited in the extent to which they can provide evidence of causal relationships between measured variables, even when confounding factors are taken into account. Measurements based on circulating and tissue levels of fatty acids also vary in relation to the validity of the markers as an index of dietary intake levels, depending on the fatty acid of interest.

Very few intervention studies have evaluated effects of diets on insulin sensitivity measured directly, though a number provide data in relation to impact of diet on fasting glucose and insulin values and surrogate measures based around fasting measurements, such as the homeostasis model (HOMA). In ten studies that compared response to low fat and high monounsaturated fat diets in subjects with type 2 diabetes, only two undertook direct measurement of insulin sensitivity and these demonstrated no marked effect on insulin sensitivity of feeding either diet for a period of three weeks (Garg et al., 1992; Parillo et al., 1992). The KANWU study (Vessby et al., 2001) undertook a comparison of monounsaturated and saturated fatty acid diets in 165 subjects and showed a 12% increase in insulin sensitivity in subjects in whom monounsaturates replaced saturates for three months, with direct measurement of insulin sensitivity by intravenous glucose tolerance test. A crossover study that compared a low fat, high carbohydrate diet with a monounsaturates-rich diet, showed both diets improved insulin sensitivity to a similar extent in young healthy subjects (Perez-Jimenez et al., 2001), suggesting the major effect may be due to removal of saturates from the diet. However, another study which compared diets rich in monounsaturates and polysaturates found beneficial changes in glucose tolerance only with the monounsaturates diet (Louheranta et al., 2002).

Overall, these studies suggest there is insufficient evidence to draw conclusions with respect to effects of dietary saturated versus unsaturated fatty acids on insulin sensitivity (see Table 11.2 and Chapter 2, Section 2.7).

11.6.2 n-3 polyunsaturates and insulin sensitivity

There has been considerable interest in possible beneficial effects of the n-3 polyunsaturates EPA and DHA, because of epidemiological evidence for
### Table 11.2  Insulin resistance: synthesis of information on nutrition and cardiovascular disease.

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fat quality</strong></td>
<td>Replacement of SFA with predominately MUFA and/or PUFA has been shown to be beneficial in terms of lipid profile; in a study of young men, LDL-cholesterol fell by 8-10%; HDL did not change. Only 2/10 studies that have compared low fat and high MUFA diets in type 2 diabetes have measured insulin sensitivity directly, and these demonstrated no marked effect after 3 weeks. However, improved insulin sensitivity has been demonstrated in some studies, e.g. the KANWU study (12% increase with MUFA compared with SFA). It is unclear whether PUFA convey a similar effect to MUFA. But in one cross-over study (similar design to KANWU study though shorter diet intervention period of 5 weeks vs. 3 months), which compared the effect of diets enriched with saturated vs. polyunsaturates, hyperinsulinaemic–euglycaemic clamp measurements of insulin sensitivity showed this to be greater after the PUFA-rich diet (Summers et al., 2002). Also, subcutaneous abdominal fat was reduced on the PUFA-rich diet.</td>
<td>In general, observational and prospective studies support the hypothesis that unsaturated fatty acids are protective and saturates are potentially harmful with respect to risk of type 2 diabetes, but methodological difficulties exist. Overall, there is insufficient evidence to draw conclusions about the effect of fat quality (SFA vs. UFA) on insulin sensitivity (see Section 11.6.1).</td>
<td></td>
</tr>
<tr>
<td><strong>Fish oils</strong></td>
<td>In healthy subjects and in Asian men specifically, moderately high fish oil intakes failed to show effects on insulin sensitivity. The KANWU study found no effect on insulin sensitivity of adding fish oils to high saturates diet or high MUFA diet. There is epidemiological evidence for a protective effect of fish oils in type 2 diabetes, and improvements in insulin sensitivity reported in subjects with impaired glucose tolerance and type 2 diabetes. Also support from animal studies.</td>
<td>Currently, in relation to insulin resistance, little evidence exists that an increased population intake of long chain n-3 fatty acids (EPA &amp; DHA) is of benefit for healthy subjects. However, the evidence is modestly strong for subjects with type 2 diabetes, although this seems to be via effects on hepatic and peripheral lipid metabolism rather than insulin secretion and/or sensitivity.</td>
<td></td>
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</tbody>
</table>

***continued***
<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Dietary carbohydrate and insulin sensitivity</td>
<td>A high carbohydrate intake can increase insulin output and exacerbate insulin resistance, particularly in overweight subjects. Preliminary studies suggest that a diet with a reduced GI may improve insulin sensitivity and related risk factors.</td>
<td>This has led to the view that moderate fat intakes (33–38%), enriched with unsaturated fatty acids, may be more beneficial than low fat/high carbohydrate diets for those with insulin resistance who need to lose weight. Evidence is currently insufficient to draw conclusions about the relative benefits of low GI diets vs. other dietary modifications.</td>
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<tr>
<td>• Fructose intake</td>
<td>It has been suggested, mainly by animal studies, that high fructose (or high sucrose) diets can induce elements of the insulin resistance syndrome (see Chapter 2, Section 2.7). But methodological issues remain and the case is currently considered non-proven.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Maternal and/or fetal nutrition</td>
<td>There is a large body of epidemiological evidence that low birthweight is associated with increased insulin resistance in adulthood. This is exacerbated by rapid gain in BMI in childhood (see Chapter 10).</td>
<td>Further research is needed to clarify the mechanisms and to identify interventions to improve fetal growth and prevent excessive gain in weight during childhood.</td>
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</table>

DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; GI: glycaemic index, MUFA: monounsaturates; PUFA: polyunsaturates SFA: saturates, UFA: unsaturated fatty acids.
11.7 Amount of dietary fat

There is an inverse linear relationship between cholesterol level and CHD risk, and hence use of threshold levels is artificial (see Chapter 1, Section 1.5.1); nevertheless, these provide a point of reference. For example, in the recent National Diet and Nutrition Survey (NDNS) (Ruston et al., 2004), mean plasma total cholesterol was 5.21 mmol/l in men and 5.25 mmol/l in women (19–64 years). Fifty-two per cent had a concentration below 5.2 mmol/l (the optimum level), 34% had mildly elevated levels (5.2–6.5 mmol/l), 12% men and 11% women had moderately elevated levels and 2% men and 3% women had severely elevated levels. Concentration rose with age. A high plasma cholesterol undoubtedly increases risk of CHD, but because of the multifactorial nature of the disease, the distribution of cholesterol levels does not clearly distinguish those with/without the disease (see Chapter 1, Section 1.4.8). Typically, cholesterol levels can be reduced by about 25% by pharmacological means (e.g. statins), by about 10% using dietary means in controlled metabolic studies and by approximately 5% in a free-living population following dietary modification. Greater reductions are achievable with extremely low fat diets, but these are not generally acceptable in westernised communities. On a population basis, a 1% lowering in blood cholesterol level translates to a 2–3% reduction in CHD risk (Department of Health,
1994). Whilst effects seen with single dietary manipulations are less impressive than with drugs, the cumulative effect of a range of dietary manipulations can have more impact (see Chapter 13, Section 13.5.3). Furthermore, the effects of dietary change on other systems may also be important, e.g. insulin resistance.

11.7.1 Low fat versus moderate fat diets

Low fat diets are often advocated for weight reduction, as well as to lower the risk of CHD and certain forms of cancer (see Chapter 1, Section 1.5.1, and Section 11.3). The prevalence of overweight and obesity is rising worldwide (see Chapter 1, Section 1.4.7 and Chapter 2, Section 2.4.3) despite increased awareness and attempts at weight loss (see Section 11.3). Although energy restriction is recognised to promote weight loss, the effect of varying the macronutrient composition of the diet on weight loss has been much debated (see Section 11.3.2). The replacement of saturates with carbohydrates has been the standard advice given for both weight loss and improvements in cardiovascular health. However, relatively recently this practice has been questioned, and the issue of low fat/high carbohydrate versus moderate fat has become quite controversial (Katan et al., 1997; Astrup et al., 2002; Willett, 2002; Sanders, 2003; see Chapter 1, Section 1.5.2).

One of the principal areas of concern is the effect of low fat/high carbohydrate diets on circulating lipids. Although LDL-cholesterol falls and beneficial effects can be expected in terms of body weight and haemostasis (decrease in factor VII coagulant (FVIIc) activity and enhanced fibrinolysis, see Chapter 6), this may be accompanied by a concomitant reduction in HDL-cholesterol and/or an increase in serum triglycerides, and by an increased number of small, dense LDL and remnant particles, all recognised as adverse factors for cardiovascular disease risk (Katan et al., 1997; Katan, 1998; see Chapter 3). Furthermore, based largely on findings in animal studies, there is the possibility that in the short term a high carbohydrate/low fat diet may induce elements of the insulin resistance syndrome (Sanders, 2003; see Chapter 2, Section 2.7), although the existence of such an effect in humans is yet to be clearly demonstrated.

The effect of dietary restriction may be relatively transient. In the Carmen study (see Chapter 1, Section 1.5.2), a 6-month intervention (low fat and high simple or complex carbohydrate) had no adverse effect on plasma lipids and resulted in modest but significant weight reduction (Saris et al., 2000).

In support of low fat diets, meta-analyses of randomised trials reveal that ad libitum low fat diets in intervention trials consistently demonstrate a highly significant weight loss of 3–4 kg in normal weight and overweight subjects, with the most compliant subjects losing about 6 kg (Astrup et al., 2002; Table 11.1) (see Section 11.3.2 for information on the sustainability of weight loss). There is also a dose–response relationship, i.e. the greater the reduction in percentage energy as fat, the greater the weight loss. Weight loss is also positively related to initial weight, with a 10% reduction in dietary fat predicting a loss of 4–5 kg in obese subjects with body mass index (BMI) > 30 (Astrup et al., 2002). This weight loss is clinically significant because it is sufficient to reduce the incidence of type 2 diabetes by 50% (Astrup, 2002b). There is also recent evidence from diabetes-prevention studies that this weight loss can be maintained if a low fat diet is combined with increased physical activity, and diabetes risk can be reduced by 58% (Tuomilehto et al., 2001; Diabetes Prevention Program Research Group, 2002). Astrup (2002b) suggests that a diet high in monounsaturates may actually have an adverse effect on both postprandial blood lipid coagulation factors and endothelial dysfunction and calls for further evidence (see Table 11.1).

In contrast, the benefits of a moderate fat diet, rich in unsaturated fatty acids, are strongly supported by others (Willett, 2002). Not only is compliance on such diets more likely for Western populations, but the author challenges that the emphasis on total fat reduction has been a serious distraction in efforts to control obesity and improve health in general; he suggests that compensatory mechanisms come into play when people adopt a low fat diet in the long term (Willett, 2002).

To illustrate this, weight loss has been achieved on a low fat/high carbohydrate diet among well motivated, compliant participants in residential studies (Prewitt et al., 1991; Stubbs et al., 1995; Poppitt et al., 1998), but with a few exceptions (Saris et al., 2000; Poppitt et al., 2002b) these results have not been replicated in larger long-term community trials (Sheppard et al., 1991; Jeffery et al., 1995; Willett, 1998; see Table 11.1).
Interest has subsequently focused on approaches that modulate the fatty acid composition of a diet containing a moderate amount of fat. A number of trials have shown that replacement of saturates with predominately monounsaturates and/or polyunsaturates can be beneficial in terms of serum lipid profile (see Poppitt et al., 2002a). Poppitt’s study (four-week intervention replacing saturates with mono- and polyunsaturates) reduced total and LDL-cholesterol by 8–10% in a population of young healthy men with a mean cholesterol level of about 4.5 mmol/l cholesterol; HDL-cholesterol did not change. Poppitt et al. (2002a) report that this reduction could translate into a fall in CHD risk of up to 27% and in stroke of up to 24% if the criteria associated with the Multiple Risk Factor Intervention Trial (MRFIT) study (Law et al., 1994) were to be applied.

It has been proposed that low fat diets would beneficially affect postprandial endothelium-dependent dysfunction (see Chapter 4, Section 4.9.1) by reducing postprandial excursions in triglycerides. However, if HDL-cholesterol levels, which are positively related to endothelium-dependent relaxation, remain persistently low over time, this may negate/counteract the postulated beneficial postprandial effects on the endothelium. Should oxidative stress prove to be the link between dyslipidaemia, postprandial hypertriglyceridaemia and impaired endothelial function (see Chapter 4), the antioxidant status of individuals in such trials would be important to determine. To resolve the controversy, further studies are required to investigate the effects of low fat diets on postprandial lipaemia using small to moderate amounts of fat in test meals, as well as the effects of such diets on disease endpoints.

In the interim, for those who are overweight or obese, low fat diets are advisable, but an increase in physical activity is important to counteract the potential negative effects on the blood lipid profile. However, for some individuals, a diet moderate in fat in which saturates are partially replaced by unsaturated fatty acids can play a role in controlling body weight because overall compliance is often greater (see Section 11.3.3 for a discussion of high protein, low carbohydrate diets).

It is interesting to note that recent dietary fat recommendations from The Netherlands distinguish between normal weight and overweight people (see Chapter 13, Table 13.6).

11.7.2 Energy density

The energy density of a meal is particularly influenced by its fat content on the one hand, as fat provides over twice the energy per gram as either protein or carbohydrate, and by fibre and water content on the other hand, as foods rich in fibre or water are bulky yet the fibre itself contributes little to energy intake and the water contributes no energy. Consequently, diets rich in fruit and vegetables (which provide both water and fibre) and cereal foods and pulses (for fibre) have a lower energy density.

The energy density of the diet is considered an important factor in terms of the likelihood of weight gain, over-consumption being more likely with energy-dense diets (Prentice & Jebb, 2003). This is demonstrated by a study using a whole body calorimeter in which lean men were given ad libitum access to food that had been covertly manipulated to provide 20%, 40% or 60% of energy as fat (protein constant and reciprocal carbohydrate level). There was spontaneous weight loss on the 20% diet but substantial weight gains on the 60% fat diet (Stubbs et al., 1996). Similar effects have been reported in free-living subjects, suggesting that appetite control can be undermined by passive over-consumption of energy-dense foods, particularly in sedentary individuals (Murgatroyd et al., 1999).

In a recent analysis, Prentice and Jebb (2003) suggest that consumption of a constant volume of food is an evolutionary response to diets of low energy density, and that consumption of energy-dense foods may overcome natural satiety mechanisms, particularly in children. Rural subsistence farmers eat a diet of low energy density, typically 108 kcal/100 g (451 kJ/100 g) for rural Gambian women compared with 161 kcal/100 g (674 kJ/100 g) for UK women. Women in the UK consuming a diet that meets healthy eating guidelines (below 35% from fat and at least 400 g fruit and vegetables per day), receive 126 kcal/100 g (527 kJ/100 g) (Prentice & Jebb, 2003).

11.8 Type of dietary fat

There is accumulating evidence that it is fat quality (the type of dietary fat), rather than the total amount of fat, that is particularly important for cardiovascular disease (Astrup, 2002a; see Table 11.3).
<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat</td>
<td>It is well established that a diet low in fat, especially saturates, will reduce LDL-cholesterol levels (Chapter 3). A reduced fat intake also reduces body weight (see Table 11.1). A 4 week intervention (obese men with type 2 diabetes) with a hypocaloric/low fat diet (combined with physical activity) decreased body weight, plasma triglycerides and small, dense LDL.</td>
<td>In most countries, France being an exception, a high saturates intake (typically a high fat diet) is associated with high mortality rates (see Section 1.5.3, Chapter 1). Small, dense LDL are clearly regulated by the amount and type of fat (see Section 3.4). No long-term interventions have measured RLP, but in the short-term the effect on RLP of a low fat diet was reversed by physical activity (Chapter 3). Postprandial triglyceride response (and that of RLP and small, dense LDL) is modulated by dietary carbohydrate – low fat diets that are high in carbohydrate have a tendency to increase triglycerides (fasting and postprandial) (see Section 11.7.1). However, the diets used have tended to be extreme. The effect can be counteracted by physical activity or n-3 PUFA (and to a lesser extent n-6 PUFA).</td>
<td>Most of the studies that have compared high fat/low carbohydrate diets and low fat/high carbohydrate diets with respect to an effect on fasting or postprandial blood lipids have used extreme levels of fat or carbohydrate over relatively short time periods, e.g. 45–50% fat compared to 20–25% fat. It remains unclear whether potential adverse effects of high carbohydrate levels are seen at more realistic intakes. The postprandial hypertriglyceridaemic effect of a low fat diet can be counteracted by moderate physical activity or by the inclusion of long chain n-3 PUFA (EPA &amp; DHA). Further studies are needed to investigate effects of low fat diets on postprandial lipaemia, using small to moderate amounts of fat in test meals and incorporating disease endpoints.</td>
</tr>
<tr>
<td>Fatty acid profile</td>
<td>Saturates increase, and polyunsaturates decrease, total and LDL-cholesterol. Saturates, monounsaturates and polyunsaturates all raise HDL-cholesterol when they replace carbohydrate, and this effect is slightly greater with saturates (Chapter 1, Section 1.5.2 and Chapter 3, Section 3.2.1). Plasma triglyceride levels rise when dietary fat is replaced by carbohydrate. Replacement of dietary saturates with carbohydrate may have a minimal effect on CHD risk (perhaps dependent on the type of carbohydrate), whereas replacement of saturates by mono- or polyunsaturates causes LDL-cholesterol to fall, and HDL changes little (especially with monounsaturates) (see Section 11.11). HDL levels are positively related to endothelium dependent relaxation (see Chapter 4). In addition, substituting polyunsaturates for saturates may be beneficial with respect to insulin sensitivity and type 2 diabetes (see Section 11.6).</td>
<td>Although the triglyceride-lowering effect is not so marked as with n-3 PUFA, n-6 fatty acids may decrease accumulation of VLDL in the postprandial state. It has been proposed that suppression of triglyceride levels may beneficially affect postprandial endothelium-dependent dysfunction (Chapter 4).</td>
<td></td>
</tr>
</tbody>
</table>

### Table 11.3 Lipid-related factors: synthesis of information on nutrition and cardiovascular disease.
Habitual high MUFA diets have little effect on postprandial lipaemia *per se*, but may influence circulating concentrations of remnant particles.

- **Fish oils**
  
  Effects of long chain *n*-3 polyunsaturates on triglycerides, small, dense LDL & RLP are only seen at high concentrations of long chain *n*-3 polyunsaturates (2–4 g/day). Generally, studies that employ <1.5 g/day do not show effects on triglycerides. Notably the GISSI trial did not show significant effect on triglycerides at an intake of 0.85 g/day. A recent study comparing effects of control, 0.8 g/day and 1.5 g/day EPA/DHA showed only a non-significant reduction in triglycerides in the 1.5 g/day group (Finnegar et al., 2003) (see Section 11.8.3 and Table 11.1).
  
  In some people, fish oils result in a rise in LDL-cholesterol, despite marked reductions in triglycerides. This is thought to be due to gene–nutrient interactions (see Section 11.8.4).
  
  In the GISSI-Prevenzione trial, the decrease in mortality was largely due to a reduction in sudden death, suggesting that effects other than those involving lipids might be important, e.g. arrhythmia. In the DART trial, no reduction in non-fatal myocardial infarction occurred (see Section 11.8.3). (For a review see Jehangir et al., 2004.)

  In animal models, fish oil protects against ventricular fibrillation after surgical occlusion of the coronary artery and EPA can prevent or terminate pharmacologically induced arrhythmias in cultured heart cells (For a review see Jehangir et al., 2004).

  It is currently unclear whether triglycerides and/or small, dense LDL reduction have anything to do with cardioprotective effects of fish oils.

  Therefore, evidence that diet can influence ALP (see Chapter 3, Section 3.2 for description) is moderately strong for high intakes of fish oils but not for levels that would be easily achieved (in healthy populations) through currently available foods.

  Benefits of fish oil were originally thought to be linked to anti-thrombotic effects but recent evidence suggests that the predominant effect may be antiarrhythmic (Jehangir et al., 2004; Leaf et al., 2003). Evidence of a direct effect in humans is still needed (trials underway).

- **Physical activity**
  
  See Chapter 12.

  A positive association between physical activity and HDL is well established, although much of the evidence is cross-sectional in nature (see Chapter 12, Table 12.2).

- **Trans fatty acids**
  
  Trans fatty acids are thought to have a similar impact on plasma cholesterol as saturates.

  Very high intakes may increase Lp(a), particularly in those with high Lp(a) levels; not all data are supportive (see Section 11.8.6).

  In the UK, current *trans* intakes are low at 1.2%E (DRV = 2%E) but some subgroups who eat diets rich in hydrogenated fats may have a high intake (see Sections 11.8.6 and 11.11.1).

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%E: percentage of energy; ALP: atherogenic lipoprotein phenotype; DRV: Dietary Reference Value; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; GISSI: GISSI-Prevenzione Trial; HDL: high-density lipoprotein; LDL: low-density lipoprotein; Lp(a): lipoprotein(a); PUFA: polyunsaturates; RLP: remnant-like particles; VLDL: very-low density lipoprotein.
11.8.1 Monounsaturates

Progress towards achieving recommendations for reductions in dietary saturates has been slow (see Chapter 13), and alternative dietary strategies have been proposed. One example is to replace a proportion of saturates with monounsaturates whilst maintaining total fat intake, as was conducted in the study by Poppitt et al. (2002a) referred to above; see also Williams et al. (1999). Current UK dietary guidelines do not specify the need to substitute monounsaturates for saturates, perhaps because of the concern about the increasing prevalence of overweight and obesity in the population, and the uncertainty about whether monounsaturates or carbohydrate should be the substitute. The optimal dietary intake of monounsaturates is unknown because it is conditional upon optimal total fat intake, which itself is not defined. A recent Food Standards Agency workshop on monounsaturates has considered future research requirements (Sanderson et al., 2002b).

One of the concerns about potential adverse effects of monounsaturate-rich diets results from postprandial studies that suggest that single meals containing large amounts of monounsaturates cause an exaggerated postprandial lipoaemia and increased activation of factor VII (Larsen et al., 1999; Sanders et al., 1999). This has not been demonstrated in all acute meal studies (Freese & Mutanen, 1995; Larsen et al., 1997; Roche & Gibney, 1997) and adverse effects have not been shown when subjects become habituated to a background diet that is high in monounsaturates. Indeed, the findings of these longer-term studies are the opposite of those observed in acute meal studies. Three separate long-term dietary studies, which substituted saturates with monounsaturates at levels found in the Mediterranean diet, showed no adverse impact on postprandial lipoaemia of the high monounsaturates diet, and in addition showed attenuation of the postprandial activation of factor VII (Roche et al., 1998; Larsen et al., 1999; Silva et al., 2003). In the last study, there was an adaptation to produce larger chylomicrons when increased amounts of monounsaturates were fed. The attenuation of factor VII activation may be as a consequence of the reduction in the number of chylomicron particles, an effect that is seen only when subjects become habituated to high monounsaturates intakes. The beneficial change in blood clotting is pertinent because of the rise seen in factor VIIc (coagulant activity) with increased fat intake (Miller et al., 1989; see Chapter 6, Section 6.9.3). A significant decrease in monocyte derived adhesion molecule expression has also been reported in those on high monounsaturates diets (Yaqoob, 1998). Inflammatory events are a recognised component in the development and progression of atherosclerosis (see Chapter 7). Studies such as these, which address the mechanistic effects of monounsaturates, are important, and further studies of this type are sought (Sanderson et al., 2002b).

In summary, at least for those with healthy body weights, the partial substitution of saturates with monounsaturates appears a pragmatic approach in terms of achieving a beneficial lipid and haemostatic profile.

11.8.2 n-6 versus n-3 polyunsaturated fatty acids

Over the past 20 years or so, campaigns to increase the public’s awareness of the benefits of n-6 polyunsaturates on total and LDL-cholesterol have been largely successful and have resulted in increased use of vegetable oils and spreads rich in linoleic acid. A consequence of this has been a shift in the total n-6/n-3 ratio (see Chapter 13, Section 13.4.2). In 1994, COMA recommended that average intakes of n-6 polyunsaturates need not increase above current levels (Department of Health, 1994).

This shift in balance between n-6 and n-3 fatty acids may provide cause for concern because both groups of fatty acids include essential fatty acids and the members of the two groups share common metabolic pathways. For example, an increase in the consumption of n-3 fatty acids results in a concomitant reduction in n-6 fatty acids in cell membranes, suggesting that the n-6/n-3 fatty acid ratio is important. However, the issue is complicated by factors discussed in Chapter 13, Section 13.4.2.

Although the benefits of n-6 polyunsaturates on LDL-cholesterol are greater than the impact of monounsaturates, intakes of n-6 polyunsaturates above 10% of energy (the recommended individual maximum level) may have adverse effects on HDL-cholesterol (Clarke et al., 1997). In addition, polyunsaturates, in comparison to monounsaturates, are more easily oxidised both in foods during cooking and processing, and in the body, where oxidised lipids are implicated in protein and DNA damage.
This may swing the balance in favour of mono-unsaturates as a replacement for saturates, although further work on the potential detrimental effects of high intakes of polyunsaturates is required.

### 11.8.3 Long chain n-3 fatty acids

An overview of n-3 fatty acids and health is available in a BNF Briefing Paper (British Nutrition Foundation, 1999b). There is currently considerable interest in the ability of the long chain n-3 fatty acids EPA and DHA to influence cardiovascular health (Kris-Etherton et al., 2002; Leaf et al., 2003; Thies et al., 2003; Jehangir et al., 2004). Randomised controlled trials have demonstrated that long chain n-3 fatty acids, taken as oil-rich fish (Burr et al., 1989) or as supplements (GISSI-Prevenzione Investigators, 1999) can reduce cardiac events (e.g. death, non-fatal myocardial infarction and non-fatal stroke), and some prospective studies (e.g. the Nurses’ Health Study) have also shown an inverse association between fish consumption and n-3 fatty acids and CHD deaths (Hu et al., 2002). Importantly, the Physicians’ Health Trial, a prospective study of over 20 years duration, has reported a strong inverse dose relationship between blood phospholipid long chain n-3 polyunsaturates content at baseline and subsequent mortality from CHD (Albert et al., 2002). These data may be valuable in identifying dietary intake levels of long chain n-3 polyunsaturates that can sustain circulating and tissue levels of these fatty acids which are protective in healthy individuals. Conflicting data have recently been reported for men with angina, in whom advice to take fish oil capsules resulted in a higher risk of cardiac death (Burr et al., 2003).

Various mechanisms, individually or in combination, have been considered to explain cardioprotective actions of long chain n-3 polyunsaturates, including blood lipid lowering, decreased inflammation, a fall in growth factor production, decreased inflammation, anti-thrombotic effects, improved endothelial function or anti-arrhythmic actions (Thies et al., 2003; Jehangir et al., 2004). A major problem has been that despite evidence for cardioprotection at low intake levels from secondary prevention trials, very few of these putative mechanisms have been demonstrable at intake levels below 1.5 g/day (Table 11.3). A recent randomised controlled study has provided evidence that an anti-inflammatory response might be involved by demonstrating in patients awaiting surgery to remove atherosclerotic plaques in the carotid artery an association between intake of long chain n-3 polyunsaturated fatty acids as a supplement (1.4 g/day, an amount that could be achieved by diet) and the stability of atherosclerotic plaques (Thies et al., 2003). This improved stability was associated with increased plaque content of the long chain n-3 fatty acids. A further question raised by this important study is whether anti-inflammatory effects that stabilise plaque stability can be used as a basis for recommending greater intakes in healthy populations, in whom such effects may not be relevant. Others have made a case for arrhythmia (see text in Table 11.3).

In addition, interest has rapidly developed in the potential of long chain n-3 fatty acids to influence type 2 diabetes and associated risks (Lovejoy, 1999) because of reported effects on insulin sensitivity (Roche & Gibney, 1999; see Section 11.6 and Table 11.2).

In summary, the evidence for a beneficial effect on cardiovascular disease of the n-3 polyunsaturates (i.e. EPA and DHA) is strong, but is largely based on epidemiological data and secondary prevention trials. Ability to make strong recommendations for increased intakes of long chain n-3 polyunsaturates would be strengthened by a better understanding of the mechanisms involved and the intake levels at which optimal cardioprotection can be achieved. However, the existing trials are clearly valuable in demonstrating increased fish consumption (two portions of oil-rich fish per week) or fish oil supplementation (1 g/day) would decrease the incidence of CHD in an at risk group. The benefits are achievable at intakes of 0.85 g long chain n-3 polyunsaturates per day, equivalent to 2–3 portions of oil-rich fish per week. The richest dietary source is oil-rich fish (see Table 11.4); a typical 100 g portion provides about 2 g long chain n-3 polyunsaturates. In the UK, COMA recommended in 1994 that people eat at least two portions of fish weekly, of which one should be oil-rich (Department of Health, 1994). SACN has reviewed the topic and noted that the evidence has strengthened since 1994 (www.sacn.gov.uk). Revised recommendations published in 2004 have compared the risks and benefits of eating oily fish, and conclude that despite the presence in oily fish of contaminants such as dioxins and polychlorinated biphenyls, the benefits to heart health far outweigh the risks.
Even high risk groups such as pregnant women can benefit from one or two servings per week, and other groups can consume even more (up to four servings per week for men, boys and women past child bearing age). See Chapter 13, Table 13.11 for further details. Despite the push for increased consumption, there are many who dislike or find these fish troublesome to cook, and intake levels have increased only in subgroups of the population in recent years. During the period 1986/7 to 2000/1, average intake of oil-rich fish in the UK has risen from a quarter to a third of a portion per week (Henderson et al., 2002; see Chapter 13, Section 13.4.2).

Supplements of fish oil have been used as a tool to study the effects and mechanisms of action of the long chain n-3 polyunsaturates, and fish oils may be an alternative approach for some sectors of the population. The use of fish oils (>1.5 g long chain n-3 polyunsaturates daily) as a non-dietary alternative to oil-rich fish in the UK has risen from a quarter to a third of a portion per week (Henderson et al., 2002; see Chapter 13, Section 13.4.2).

Table 11.4 Typical n-3 fatty acid content of fish, fish oil and seafood.

<table>
<thead>
<tr>
<th>Food</th>
<th>Portion size (g)*</th>
<th>Total n-3 per portion (g)</th>
<th>Total long chain n-3 per portion (g)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod</td>
<td>120</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>Haddock</td>
<td>120</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>Plaice</td>
<td>130</td>
<td>0.42</td>
<td>0.39</td>
</tr>
<tr>
<td>Herring</td>
<td>119</td>
<td>2.18</td>
<td>1.56</td>
</tr>
<tr>
<td>Mackerel</td>
<td>160</td>
<td>4.46</td>
<td>3.09</td>
</tr>
<tr>
<td>Kippers</td>
<td>130</td>
<td>4.37</td>
<td>3.37</td>
</tr>
<tr>
<td>Salmon</td>
<td>100</td>
<td>2.50</td>
<td>2.20</td>
</tr>
<tr>
<td>Trout</td>
<td>160</td>
<td>2.03</td>
<td>1.84</td>
</tr>
<tr>
<td>Pilchards (canned in tomato sauce)</td>
<td>110</td>
<td>3.16</td>
<td>2.86</td>
</tr>
<tr>
<td>Sardines (canned in tomato sauce)</td>
<td>100</td>
<td>2.02</td>
<td>1.67</td>
</tr>
<tr>
<td>Salmon (canned in brine)</td>
<td>100</td>
<td>1.85</td>
<td>1.55</td>
</tr>
<tr>
<td>Crab (canned)</td>
<td>85</td>
<td>0.91</td>
<td>0.85</td>
</tr>
<tr>
<td>Mussels (boiled)</td>
<td>40</td>
<td>0.26</td>
<td>0.24</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>5 ml</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Cod liver oil capsules***</td>
<td>1 capsule ~500 mg</td>
<td>0.12–0.16</td>
<td>0.08–0.14</td>
</tr>
<tr>
<td>Fish oil concentrate***</td>
<td>1 capsule ~500 mg</td>
<td>0.13–0.30</td>
<td>0.11–0.21</td>
</tr>
</tbody>
</table>

*Portion sizes are based on those for average/medium servings (Ministry of Agriculture Fisheries and Food, 1993).
**Chain length 20 carbons or more: includes 20:5 (EPA), 22:5 and 22:6 (DHA).
***Based on selected products on the market in 2004; capsule size and quantity contained are variable.


(SCientific Advisory Committee on Nutrition, 2004). Even high risk groups such as pregnant women can benefit from one or two servings per week, and other groups can consume even more (up to four servings per week for men, boys and women past child bearing age). See Chapter 13, Table 13.11 for further details. Despite the push for increased consumption, there are many who dislike or find these fish troublesome to cook, and intake levels have increased only in subgroups of the population in recent years. During the period 1986/7 to 2000/1, average intake of oil-rich fish in the UK has risen from a quarter to a third of a portion per week (Henderson et al., 2002; see Chapter 13, Section 13.4.2).

Supplements of fish oil have been used as a tool to study the effects and mechanisms of action of the long chain n-3 polyunsaturates, and fish oils may be an alternative approach for some sectors of the population. The use of fish oils (>1.5 g long chain n-3 polyunsaturates daily) as a non-dietary alternative to oil-rich fish in the UK has risen from a quarter to a third of a portion per week (Henderson et al., 2002; see Chapter 13, Section 13.4.2).

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The likely benefit of increased consumption of long chain n-3 fatty acids is supported by data relating to beneficial effects on blood lipids markers (triglyceride levels, small dense LDL and remnant-like particles), and on markers of endothelial, platelet and inflammatory function (see Chapters 4, 6, 7). However, these effects are generally observed at high doses (2–4 g n-3 fatty acids) and do not explain the beneficial effects of relatively low dose fish oil supplementation or increased fish consumption in secondary prevention trials. For example, studies that employ less than 1.5 g/day do not show effects on blood lipids. Notably, the GISSI trial did not show significant effects on triglycerides at an intake of 0.85 g/day (GISSI-Prevenzione Investigators, 1999). A recent study that compared 0.8 g/day and 1.5 g/day EPA/DHA showed only a non-significant reduction in triglyceride concentration at the higher dose (Finnegan et al., 2003). This brings into question whether triglyceride/small, dense LDL reduction has anything to do with the cardioprotective effects of fish oils. Therefore, evidence that diet can influence the atherogenic lipoprotein phenotype (ALP) is
moderately strong for high intakes of fish oils, but not for levels that would be easily achieved in healthy populations.

It is now considered likely that the effects seen in secondary prevention trials result from an anti-arrhythmic effect (see Table 11.4; Marchioli et al., 2002; Jehangir et al., 2004). The observed reduction in death rate may partly be attributable to suppression of platelet activation via attenuation of thromboxane A2 (TXA2) synthesis (see Chapter 6), even though there is little evidence for a major anti-thrombotic effect of diet-related doses of long chain fatty acids on coronary thrombosis (Kristensen, 2001). Irrespective of the dose, the effects of fish oils on the risk markers discussed are likely to be a combination of eicosanoid-dependent and eicosanoid-independent mechanisms.

Suggestions have been made that high doses of fish oil (>5 g/day n-3 polyunsaturates) without adequate protection by antioxidant vitamins might induce pro-inflammatory responses and adversely affect endothelial function (see Chapter 7, Section 7.8.1 and Johansen et al. (1999)). Hence, where high doses are used the antioxidant status of patients should be evaluated. This could be done by measuring plasma or lymphocyte antioxidant concentrations, which provide useful information of the nutritional status of individuals. But it should be noted that the best measurable product of lipid peroxidation (F2 isoprostanes) may not even respond to increased fish oil intake (see Chapter 5).

### 11.8.4 Gene variability

It has been established for some time that a proportion of individuals respond to fish oil supplementation with an increase in LDL-cholesterol, despite marked reduction in triglycerides. A recent study showed that long chain n-3 fatty acids at intakes of 3 g/day caused a 15% increase in LDL-cholesterol concentrations in subjects who carry the apolipoprotein E4 (apoE4) allele, compared to individuals homozygous for the apoE3 allele, suggesting that effects of long chain n-3 polyunsaturates on blood lipids are genotype dependent. In the UK population, 25–30% of people carry the apoE4 allele, and have a higher total and LDL-cholesterol concentration relative to individuals homozygous for the apoE3 allele. Such effects could be important, and may influence the certainty with which population recommendations for increased long chain n-3 fatty acid intakes can be made. However it should be noted that the study employed daily intakes of long chain n-3 fatty acids of more than 3 g/day, genotyping was undertaken retrospectively and involved individuals with the atherogenic lipoprotein phenotype (Minihane et al., 2000; see Chapter 3, Section 3.8).

Although most studies use fish oil as the source of long chain n-3 fatty acids, there are other sources; Theobald et al. (2004) have demonstrated that consumption of 0.7 g of n-3 fatty acids per day as DHA (derived from algal rather than fish oil) also leads to a significant increase in LDL-cholesterol, without a decrease in triglyceride levels. In post hoc analysis, carriers of the apoE4 allele showed a non-significant increase in LDL-cholesterol relative to non-carriers when adjusted for gender, age and BMI (i.e. subjects homozygous for the E3 allele displayed a 5.9% increase in LDL-cholesterol concentration whereas those heterozygous for E3:E4 displayed a 5.7% increase). Potential heterogeneity of response warrants further investigation, given that 80% of the population carry either or both of the E3 and E4 alleles.

### 11.8.5 Alpha-linolenic acid

An alternative dietary source of n-3 polyunsaturates is plant oils, such as linseed, rapeseed and nut oils, which are relatively rich in alpha-linolenic acid (ALNA) but do not contain the long chain n-3 fatty acids present in fish oil. Theoretically, ALNA can be elongated and desaturated to EPA and DHA, although in man the extent and regulation of this conversion is unclear. In dietary studies, ALNA-rich oils do not appear to reproduce fish-oil-like effects on cardiovascular disease risk factors, including blood lipids, haemostatic factors, or immune, inflammatory and endothelial function (Sanderson et al., 2002a; Finnegan et al., 2003) or platelet function (see Chapter 6). It appears that, in adults, dietary ALNA can increase circulating levels of EPA, but not DHA (Burdge et al., 2003), which may explain the absence of the anticipated beneficial effects. There is the possibility of feeding livestock ALNA-rich oils to provide a means of increasing the amount of n-3 fatty acids present in the meat, milk, eggs and other products, and hence increase human intake of EPA and DHA. However, the viability of this approach will depend on the extent to which
various animal species can complete the conversion and also the costs to industry (Wood et al., 1999), the willingness of the consumer to pay a premium price and, of course, a strong scientific rationale.

As well as looking at opportunities to influence the fatty acid profile of cows’ milk, a five-year EU funded Integrated Project (2004–2008) is considering opportunities to incorporate the biochemistry responsible for EPA synthesis into linseed (www.lipgene.tcd.ie; www.nutrition.org.uk/lipgene).

11.8.6 Trans fats

High intakes of trans fatty acids have been shown to adversely influence the ratio of LDL-cholesterol to HDL-cholesterol (Mensink & Katan, 1990). However, current European diets are generally sufficiently low in these fatty acids not to warrant concern (Hulshof et al., 1999). An exception to this are population groups, often low-income groups, who eat relatively large quantities of foods made with fats derived from hydrogenated oils (which are a source of trans), e.g. block margarines. In Britain, current intake is low at 1.2% of energy (Henderson et al., 2003a; Dietary Reference Value is 2%), but reductions in intake are less apparent in North America.

Individuals with raised lipoprotein(a) (Lp(a)) levels may benefit from advice to restrict products rich in artificially produced trans fatty acids, e.g. hydrogenated oils and spreads (see Chapter 3, Section 3.7).

11.9 Other dietary components that influence blood cholesterol

11.9.1 Soya

Legumes, particularly soya and its products, have been associated with decreased blood cholesterol levels. In a meta-analysis of 38 studies, which provided an average of 47 g/day (range 17–124 g) soya protein, there was an average reduction in total and LDL-cholesterol of 0.6 mmol/l and 0.56 mmol/l, respectively (Anderson et al., 1995). The mechanism of action remains unclear and there was no evidence of a dose–response effect. It is not yet clear whether this is a direct (causal) association or whether it results from the substitution of soya for other foods, or is influenced by other aspects of the diet or lifestyle of people who choose soya (see Table 11.5).

In the UK, the Joint Health Claims Initiative (www.jhci.co.uk) has concluded that ‘the inclusion of at least 25 g of soya protein per day, as part of a diet low in saturates, can help reduce blood cholesterol levels’. To carry the claim, a minimum of 5 g soya protein (natural isoflavones intact), must be present per serving of a food. A similar health claim is also permitted in the US (www.cfsan.fda.gov).

Based on the findings of Anderson et al. (1995), 25 g/day might be expected to reduce total cholesterol by 0.23 mmol/l on average; individuals with a higher initial cholesterol level are likely to experience the greatest reduction. Achievement of an intake of 25 g soya protein is likely to require inclusion of soya derived foods at each meal (see British Nutrition Foundation (2003b) for more details). There is also limited evidence to suggest that soya may exert effects other than cholesterol lowering (see British Nutrition Foundation, 2003b for more details). For information on soya isoflavones and blood pressure see Table 11.1.

11.9.2 Dietary fibre

One of the potential ways by which soya might exert its effect on blood cholesterol is via its fibre content (about 6 g as non-starch polysaccharide per 100 g boiled beans), which is primarily soluble fibre. Soluble fibre (e.g. from oats) has been shown to lower plasma total and LDL-cholesterol (see www.jhci.co.uk), although the effect is small for those consuming moderate amounts (Truswell, 2002). In the meta-analysis by Brown et al. (1999) 2–10 g/day of soluble fibre was associated with a small but significant fall in total cholesterol (0.045 mmol/l per g fibre) and LDL-cholesterol (0.057 mmol/l per g fibre). Three apples or three (28 g) servings of oatmeal, providing 3 g soluble fibre, decreased total and LDL-cholesterol by about 0.13 mmol/l. The effects on plasma lipids were not significantly influenced by source of the fibre (oats, psyllium or pectin) and were independent of background dietary fat intake. One of the components attracting interest is beta glucan, found in oats (Amundsen et al., 2003), although not all studies show an effect (Lovegrove et al., 2000).

The mechanism of this effect remains undefined. Suggestions include bile acid binding, resulting in an up-regulation of LDL receptors and thus increased clearance of LDL-cholesterol; inhibition of hepatic
**Evidence from RCTs**

In a large 6-month RCT in healthy subjects, an increased fruit and vegetable intake (about five portions per day was achieved) had no effect on plasma cholesterol, but a clinically significant fall in blood pressure was recorded (John *et al.*, 2002; see Section 11.4).

Modest cholesterol lowering demonstrated in meta-analysis (0.23 mmol/l with intakes of >25 g soya protein per day).

In a meta-analysis, 2–10 g/day soluble fibre was associated with a small but significant fall in total cholesterol (0.045 mmol/l/g fibre). In this analysis the effect was not significantly influenced by source of fibre.

The findings of a review of clinical trials on the effects of nut-containing diets on blood lipids was inconclusive.

**Plant stanol and sterol esters**

Plant stanols and sterol esters seem to be equally effective in lowering blood cholesterol; 2 g/day, taken as a number of doses over the day via spreads and other foods, achieves a reduction in LDL-cholesterol of 10–15%.

---

**Table 11.5** Other dietary factors that may influence lipids: synthesis of information on nutrition and cardiovascular disease.

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fruit and vegetables</td>
<td>In a large 6-month RCT in healthy subjects, an increased fruit and vegetable intake (about five portions per day was achieved) had no effect on plasma cholesterol, but a clinically significant fall in blood pressure was recorded (John <em>et al.</em>, 2002; see Section 11.4).</td>
<td>There is consistent epidemiological data (observational, cohort, case-control) that diets rich in fruit and vegetables are associated with favourable blood lipids, but this may be an indirect effect associated with dietary substitution effects.</td>
<td>See Section 11.10.2.</td>
</tr>
<tr>
<td>• Soya protein</td>
<td>Modest cholesterol lowering demonstrated in meta-analysis (0.23 mmol/l with intakes of &gt;25 g soya protein per day).</td>
<td>Supportive epidemiological data exist, e.g. in Japanese populations where soya intake is high and typically lifelong.</td>
<td>Legumes, particularly soya and its products, have been associated with cholesterol lowering, but substantial intakes, equating to soya products at each meal, are required to achieve modest effects. Not yet clear if this is a causal relationship, at least in part, or an indirect effect associated with diet substitution effects. A JHCI approved health claim for soya in relation to cholesterol lowering now exists (as does a FDA-approved claim in the USA).</td>
</tr>
<tr>
<td>• Whole-grain cereals and fibre</td>
<td>In a meta-analysis, 2–10 g/day soluble fibre was associated with a small but significant fall in total cholesterol (0.045 mmol/l/g fibre). In this analysis the effect was not significantly influenced by source of fibre.</td>
<td>Diets rich in whole-grain cereals are associated with a lower risk of cardiovascular disease. In US male health professionals, the highest quintile for total fibre intake had an age-adjusted RR of 0.59 compared with the lowest quintile.</td>
<td>Diets rich in whole-grain cereals are associated with heart health, but the mechanism is unclear. Part of the effect may be associated with fibre, although other components may have a role to play (see Section 11.9.2).</td>
</tr>
<tr>
<td>• Nuts</td>
<td>The findings of a review of clinical trials on the effects of nut-containing diets on blood lipids was inconclusive.</td>
<td>An inverse association between nut intake and reduced risk of heart disease has been shown.</td>
<td>Whether nuts lower blood lipids independently of other factors has yet to be determined (see Section 11.9.3).</td>
</tr>
<tr>
<td>• Plant stanol and sterol esters</td>
<td>Plant stanols and sterol esters seem to be equally effective in lowering blood cholesterol; 2 g/day, taken as a number of doses over the day via spreads and other foods, achieves a reduction in LDL-cholesterol of 10–15%.</td>
<td>Plant phytosterols are an effective means of lowering blood cholesterol (see Section 11.9.4).</td>
<td></td>
</tr>
</tbody>
</table>
Table 11.5  (continued)

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Other aspects of diet</td>
<td>No RCTs.</td>
<td>There is some evidence of a cholesterol lowering effect with mycoprotein (see Section 11.9.5).</td>
<td>Whether mycoprotein lowers blood lipids independently of other factors has yet to be determined. With the exception of boiled coffee, other forms of coffee seem to have little effect on blood lipids (Section 11.9.6). The evidence for an effect of CLA on blood cholesterol is weak (Section 11.9.7).</td>
</tr>
<tr>
<td>Infant feeding</td>
<td>No RCTs.</td>
<td>There is some evidence that breastfeeding protects against raised total and LDL-cholesterol in adult life.</td>
<td>The evidence is inconclusive. However, breastfeeding in infancy can be recommended for a number of other reasons.</td>
</tr>
<tr>
<td>Alcohol</td>
<td>A meta-analysis of interventions with moderate alcohol (30 g/day as ethanol, beer, wine or spirits) showed that alcohol is causally related to lower risk of CHD (estimated decrease of 24.7%) through changes in lipids (increased HDL-cholesterol) and haemostatic factors (Rimm et al., 1999).</td>
<td>Moderate alcohol intake increases HDL, but this should be seen in the context of the adverse health effects of higher intakes. Mechanisms are summarised in Section 11.9.8.</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoking increases triglycerides and LDL-cholesterol, and reduces HDL-cholesterol (Freeman &amp; Packard, 1995).</td>
<td>Smoking is a major risk factor for CHD.</td>
<td></td>
</tr>
</tbody>
</table>

CHD: coronary heart disease; CLA: conjugated linoleic acid; HDL: high-density lipoprotein; IHCI: Joint Health Claims Initiative; FDA: Food and Drug Administration; LDL: low-density lipoprotein; RCT: randomised controlled trial; RR: relative risk.
fatty acid synthesis by products of fermentation in the large bowel (e.g. propionate, acetate, butyrate); changes in motility or satiety; or slowed absorption of macronutrients resulting in improved insulin sensitivity (see Brown et al., 1999 and Section 11.6.3).

In a prospective study of 45,000 US male health professionals, followed up for six years, the age adjusted relative risk was 0.59 among men in the highest quintile of dietary fibre (soluble and insoluble) intake compared with men in the lowest quintile (Rimm et al., 1996). The strongest association (RR = 0.45) was for fatal coronary disease.

Consumption of diets rich in whole-grain cereals (e.g. whole-wheat cereals, wholemeal bread and brown rice) has been associated with a lower risk of cardiovascular disease (Pietinen et al., 1996; Jacobs et al., 1999; Liu et al., 1999; Truswell, 2002). It is as yet unclear whether particular constituents are responsible for this effect. Vitamin E, dietary fibre (Richardson, 2000), resistant starch and oligosaccharides (Cummings et al., 1992), as well as plant sterols (see Section 11.9.4; Jones et al., 1997) are some of the components of whole-grain cereals that may contribute to a reduced risk of heart disease (see Chapter 13, Section 13.4.5 and McKeith (2004) for more information on cereals and health).

11.9.3 Nuts
A review of published epidemiological studies (Kris-Etherton et al., 2001) showed that in five large cohort studies, eating nuts more than once per week was associated with a decreased risk of CHD in both men and women (relative risks ranged from 0.45 to 0.75). In four clinical trials comparing the lipid lowering effects of diets with and without nuts, the diets with nuts seemed to have the greater effect. In a further 11 studies they found it was not possible to attribute the lipid lowering effects to nuts specifically because of the complex nature of most of the dietary interventions. They concluded that nuts may contain cholesterol lowering bioactive substances (see Table 11.5).

11.9.4 Plant phytosterol-enriched foods
In an attempt to help achieve reductions in blood cholesterol levels, a number of specific foods have been developed. However, to positively influence cardiovascular disease risk, consumption of such foods should be an adjunct to other dietary and life-style modifications, such as an increase in physical activity and cessation of smoking. Examples of these foods are spreads and other products that contain plant sterols or stanols, which have been shown to reduce LDL-cholesterol by approximately 10–15% (Law, 2000) when consumed periodically during the day, over a period of time. Plant sterol and stanol esters seem to be equally effective, and a daily dose of 2 g is effective; larger doses do not increase efficacy. More details can be found in a report from the EU Scientific Committee on Food (European Commission Scientific Committee on Food, 2002; see Table 11.5).

Cholesterol uptake is inhibited by these phytosterols and its elimination facilitated. The proposed mechanisms of action have been reviewed by Trautwein et al. (2003).

11.9.5 Mycoprotein
There is some evidence that mycoprotein, a food produced by continuous fermentation of Fusarium venenatum on a carbohydrate substrate, exerts a beneficial effect on total and LDL-cholesterol (Turnbull et al., 1990, 1992). A potential mechanism for this effect might concern the high fibre content of mycoprotein, which includes chitin and beta-glucans. For a review of the nutritional properties of edible fungi in general, see Sadler (2003).

11.9.6 Coffee
With the exception of boiled coffee, which has been shown to increase plasma cholesterol (Gross et al., 1997), there is little evidence that moderate coffee intake either increases or decreases cardiovascular risk (Woodward & Turnstall-Pedoe, 1999). Homocysteine levels are raised in heavy coffee drinkers (Grubben et al., 2000; see Chapter 8, Section 8.6).

11.9.7 Conjugated linoleic acid (CLA)
Conjugated linoleic acid (CLA) is a series of isomers of linoleic acid found predominantly in the meat and milk of ruminant animals. Preliminary studies, mainly in animals, suggested a role in beneficially modifying blood lipid profiles, but the results of subsequent studies have been inconsistent, which may be attributed to the variability of the dose level and/or the mix of CLA isomers used, particularly as results
from animal studies show that specific isomers of CLA may be responsible for specific biological effects (De Deckere et al., 1999; Park et al., 1999).

More convincingly, the ability of CLA to influence the lean/fat tissue ratio in favour of lean tissue, has been shown in animal models (Roche et al., 2001). There is preliminary evidence that CLA may also decrease body fat in humans. After weight loss, regain of fat-free mass was favourably and independently influenced by 13-week consumption of 1.8 or 3.6 g/day CLA, which subsequently increased metabolic rate but did not improve body weight maintenance (Kamphuis et al., 2003). However, unfavourable effects of one of the CLA isomers (t10c12 CLA, 3.4 g/day) have been reported in middle-aged obese men (Risérus et al., 2001), related to increased insulin resistance and increased oxidative stress and inflammatory biomarkers, indicating the need for caution.

The main sources of CLA in the human diet are meat from ruminant animals and dairy products, particularly cheese. The amount present varies with the breed and the animals’ diet, being high in grass-fed animals (Lawson, 2001). As CLA is present in milk fat, the amount present is also directly affected by processing. More than 90% of the CLA present in cows’ milk is in the cis-9, trans-11 form, and the majority is produced within the mammary gland itself from the rumen-derived substrate vaccenic acid, although small amounts derive directly from biohydrogenation of linoleic acid in the rumen.

11.9.8 Alcohol

Drinking moderately (about two drinks per day) may provide some protection against cardiovascular disease, particularly in post-menopausal women and middle-aged men (>40 years). Data are emerging that alcohol, rather than some bioactive component within alcoholic drinks, is the predominant protective agent (Hines, 2001; British Nutrition Foundation, 2003a). An increase in HDL-cholesterol (Table 11.5) and changes in platelet function and fibrinolytic parameters (Redmond et al., 2000) are some of the suggested mechanisms of action. Although experimental studies suggest alcohol influences haemostasis, there are discrepancies in the literature as a result of the factor measured and study design. Increasing alcohol intake above moderate levels increases the risk of cardiovascular disease; mech-

11.10 Relationship of diet with other risk factors

Table 11.6 summarises the information presented within the Task Force report linking diet with other risk factors for cardiovascular disease, e.g. endothelial function, haemostasis, oxidative stress, inflammation-related factors and homocysteine.

11.10.1 Diet and endothelial function

For some nutrients, e.g. long chain n-3 fatty acids, there is evidence of a consistent benefit from a clinical perspective, but for others the data are inconsistent (see Table 11.6). Irrespective of this, improvement in endothelial function should not be taken to infer a guarantee of reduced vascular risk (see Chapter 4).

11.10.2 Oxidative stress

Diets rich in fruits and vegetables are associated with reduced risk of chronic disease at the population level. The mechanism is not understood, yet it has been assumed that antioxidant effects may be involved. Although the scientific rationale and results from observational studies have been convincing (e.g. Joshipura et al. (2001); see Chapter 5), randomised clinical trials are not consistently supportive of cardiovascular disease risk reduction with antioxidant supplementation (vitamin C, vitamin E or beta-carotene) (Marchioli, 1999; Chapter 5). These trials have usually involved the administration of one or two antioxidant nutrients given at relatively high doses. Moreover, the interaction between antioxidants, the doses employed, the laboratory tests performed and the subjects involved, may partly explain the findings of these studies. This area has been reviewed recently by Stanner et al. (2004); the results of trials such as SU.VI.MAX, which have investigated the effect of a balanced combination of antioxidants at levels present in foods, have not demonstrated a significant effect.
Evidence from RCTs

High fat meals have been shown to impair endothelial function. A high intake of saturates increases LDL-cholesterol and reduces HDL-cholesterol, and by this mechanism can chronically impair endothelial function. Acute effects: A balance of evidence indicates that endothelial dependent dysfunction occurs soon after a fatty meal. The effect perhaps occurs via a pro-oxidant effect of triglyceride-rich lipoproteins as it may be attenuated by simultaneous antioxidant intake. It is not yet certain whether this effect represents a physiological or pathological response but it is relevant that the extent of the ‘impairment’ reflects the magnitude of the postprandial triglyceride rise.

There is some evidence that diets rich in monounsaturates may improve endothelial function.

Acute intake enhances endothelial function, presumably through insulin release. But a chronically high (refined) carbohydrate intake can lead to hypertriglyceridaemia and associated impairment of vascular response (see above).

Numerous studies have suggested a beneficial effect of antioxidants on vascular function (see Chapter 4, Section 4.9.2). These studies need to be considered in the context of RCTs that show no beneficial effect of antioxidant supplements on heart health endpoints (see Section 11.10.2 and Chapter 5, Section 5.10).

### Table 11.6 Other risk factors: synthesis of the available information on diet and cardiovascular disease.

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endothelial dysfunction</strong></td>
<td></td>
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</tr>
<tr>
<td>Dietary fats</td>
<td>High fat meals have been shown to impair endothelial function. Chronic effects: A high intake of saturates increases LDL-cholesterol and reduces HDL-cholesterol, and by this mechanism can chronically impair endothelial function. Acute effects: A balance of evidence indicates that endothelial dependent dysfunction occurs soon after a fatty meal. The effect perhaps occurs via a pro-oxidant effect of triglyceride-rich lipoproteins as it may be attenuated by simultaneous antioxidant intake. It is not yet certain whether this effect represents a physiological or pathological response but it is relevant that the extent of the ‘impairment’ reflects the magnitude of the postprandial triglyceride rise. There is some evidence that diets rich in monounsaturates may improve endothelial function.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fish oils</td>
<td>Strong and generally consistent data suggest that fish oils (rich in EPA and DHA) have a beneficial effect on vascular function. This effect may be shared by plant-derived n-3 fatty acids (though endpoint studies are lacking).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>Acute intake enhances endothelial function, presumably through insulin release. But a chronically high (refined) carbohydrate intake can lead to hypertriglyceridaemia and associated impairment of vascular response (see above).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dietary antioxidants</td>
<td>Numerous studies have suggested a beneficial effect of antioxidants on vascular function (see Chapter 4, Section 4.9.2). These studies need to be considered in the context of RCTs that show no beneficial effect of antioxidant supplements on heart health endpoints (see Section 11.10.2 and Chapter 5, Section 5.10).</td>
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</tr>
</tbody>
</table>

### Other evidence

**Endpoint/risk factor**

- **Dietary fats**
  - High fat meals have been shown to impair endothelial function.

**Endothelial dysfunction**

- Dietary fats: High fat meals have been shown to impair endothelial function. Chronic effects: A high intake of saturates increases LDL-cholesterol and reduces HDL-cholesterol, and by this mechanism can chronically impair endothelial function. Acute effects: A balance of evidence indicates that endothelial dependent dysfunction occurs soon after a fatty meal. The effect perhaps occurs via a pro-oxidant effect of triglyceride-rich lipoproteins as it may be attenuated by simultaneous antioxidant intake. It is not yet certain whether this effect represents a physiological or pathological response but it is relevant that the extent of the ‘impairment’ reflects the magnitude of the postprandial triglyceride rise.

- There is some evidence that diets rich in monounsaturates may improve endothelial function.

- Fish oils: Strong and generally consistent data suggest that fish oils (rich in EPA and DHA) have a beneficial effect on vascular function. This effect may be shared by plant-derived n-3 fatty acids (though endpoint studies are lacking).

- Carbohydrate: Acute intake enhances endothelial function, presumably through insulin release. But a chronically high (refined) carbohydrate intake can lead to hypertriglyceridaemia and associated impairment of vascular response (see above).

- Dietary antioxidants: Numerous studies have suggested a beneficial effect of antioxidants on vascular function (see Chapter 4, Section 4.9.2). These studies need to be considered in the context of RCTs that show no beneficial effect of antioxidant supplements on heart health endpoints (see Section 11.10.2 and Chapter 5, Section 5.10).
Table 11.6 (continued)

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
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</tr>
</thead>
<tbody>
<tr>
<td>● Folic acid and B vitamins</td>
<td>Relevance largely linked to the ability of</td>
<td>these vitamins to reduce circulating homocysteine levels, which harm the</td>
<td>* Evidence from RCTs. Some RCTs have looked at the effect on biomarkers of antioxidant stress (Buttriss et al., 2002b) but shown inconsistent effects.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>endothelium (see Chapter 8).</td>
<td>* People who consume a diet rich in fruits and vegetables have a lower risk of</td>
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<td></td>
<td></td>
<td></td>
<td>cardiovascular disease. In a large study (84 251 women and 42 148 men), Joshipura</td>
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<td>et al. (2001) reported a significant inverse association between consumption of fruits</td>
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<td></td>
<td></td>
<td>and vegetables, particularly green leafy vegetables and vitamin C rich fruits and</td>
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<td></td>
<td></td>
<td></td>
<td>vegetables, and risk of CHD.</td>
</tr>
<tr>
<td>● L-Arginine</td>
<td>Data are inconsistent, although it is plausible</td>
<td>that L-arginine, a precursor for nitric oxide, might influence endothelial</td>
<td>* Diets rich in fruit and vegetables are associated with reduced risk of chronic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>function.</td>
<td>disease at a population level; the mechanism for this is not understood.</td>
</tr>
<tr>
<td>● Smoking</td>
<td>Arterial endothelial relaxation is impaired</td>
<td>by smoking (clinical and animal data); effect may be attenuated by vitamin C and</td>
<td>* Unclear whether effects are restricted to red wine.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>by smoking cessation.</td>
<td>* Clinical perspective on nutrient–vascular function studies. Whilst evidence to support</td>
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<td></td>
<td></td>
<td>a consistent benefit exists for some nutrients, others are associated with both</td>
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<td></td>
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<td></td>
<td>positive and negative studies. Irrespective of this, improvement in endothelial</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>function should not be taken to infer a guarantee of reduced vascular risk.</td>
</tr>
<tr>
<td>● Alcohol</td>
<td>There are some human and supporting in vitro</td>
<td>linking alcohol intake with better endothelial function.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>data linking alcohol intake with better endothelial function.</td>
<td></td>
</tr>
<tr>
<td>● Maternal and/or fetal nutrition</td>
<td>No RCTs.</td>
<td>There is some evidence that low birthweight is associated with impaired</td>
<td>* Further confirmation is needed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>endothelial function in children and adults.</td>
<td></td>
</tr>
</tbody>
</table>

Oxidative stress

● Fruit and vegetables

People who consume a diet rich in fruits and vegetables have a lower risk of cardiovascular disease. In a large study (84 251 women and 42 148 men), Joshipura et al. (2001) reported a significant inverse association between consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C rich fruits and vegetables, and risk of CHD.
Despite the positive findings from observational and case-control studies, systematic reviews and meta-analyses of the trial data show that supplementation with single or several antioxidants (at high dose) does not reduce risk in either primary or secondary prevention studies; indeed, increased risk was seen in some studies (Asplund, 2002; Clarke & Armitage, 2002).

There is also evidence from observational studies and case-control studies that populations with a high occurrence of cardiovascular disease often have low intakes or plasma levels of antioxidant nutrients, e.g. vitamins C and E and beta-carotene. This has been suggested to increase vulnerability to oxidative stress, particularly in high risk groups such as smokers. Little information exists about the impact on markers of oxidative damage.

In one study, enhanced fruit and vegetable consumption in young male smokers improved plasma antioxidant status and partially offset a reduction in oxidative stability of LDL triggered by fish oil supplementation (4 g/day) (Robert et al., 2003).

No effect of antioxidant supplementation was found in a study looking at the effect of antioxidant supplementation on biomarkers of oxidative damage in healthy men with low fruit and vegetable intake (Jacob et al., 2003).

Low plasma concentrations of antioxidants are observed in smokers (Margetts & Jackson, 1996).

Diets rich in antioxidants can modify the markers of oxidative damage, usually in a beneficial way. However, this does not necessarily mean that cardiovascular disease risk is reduced, and major studies in this area have not specifically tested whether the dietary regimen used actually lowered oxidation, using robust biomarkers (see Chapter 5).

RCTs have generally had either neutral or in some cases adverse effects on cardiovascular disease events.

Findings about the ability of antioxidants to attenuate the effects of (severe) physical activity on the production of oxidation products are mixed.

---

**Antioxidant supplements**

Despite the positive findings from observational and case-control studies, systematic reviews and meta-analyses of the trial data show that supplementation with single or several antioxidants (at high dose) does not reduce risk in either primary or secondary prevention studies; indeed, increased risk was seen in some studies (Asplund, 2002; Clarke & Armitage, 2002).

Much epidemiological evidence and many plausible mechanisms exist (e.g. re. oxidation of LDL-cholesterol and atherosclerosis). No effect of antioxidant supplementation was found in a study looking at the effect of antioxidant supplementation on biomarkers of oxidative damage in healthy men with low fruit and vegetable intake (Jacob et al., 2003).

Diets rich in antioxidants can modify the markers of oxidative damage, usually in a beneficial way. However, this does not necessarily mean that cardiovascular disease risk is reduced, and major studies in this area have not specifically tested whether the dietary regimen used actually lowered oxidation, using robust biomarkers (see Chapter 5).

RCTs have generally had either neutral or in some cases adverse effects on cardiovascular disease events.

---

**Smoking**

Low plasma concentrations of antioxidants are observed in smokers (Margetts & Jackson, 1996).

---

**Physical activity**

See Chapter 12.
Table 11.6 (continued)

<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemostatic factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Fish oils</td>
<td>The effect of fish oil in modulating platelet function, through attenuation</td>
<td>With regard to coagulation, fish oils appear not to activate fasting or postprandial FVII levels, although most studies have been confined to the fasting state.</td>
<td>The evidence for an effect of long chain $n$-3 PUFA is moderately strong. But many studies have used doses &gt;1.5 g/day in order to observe an effect, though a few studies have observed effects at lower doses. Questions remain to be answered. For example, why is there no benefit of long chain $n$-3 PUFA on FVII, given that they lower triglyceride levels? The underlying mechanism for activation of FVII and its meaning for CHD risk are not fully understood. See Chapter 6.</td>
</tr>
<tr>
<td></td>
<td>of thromboxane A₂ synthesis was clearly demonstrated in the GISSI trial (men</td>
<td>With regard to fibrinolysis, the majority of studies suggest that supplementation of the diet with $n$-3 fatty acids (supplement or oil-rich fish) increases PAI-1 levels (see Chapter 6, Section 6.10.6).</td>
<td></td>
</tr>
<tr>
<td></td>
<td>recently recovered from a heart attack). Long-term fish oil supplementation (1 g/day $n$-3 PUFA) significantly reduced the incidence of cardiovascular deaths, non-fatal events and strokes. Another secondary prevention trial (the DART study) demonstrated that oil-rich fish 2–3 times per week for 2 years lowered mortality by 29%. Among men with angina, advice to take fish oil capsules resulted in a higher risk of cardiac death. Plant $n$-3 fatty acids seem to be less effective.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Dietary fat intake and fat quality</td>
<td>An increase in total fat (acute or chronic) causes postprandial activation of FVII. Prior exercise reduces postprandial lipaemia after a fatty meal, but not the accompanying activation of FVII. A background diet with a higher proportion of polyunsaturates or monounsaturates to saturates may attenuate the acute pro-coagulant effects of fatty meals. With respect to postprandial FVII, evidence is now accumulating to suggest that for the background diet, SFA &gt; PUFA &gt; MUFA. Acutely, meal fat effect may be different MUFA &gt; SFA &gt; PUFA. Dietary saturates increase platelet activity (polyunsaturates have the opposite effect and the effect of monounsaturates is as yet unclear). Oxidised LDL enhances platelet activity, whereas high levels of HDL tend to suppress platelet activation.</td>
<td>Evidence is emerging that diets with a high MUFA/SFA ratio may possibly be beneficial in terms of coagulation. The same applies to low fat diets. A high fat diet appears to suppress fibrinolysis but little is known about the effect of fat quality, either acutely or chronically. See Chapter 6.</td>
<td></td>
</tr>
</tbody>
</table>
### Diet and Cardiovascular Disease: Where Are We Now?  

**Alcohol**  
Reports of the effects of acute and chronic alcohol consumption on haemostatic markers have been inconsistent. Regular alcohol seems to reduce fibrinogen, FVII and FVIII, but heavy drinking has been associated with reduced fibrinolytic activity. The effect on platelet function is unclear (may vary with the type consumed and the intensity and duration of consumption).

**Smoking**  
Cessation decreases fibrinogen and platelet aggregation.

**Antioxidant supplements**  
Studies suggest a potential role for retinol, vitamin C and folate/vitamin B6 in haemostasis, but much more research is needed before dietary intervention trials are warranted. The role for vitamin E, particularly on platelet function, is stronger. The efficacy of vitamin E supplementation on thrombosis risk is unknown.

**Non-rapid weight loss**  
Diets that result in gradual weight loss, e.g. low fat/high fibre diets, improve fibrinolytic activity. The effect of energy restriction on fibrinogen and free fatty acids (which stimulate fibrinogen output) vary depending on whether weight loss is rapid or gradual. Levels rise if weight loss is rapid and fall with more gradual loss.

**Physical activity**  
See Chapter 12, Table 12.2.

### Inflammation-related factors

**Weight loss**  
Circulating CRP is positively associated with body mass, and weight loss has been demonstrated to result in decreased levels of CRP in the circulation (Chapter 7).

**Smoking**  
Cessation decreases fibrinogen.

As well as exerting beneficial effects on HDL-cholesterol, moderate alcohol intake may also be beneficial in terms of haemostasis (see Table 11.1 for information on alcohol and blood pressure).
<table>
<thead>
<tr>
<th>Endpoint/risk factor</th>
<th>Evidence from RCTs</th>
<th>Other evidence</th>
<th>Additional comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish oils</strong></td>
<td>No effect of fish oil on CRP shown by several studies. Evidence for effects of fish oil on inflammatory cytokines and soluble adhesion molecules is inconsistent. Some emerging evidence exists that fish oil may improve plaque stability.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antioxidants</strong></td>
<td>High dose vitamin E lowers CRP in type 2 diabetes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td>U-shaped association with CRP in men; non-drinkers and heavy drinkers have highest levels.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Homocysteine**

- **Folic acid**: The evidence that supplementation with folic acid and other B vitamins can reduce plasma homocysteine is strong. Plausible mechanistic data exist (see Chapter 8).

  - **Other B vitamins**: Supplementation with folic acid and vitamin B₁₂ reduced homocysteine levels by about 30%.

**Adipocyte-related factors**

- **Low energy diets/weight change**: No RCTs. Secretion influenced by weight loss and fasting: leptin, IL-6 and ASP fall (they rise with weight gain and excess energy intake). But adiponectin increases with weight loss and decreases with obesity. Animal data suggest an influence of acute and longer duration exercise on several adipokines. Detailed information on the effects on adipokines of macro- and micronutrients is lacking.

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ASP: acylation stimulation protein; CHD: coronary heart disease; CRP: C-reactive protein; DART: Diet and Reinfarction Trial; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FVII: factor VII; FVIII: factor VIII; GISSI: GISSI-Prevenzione Trial; HDL: high-density lipoprotein; IL-6: interleukin-6; LDL: low-density lipoprotein; MUFA: monounsaturates; PAI-1: plasminogen activator inhibitor-1; PUFA: polyunsaturates; RCT: randomised controlled trial; SFA: saturates.
Antioxidant supplements cannot therefore be recommended as a tool for reducing risk of cardiovascular disease. Because there is no evidence that any particular nutrient or bioactive agent present in fruit and vegetables makes a special contribution, currently the most prudent public health advice is to increase vegetable and fruit consumption. For reviews see Buttriss (2003) and Stanner et al. (2004). The current advice to consume at least five portions of a variety of fruits and vegetables on a daily basis is therefore appropriate and to be encouraged.

It is likely that the effects of foods such as fruits and vegetables, recognised as rich sources of antioxidant vitamins, are only partly mediated by their antioxidant properties. For example, vitamins C and E tend to show anti-inflammatory effects and an improvement in endothelial function, and the literature also tends to support an effect of vitamin E on platelet function (see Chapter 5).

Apart from the antioxidant vitamins, in plants there are many thousands of other substances with antioxidant properties, such as the flavonoid family (British Nutrition Foundation, 2003a). Flavonoids are found throughout the plant kingdom and are present in foods as diverse as wine, tea, fruit, onions and green vegetables. Flavonoid intake has been shown to be associated with a reduced risk of CHD (but not stroke) in most but not all studies (e.g. Hollman & Katan, 1999; Yochum et al., 1999).

It remains unclear whether it is the antioxidant properties of these substances that underlie their apparent effect, or whether some other mechanism is responsible, e.g. influence on cell signalling cascades (Buttriss et al., 2002b).

11.10.3 Diet and haemostatic factors

The evidence for an effect of EPA/DHA is moderately strong, but many studies have used high doses in excess of 1.5 g/day (see Table 11.6). Plant n-3 fatty acids, i.e. alpha-linolenic acid, seem to be less effective, although more research is required. Evidence is emerging that fat quality may be important in terms of coagulation (see Chapter 6).

11.10.4 Diet and inflammation

Current knowledge is summarised in Table 11.6 (see also Chapter 7).

11.10.5 Folate and B vitamins

It is likely that elevated blood homocysteine will moderately increase the risk of cardiovascular disease. There is also increasing evidence that folic acid supplementation has a beneficial effect on endothelial function as measured by flow-mediated vasodilation or haemostatic markers (see Brown & Hu, 2001), which is mediated through the homocysteine-lowering effect of folic acid, although other mechanisms may contribute to the benefit. Inclusion of vitamins B6 and B12 has been shown to amplify the homocysteine-lowering effect of folic acid (Chapter 8). Investigations in South Asians indicate that hyperhomocysteinaemia is related to B12 deficiency, which is particularly prevalent in this population (Antony, 2003).

Large-scale trials are underway to confirm the association between blood homocysteine and risk of cardiovascular disease, and to determine if it is reversible by folic acid, vitamin B12 and/or vitamin B6. Meanwhile, it is not currently recommended that the general population supplements its diet with folic acid or other B vitamins in order to reduce the risk of cardiovascular disease (Chapter 8).

11.11 Conclusions

The original diet–heart hypothesis was overly simplistic: it is now recognised that the effects of diet on CHD can be mediated through multiple biological pathways other than serum total cholesterol or LDL-cholesterol (Fig. 11.3). Nevertheless, the influence of...
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11.11.1 Dietary fat

Epidemiological evidence has established that the amount and type of dietary fat consumed by populations is related to blood cholesterol levels and mortality rates from CHD; on the basis of this, restriction of total fat, saturates and cholesterol has been advocated. However, the relevance of this combination has been questioned, partly because of the difficulty of achieving compliance and partly because of the uncertainty of what should replace the saturates (Clarke & Frost, 2001; see also Sections 11.7 and 11.8).

Saturates increase and polyunsaturates decrease total and LDL-cholesterol. All three classes of fatty acids (saturates, monounsaturates and polyunsaturates) elevate HDL-cholesterol when they replace carbohydrates in the diet, and this effect is slightly greater with saturates. Also, triglyceride levels increase when dietary fatty acids are replaced by carbohydrate. Because replacement of saturates with carbohydrates proportionally reduces both LDL- and HDL-cholesterol and increases triglycerides, this change would be expected to have minimal benefit on CHD risk. However, when monounsaturates or polyunsaturates replace saturates, LDL-cholesterol falls and HDL-cholesterol changes only slightly (Hu & Willett, 2002). In addition, substituting polyunsaturates for saturates may have beneficial effects on insulin sensitivity and type 2 diabetes.

Using estimates derived from a meta-analysis of metabolic ward studies (Clarke et al., 1997), Clarke and Frost (2001) have estimated the impact of dietary change in a free-living UK population, using data from the 1986/7 survey of British adults (Gregory et al., 1990). The combined effect of a change in the type, but not the amount, of dietary fat that involves a 10% reduction in energy from saturates, a 5% increase in energy intake from monounsaturates, a 5% increase in energy from polyunsaturates, together with a 200 mg reduction in dietary cholesterol would be a fall in total cholesterol of about 0.8 mmol/l, primarily due to a fall in LDL-cholesterol with only a small reduction in HDL-cholesterol. Furthermore, they estimated that isoenenergetic replacement of saturates by unsaturates would result in about three times the reduction in cholesterol level compared with replacement by complex carbohydrate (i.e. a moderate versus low fat diet). Their findings were not influenced by gender, total energy intake or body weight.

Trans fatty acids are thought to have a similar effect on blood cholesterol as saturates. Additionally, they increase plasma levels of lipoproteins and triglycerides, and may reduce endothelial function by impairing flow-mediated dilatation (Chapter 4). There is also evidence that they adversely affect essential fatty acid metabolism and prostaglandin balance (see also Section 11.8.6).

Only a few dietary trials with CHD endpoints have been conducted, and most were among patients with CHD, e.g. the n-3 fatty acid trials. In a systematic review of cohort studies, the inverse association for n-3 fatty acids was stronger for fatal CHD than for non-fatal myocardial infarction, and the benefit is most evident in populations with higher than average risk of CHD (Marckmann & Gronbaek, 1999). Information published subsequently has supported this conclusion.

The overall evidence to support the benefit of long chain n-3 fatty acids in cardiovascular disease appears more convincing than that for other nutrients or food components, largely due to the positive results from the DART and GISSI-Prevenzione trial. The effects of monounsaturates are likely to be subtler but are of potential benefit long-term. It is likely that the effects are mediated by multiple and complex mechanisms.

In conclusion, for the vast majority of the population, moderation in fat intake (30–35% of energy) needs to be emphasised, with the partial replacement of saturates by unsaturated fatty acids being implicit within this message. Also, inherent in this message is the importance of sources of long chain n-3 polyunsaturates in the diet. n-3 fatty acids at intakes of 1 g/day, whether via supplements or food sources, are likely to be beneficial for those at risk of cardiovascular disease. A recent meta-analysis of randomised controlled trials (RCTs) concluded that dietary and supplemental intake of n-3 polyunsaturates (0.3–6.0 g EPA, 0.6–3.7 g DHA) reduces overall mortality, mortality due to myocardial infarction, and sudden death in patients with CHD (Bucher et al., 2002). These fatty acids should now be considered to be a recommended component of secondary prevention, and a dose of approximately 1 g of EPA and DHA combined has recently been recommended by the American Heart Association.
Diet and Cardiovascular Disease: Where Are We Now?

(Kris-Etherton et al., 2003). It is also likely that the general (healthy) population, many of whom have some form of cardiovascular disease, would benefit from reductions in risk of cardiovascular disease if intakes were increased to the recent recommendation (Scientific Advisory Committee on Nutrition, 2004) for long chain \( n-3 \) fatty acids of 0.45 g/day. It may be possible to derive a definitive figure once RCTs linking fish consumption or fish oil supplementation with primary prevention of CHD have been completed. A key challenge is how to increase intakes of oil-rich fish, which currently fall below targets (see Chapter 13, Section 13.4.2).

11.11.2 Other dietary components

Regular consumption of a variety of plant foods (e.g. fruit and vegetables, whole-grain cereals, soya and nuts) has been associated with cardiovascular health. Joshipura et al. (2001) have reported a significant inverse association between consumption of fruits and vegetables, particularly green leafy vegetables and vitamin C rich fruits and vegetables, and risk of CHD. The mechanism is not understood. Robust evidence for promoting specific fruits or vegetables, or specific components is lacking, emphasizing the importance of variety when selecting from this food group.

In epidemiological studies, higher consumption of whole-grain cereals has been associated with lower risk of CHD. Also, prospective cohort studies have consistently found an inverse association between fibre intake and CHD risk. The latter cannot be fully explained by a cholesterol lowering effect. The low GI of foods rich in fibre, and numerous micronutrients and phytochemicals present in whole-grain cereals may also contribute to benefit. In several controlled clinical studies, feeding low GI meals to diabetic patients led to significant improvements in glycaemic control and lipid profile, but larger studies are needed, especially in non-diabetic subjects. Interest has focused on the soluble fibre content of grains such as oats (see Section 11.9.2). Plant phytosterols are an effective way of reducing blood cholesterol, indicating a possible role for functional foods.

An inverse association between nut consumption and risk of CHD has been seen consistently in prospective studies, but it needs to be determined whether this effect is independent of other dietary and lifestyle factors.

Folic acid has been shown to reduce homocysteine, and trials are underway to establish whether or not this is associated with a reduction in cardiovascular risk. The role of antioxidant nutrients in cardiovascular health remains unclear. Alcohol in moderation may be beneficial, especially for middle-aged men and post-menopausal women.

Most is known about the impact of dietary components on lipid metabolism; however, it is becoming clear that many other mechanisms are important, as illustrated in Fig. 11.3 and by the example of sodium, which influence blood pressure rather than plasma lipids.

11.11.3 Dietary patterns

Recently, several studies have reported the role of overall dietary pattern in predicting long-term risk of CHD. These are discussed in Chapter 13, Section 13.5.3.

In conclusion, substantial evidence shows that diets incorporating non-hydrogenated unsaturated fatty acids as the predominant form of fat, whole-grain cereals as the main form of carbohydrate, an abundance of fruits and vegetables, and adequate long chain \( n-3 \) fatty acids can offer significant protection against CHD. Such diets, together with regular physical activity, avoidance of smoking and maintenance of a healthy body weight, may prevent the majority of cardiovascular disease in Western populations (see Chapter 13 for details). We are beginning to learn about the interactions that occur between diet and lifestyle factors such as physical activity. For example, whilst low fat diets effectively reduce LDL-cholesterol, they can simultaneously raise triglyceride levels, but this latter effect can be offset by moderate physical activity or the inclusion of \( n-3 \) long chain fatty acids in the diet, demonstrating the need for a holistic approach.

11.11.4 Areas of uncertainty

There remain some areas of uncertainty:

- The optimal amounts of monounsaturates and polyunsaturates remain to be established.
- The optimal balance of \( n-3 \) and \( n-6 \) has not been clearly defined.
- The role of phytochemicals in prevention of CHD is promising but unsettled. Six prospective cohort
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studies have evaluated the association of flavonoid intake with risk of CHD: a significant inverse association is shown in some \( n = 4 \) but not others.

- The effect of plant sterols has been well documented, but long-term effects are as yet unknown.
- Although a body of experimental data has demonstrated the role of antioxidant vitamins in reducing oxidative stress and substantial epidemiological evidence has linked intake of vitamin E with lower CHD risk, results of supplementation trials have not supported these findings, and in some cases adverse effects have been reported. Primary prevention trials may provide more insight.
- Studies of the role of minerals in reducing CHD risk have largely been ecological or case control, and have yielded inconclusive results. Large prospective studies or randomised controlled trials with clinical endpoints are required to resolve the role of individual minerals from the diet and from supplements.

11.12 Key points

- Since the UK recommendations for cardiovascular disease were published in 1994, knowledge of the pathophysiology and risk factors for heart disease has progressed, providing the impetus to re-examine current dietary guidelines, the main emphasis of which is reduction in total fat and saturates.

- The Task Force’s analysis of the interaction of diet with emerging risk factors suggests that greater emphasis on fat quality (fatty acid profile) is now justified. Furthermore, as well as considering the impact of dietary fat on blood lipids, it is equally important to take into consideration the impact on insulin sensitivity and energy balance. The strengths and weaknesses of different approaches are hotly debated (e.g. low fat versus moderate fat as percentage of energy).

- It is also now clear that attention needs to be paid to gene–nutrient interactions, as dietary measures that benefit one individual may be detrimental to another, as has now been demonstrated with fish oil in those with a particular genetic variant of apolipoprotein E.

- Also, greater attention needs to be paid to the interactive and synergistic effects of diet and activity level, both in a positive and negative sense, and the implications of maternal and fetal nutrition on future health of the offspring.

- Overall, the evidence supports the importance of dietary variety. Although the impact of individual foods on risk factors such as blood lipids may be small, if combined these individual influences have the potential to make a substantial impact.

- Given people’s reluctance to change dietary patterns, it is important that alongside provision of dietary advice is research that seeks to identify the extent to which the nutrient composition of staple foods such as meat, milk, grains, fruits and vegetables can be modified through feeding regimens and breeding programmes, as it being done in the EU-funded Lipgene project, which is focusing on ways of tackling the metabolic syndrome.

- Saturates increase and polyunsaturates decrease total and LDL-cholesterol. All three classes of fatty acids (saturates, monounsaturates and polyunsaturates) elevate HDL-cholesterol when they replace carbohydrates in the diet, and this effect is slightly greater with saturated fatty acids. Also, triglyceride levels increase when dietary fatty acids are replaced by carbohydrate. When monounsaturates or polyunsaturates replace saturates, LDL-cholesterol falls and HDL-cholesterol changes only slightly. The benefit of long chain \( n-3 \) fatty acids is convincing largely due to the positive results from secondary prevention trials. The effects of monounsaturates are likely to be more subtle, but are of potential benefit long-term.

- With regard to endothelial dysfunction, a habitually high saturates intake increases LDL-cholesterol and lowers HDL-cholesterol, and by this mechanism impairs endothelial function. Classical risk factors explain only 20% of the variation in endothelial function. Some associations with dietary factors are emerging, but smoking remains a major factor.

- With regard to oxidative stress, diets rich in antioxidant nutrients can modify the markers of oxidative damage, usually in a beneficial way,
but this does not necessarily mean that cardiovascular disease risk is reduced.

- The evidence for an effect of long chain \( n-3 \) fatty acids on haemostasis is moderately strong, but most studies have used doses (as fish oil) in excess of 3 g/day. The strongest evidence for oil-rich fish comes from secondary prevention studies in which the incidence of sudden death is reduced. Moderate alcohol intake may be beneficial for haemostasis as is moderate physical activity; smoking is harmful.

- Inflammation and adipocyte-related factors are both areas in which associations with diet are beginning to emerge, but research is at an early stage.

- Plasma homocysteine is related to CHD and stroke, but there is uncertainty about whether it is a causal relationship. Results of trials with folic acid supplements for prevention of cardiovascular disease are awaited.

- For the majority of the population, moderation in fat intake (30–35% of energy) needs to be emphasised, with the partial replacement of saturates by unsaturated fatty acids being implicit within this message. \( n-3 \) fatty acids are now recognised as important dietary factors and intakes of 1 g/day (via diet or supplements) are likely to be beneficial for those at risk of a heart attack (secondary prevention). The recommendation for the general population has recently been increased to 0.45 g/day of long chain \( n-3 \) fatty acids. Coupled with this is the need for a varied diet including a wide range of plant foods, many of which have been associated with heart health. The *Balance of Good Health* (the UK Government’s plate model depicting a healthy, balanced diet) provides a useful guide for constructing such a diet and emphasises the importance of lean meat and low fat dairy products as sources of essential nutrients.
Physical Activity: Where Are We Now?

12.1 Introduction

Two recent surveys among adults, the Health Survey for England (Department of Health, 1999a) and the National Diet and Nutrition Survey (NDNS) of adults (Ruston et al., 2004) have demonstrated the low prevalence of physical activity in the UK (Department of Health, 1999a). This situation is of concern because regular physical activity is known to benefit health (see Section 12.2 and Chapter 2, Section 2.8). In the Health Survey for England (Department of Health, 1999a), only 37% of men achieved the recommended level, i.e. taking part in physical activity of at least moderate intensity for at least 30 minutes on most days (at least five) of the week. For women the figure was 25%, and participation declined with age for both groups. In men, the proportion achieving the recommendations fell from 58% among 16–24 year olds to 7% in those over 75 years. In women in the age group 16–54 years, it was similar across age groups at about 30–32%. For men, but not women, activity levels were higher in manual social classes because of the contribution of occupational activity. A similar pattern of inactivity was evident in the NDNS (Fig. 12.1).

Overall, 6 in 10 men and 7 in 10 women aged 19–64 years do not undertake enough physical activity to benefit their health (see Section 12.3) (Ruston et al., 2004). Activity levels decrease with age: 7 in 10 men and 8 in 10 women aged 75 years are inactive (Department of Health, 2004a).

Physical activity levels are greatly affected by occupational activities (Table 12.1), a measure of which is the physical activity ratio (PAR). These days, the majority of the population lead sedentary lives, with occupations that fall within the light or moderate categories. This makes recreational or opportunistic activity all the more important. The proportion (adjusted for age) of men who walk as a leisure-time activity is 38% higher in social class I than social class V. For women, rates are 67% higher in social class I than in social class V (Department of Health, 2004a). Similar trends are observed for sports participation. In both men and women and in all age groups, low educational attainment predicts higher levels of inactivity. In the 25 years between national travel surveys (1975–6 versus 1999–2002), total miles travelled per year on foot fell by 26%, and by bicycle 24% (Department of Health, 2004a).

The level of energy expenditure associated with different types of activity can be found in the Chief Medical Officer’s (CMO) report, At Least Five a Week (Department of Health, 2004a).

In England, 16% of children aged 2–15 years are obese (Department of Health, 2004a), and activity levels among children are also a cause for concern. The NDNS revealed that with the exception of very young children (4–6 year olds), the majority of young people are largely inactive (Gregory et al., 2000). About 40% of boys and 60% of girls aged 7–14 years spent less than one hour per day in activities of at least moderate intensity, and thus failed to meet the recommendations for young people. In 15–18 year olds, the proportions rose to 56% of boys and 69% of girls (see Buttriss, 2002 for a summary). A study among Scottish children shows that a sedentary lifestyle is established during the pre-school years (Reilly et al., 2004); at ages 3 years and 5 years, almost 80% of time (excluding night time) was spent in sedentary behaviour.
12.2 Role of physical activity in promoting cardiovascular health

In countries such as the USA and the UK, the prevalence of obesity has continued to rise despite a reported reduction in the proportion of energy consumed as fat (Willett, 1998) (see Chapter 1 and Chapter 13, Section 13.4). Among the likely explanations for this is a sedentary lifestyle with a low level of energy expended on physical activities, and important interactions may exist between genetic make-up, dietary fat intake and physical fitness (Astrup, 1999).

It is difficult to measure physical activity directly, and most studies have relied on self-reported levels. However, arguably a more objective measure is cardiorespiratory fitness (US Department of Health and Human Services, 1996). Regular physical activity and higher cardiorespiratory fitness are associated with lower overall mortality in a dose–response fashion, broadly irrespective of the age group studied.
Men and women who are physically active, even at a moderate level of intensity, experience lower overall mortality rates (Lee & Skerrett, 2001) than those who are inactive. However, further research is needed to clarify the contributions made by the components, e.g. intensity, duration and frequency.

12.2.1 Cardiovascular endpoints

Men and women who are physically active experience a lower risk of cardiovascular disease in general and CHD in particular (US Department of Health and Human Services, 1996; Lee & Skerrett, 2001; Wannamethee & Shaper, 2002). For example, in a review of 27 cohort studies (Berlin & Colditz, 1990) sedentary groups have been shown to be twice as likely to experience CHD than more active groups; in middle-aged men and women, level of leisure time physical activity has also been shown to be inversely related to the risk of CHD, hypertension and diabetes (Haapanen, 1997a). Furthermore, in the Nurses’ Health Study, a large prospective study in the USA, both brisk walking and regular vigorous exercise were associated with a reduction in risk of coronary events by 30–40% (Manson et al., 1999), and sedentary women who became active in middle-age or later had a lower risk than their counterparts who remained sedentary (see Table 12.2). As with overall mortality, the epidemiological literature for CHD indicates an inverse association and a dose-response gradient between physical activity level or cardiorespiratory fitness and incidence of CHD (see Fig. 12.2).

The picture for stroke is less clear, perhaps because studies have generally not distinguished between ischaemic and haemorrhagic stroke, which have different pathophysologies. However, the Nurses’ Health Study has shown that physical activity, including moderate intensity activity such as brisk walking, was associated with a substantial fall in risk of both total and ischaemic stroke, in a dose–response manner (Hu et al., 2000b). This conclusion is supported by a review of population studies (Wannamethee & Shaper, 2002). Physically active people are also less likely to experience hypertension and type 2 diabetes than inactive people (Department of Health, 2004a).

Cohort (prospective) studies show that physical inactivity is associated with an increased risk of later developing hypertension among both men and women, and intervention trials provide evidence that moderate intensity activity may achieve a similar, or an even greater blood-pressure-lowering effect than vigorous intensity activity. However, few studies have addressed the intensity question (for details of studies, see US Department of Health and Human Services, 1996; Department of Health, 2004a; see also Table 12.2 and Section 12.2.2).

The biological mechanisms for these effects are plausible, and the benefits seen may in part be due to characteristically high concentrations of plasma high-density lipoprotein (HDL) cholesterol and low concentrations of triglycerides (and associated effects on atherosclerosis) in those who are physically active (Hardman, 1999). Even a single episode of short-term (≤1 day) physical activity can result in an improved lipid profile (for details of studies, see Hardman,
Table 12.2  Summary of influence of physical activity on risk factors for cardiovascular disease.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Findings of intervention studies§</th>
<th>Evidence from studies using other designs</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Body fatness, amount and distribution | • Reviews and meta-analyses conclude that physical activity promotes fat loss, whilst preserving or increasing lean mass.  
• Modest rate of weight or fat loss is positively related, in a dose–response manner, to the energy expended in activity.  
• Combination of increased physical activity and energy intake restriction is more effective for long-term weight regulation than dieting alone. | • Cross-sectional studies report lower weight, BMI or skinfold thicknesses among people with higher levels of physical activity or fitness.  
• Prospective studies less consistent; some show inverse association between amount of leisure time physical activity and later weight gain, others do not.  
• Several large cross-sectional studies report an inverse association between energy expenditure from physical activity and indicators of central body fat distribution. | • Findings from RCTs differ according to duration of intervention: short-term trials (≤16 weeks) report higher energy expenditures (~9.2 MJ/wk) and loss of weight and fat (averages 0.26 kg/wk and 0.25 kg/wk, respectively) than trials ≥26 weeks (expenditures ~4.6 MJ/wk; weight/fat loss 0.06 kg/wk).  
(For more information, see DiPietro, 1999; Ross & Janssen, 2001; Williamson et al., 1993; Haapanen, 1997b; Department of Health, 2004a) |
| Blood pressure | • Findings based on meta-analyses of RCTs show that aerobic exercise decreases systolic and diastolic blood pressure by 3–6 mm Hg.  
• Some evidence that moderate intensity exercise (40–50% maximal oxygen uptake) is at least as effective as vigorous exercise. | • Several cohort studies (men and women) show that physical inactivity is associated with increased risk of later developing hypertension.  
• Least active have 30% greater risk of becoming hypertensive than most active. | Single episode of exercise lowers blood pressure for some hours.  
(For more information, see Hu et al., 2000b; Fagard, 2001; Arroll et al., 1994; Kelley & McClellan, 1994; Arroll & Beaglehole, 1992; Department of Health, 2004) |
| Blood lipids | Intervention studies reveal:  
• ↑ HDL-cholesterol, average 5%  
• small, usually non-significant changes in total cholesterol  
• LDL-cholesterol ↓, average 5% (inconsistent)  
• triglycerides ↓, average 3.5% (inconsistent) | • Endurance athletes have 20–30% higher HDL-cholesterol than sedentary peers.  
• Endurance athletes often (not invariably) have ↓ LDL-cholesterol and ↓ triglycerides. | Findings of intervention studies inconsistent.  
• Most consistent change is ↑ HDL-cholesterol found in about half of more than 60 intervention studies; no influence of age or sex.  
• Sizeable weight loss through physical activity enhances favourable effects.  
• Exercise attenuates the ↓ HDL-cholesterol that accompanies substitution of dietary fat with carbohydrate.  
• Single episode of exercise reduces triglycerides.  
(For more information, see Leon & Sanchez, 2001; Durstine & Haskell, 1994; Department of Health, 2004a) |
### Table 12.2 (continued)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Findings of intervention studies</th>
<th>Evidence from studies using other designs</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Insulin sensitivity and type 2 diabetes | • Two intervention studies found that people with impaired glucose tolerance who increased their physical activity level were half as likely to develop diabetes as those in the control group. A third study also demonstrated a benefit of physical activity over and above that seen with diet alone.  
• One RCT found that, in people with impaired glucose tolerance, increased physical activity was as effective in reducing the risk of developing diabetes as metformin and was associated with fewer side effects. | • Large-scale prospective studies demonstrate that physical activity is inversely related to incidence of type 2 diabetes, even after adjusting for confounding factors. There is some evidence for a dose–response gradient.  
• Many cross-sectional studies have found physical inactivity to be positively and significantly related to type 2 diabetes.  
• Cross-sectional studies found that, after a meal, blood glucose and insulin values were significantly higher in less active than in more active people. | • A single session of exercise increases insulin sensitivity.  
• Endurance-trained athletes show greater insulin sensitivity than sedentary people.  
• Among these athletes and among people who are regularly active at a recreational level, enhanced insulin sensitivity deteriorates rapidly (over 2–3 days) when the habit of exercise is interrupted.  
(For more information, see Hu, 1999; Pan et al., 1997; Tuomilehto et al., 2001; Diabetes Prevention Program Research Group, 2002; Kelley, 2001; Department of Health, 2004a) |
| Coagulation and haemostasis          | • Evidence inconsistent on changes in fibrinogen and markers of fibrinolytic activity (tPA, PAI-1).  
• Some evidence that training at moderate intensity decreases platelet adhesion and aggregation. | • Cross-sectional studies consistently show that an episode of exercise acutely stimulates fibrinolytic activity. | • An episode of strenuous exercise activates platelets in sedentary but not in physically active, healthy subjects.  
• By contrast, an episode of moderate exercise (50–55% maximal oxygen uptake) has an inhibitory effect on platelet adhesion and aggregation.  
(For more information, see Rauramaa et al., 2001) |
| Endothelial function                 | • One small RCT found that exercise training improves endothelium-dependent vasodilatation of coronary arteries. | • Animal studies show that training increases the interior diameter of major coronary arteries, increasing maximal coronary blood flow. | • Animal studies show that training increases the interior diameter of major coronary arteries, increasing maximal coronary blood flow.  
(For more information, see US Department of Health and Human Services, 1996) |

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8 Unless otherwise stated, intervention studies have employed exercise regimens designed to improve fitness, i.e. training at 50–75% maximal oxygen uptake, 3–4 times per week, 30–60 minutes per session.  
BMI: body mass index; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PAI-1: plasminogen activator inhibitor; RCT: randomised controlled trial; tPA: tissue plasminogen activator.
1999; US Department of Health and Human Services, 1996), and other possible mechanisms include effects on blood pressure, thrombosis, arrhythmias and the availability of oxygenated blood for heart muscle (see Table 12.2).

12.2.2 Cardiovascular risk factors

In common with dietary interventions that produce weight loss (see Chapter 11, Section 11.3), increased physical activity may contribute to improvements in a number of cardiovascular risk factors, including blood pressure level, blood lipids, glucose tolerance and insulin sensitivity (Grimm, 1999), thus reducing the risk of developing type 2 diabetes (see Table 12.2 for details).

(i) Body fatness: amount and distribution

In a large cohort study (3515 men and women aged 25–74 years), low recreational physical activity was inversely related to body weight, and strongly related to major weight gain (>13 kg) in the previous 10 years (Williamson et al., 1993). A summary of the influence of physical activity on body fatness and fat distribution is provided in Table 12.2. A review of well-controlled published studies (1966–2000) revealed that increasing physical activity level is positively related to reductions in total adiposity in a dose–response fashion, particularly in short-term trials (Ross & Janssen, 2001). In these trials, although physical activity was associated with a reduction in abdominal and visceral fat, it was concluded that there was insufficient evidence to determine a dose–response relationship.

Tackling the increasing adiposity in childhood is a major challenge (see Section 12.4).

(ii) Blood pressure

As blood pressure increases, so does the risk of heart disease and stroke. A review of nine randomised controlled trials investigating chronic training effects and involving 245 people aged 29–72 years suggested that moderate (60% VO\(_{\text{max}}\)) activity is associated with a net reduction of about 4 mmHg in systolic blood pressure (Mulrow & Jackson, 1999). In another study, both a structured exercise intervention (to achieve 50–80% VO\(_{\text{max}}\) on at least three occasions for a week) and a lifestyle approach (designed to accumulate at least 30 minutes of moderate intensity physical activity on most days of the week, e.g. by brisk walking), achieved a 5 mmHg reduction in diastolic blood pressure (Dunn et al., 1999; see Table 12.2). However, even after a single exercise session, blood pressure is reduced for some hours.

(iii) Diabetes and insulin sensitivity

Considerable evidence (cohort studies and intervention trials) supports a protective effect of physical activity on the likelihood of developing type 2 diabetes (for details of studies, see US Department of Health and Human Services, 1996; Department of Health, 2004a). For example, the prospective Nurses’ Health Study has shown that greater physical activity level is associated with a substantial reduction in risk of type 2 diabetes, including physical activity of moderate intensity and duration, even after adjustment for body mass index (BMI) (Hu et al., 1999). The Chief Medical Officer (CMO) report concluded that, as with cardiovascular disease, regular physical activity can reduce risk of type 2 diabetes by up to 50% (Department of Health, 2004).

Several plausible biological mechanisms exist to explain this effect, e.g. improved insulin sensitivity (see Chapter 2, Section 2.8). Furthermore, there is some evidence of a dose–response relationship.

The ability of moderate physical activity to substantially ameliorate the risk of type 2 diabetes is independent of body weight, as demonstrated by three randomised controlled trials, which reported reductions in risk of 42–58% (Pan et al., 1997; Tuomilehto et al., 2001; Diabetes Prevention Program Research Group, 2002). In the most recent of these studies, lifestyle changes to increase physical activity levels and to lose modest amounts of weight were more effective in preventing the incidence of type 2 diabetes than treatment with metformin (58% versus 31% compared with placebo) (Diabetes Prevention Program Research Group, 2002). The results were similar in men and women, young and old, and in all racial and ethnic groups studied.

Even after a single exercise session, insulin sensitivity is improved in the short term (perhaps 1–2 days) (Thompson et al., 2001), and insulin sensitivity tends to be greater in endurance-trained athletes than in sedentary people. In athletes and people who are active at a recreational level, alike, enhanced insulin sensitivity declines rapidly once regular exercise ceases.
(iv) Blood lipid profile

Trained endurance runners (male and female) possess lipoprotein profiles consistent with a low CHD risk. Differences in HDL-cholesterol are most marked and levels are typically 20–30% higher than in comparable sedentary controls. Plasma triglycerides are also low, particularly among veteran athletes. By contrast, athletes trained specifically for strength and power do not differ from sedentary people in terms of lipid profile (Hardman, 1999).

Physically active people in the general population also show favourable lipoprotein profiles, and even ordinary walking has been linked to elevated HDL levels, with relationships between distance walked per day and HDL concentration (Cook et al., 1986; Tucker & Friedman, 1990). The pace of walking, at least in middle-aged women, does not seem to be an important determinant of the increase in HDL-cholesterol (Duncan et al., 1991).

Obesity is associated with dyslipidaemia (see Chapter 3), in particular with elevated plasma concentrations of triglycerides, low levels of HDL-cholesterol, high levels of apolipoprotein B, a preponderance of small, dense low-density lipoprotein (LDL) particles and high levels of postprandial lipaemia. Derangements are exacerbated when, for a given degree of obesity, abdominal adipose tissue distribution predominates. When people take up regular physical activity, the small amount of body fat they typically lose may contribute towards beneficial changes in lipoprotein profile.

Although there is no clear threshold of benefit, it has been suggested that most changes to HDL-cholesterol occur at distances of 11.3–22.5 km (7–14 miles) per week (Kokkinos, 1995), which is easily met by a brisk walk or slow jog for about 30 minutes on most days of the week (Hardman, 1999).

These changes in plasma lipids may be linked to improved transport and disposition of dietary fat during the hours following a fat-containing meal. Like other effects on metabolic risk factors for cardiovascular disease, such as lowering blood pressure and increasing insulin sensitivity, the effects of exercise on postprandial triglyceride metabolism are short-lived. To modify lipoprotein metabolism, regular and frequent exercise is recommended, which can be of moderate intensity so long as sufficient energy is expended (Hardman, 1999).

With regard to lipoprotein metabolism, the consensus is that aerobic activity involving an expenditure of at least 8 MJ/week results in an increase in HDL-cholesterol and probably decreases fasting triglyceride level (Hardman, 1999). These changes may be the result of alterations in the dynamics of triglyceride-rich particles, particularly in the fed state, and occur despite the spontaneous increase in the proportion of dietary energy from carbohydrate that often accompanies increased exercise. For this reason, increased physical activity may be a means of reducing the hypertriglyceridaemic and HDL-lowering effects of low fat (high carbohydrate) diets (see Table 12.2).

(v) Haemostatic factors

Little information is available on the interactions between regular moderate activity and diet and haemostatic factors. Vascular haemostasis results from a regulated interaction of blood coagulation and fibrinolysis (see Chapter 6). A review of randomised controlled studies (Rauramaa & Vaisanen, 1999) concluded that regular moderate intensity physical activities, as well as a diet rich in n-3 fatty acids, decreases platelet aggregability, but the effects of activity on plasma fibrinogen level remain contradictory. Plasminogen activator inhibitor-1 level, a possible link between metabolic syndrome and CHD, may be reduced by physical training. Rauramaa and Vaisanen (1999) hypothesised that a combination of moderate physical activity and dietary modification is likely to have a more powerful impact on blood coagulation and fibrinolysis than either of these separately. The authors identified the need for more research on the interactions of a fat-modified diet and regular moderate physical activity on haemostasis.

(vi) Endothelial dysfunction

Regular physical activity has been shown to improve endothelial function in patients with coronary artery disease (Hambrecht et al., 2003) and weight loss in combination with physical activity improves endothelial dysfunction in obese subjects (Sciaccqua et al., 2003; see Chapter 4, Section 4.7.4).

12.2.3 Summary

Physical activity is a major independent protective factor against CHD in men and women. Unfit and
inactive people have almost double the risk of dying from CHD compared with the most fit and active people. Physical activity also significantly reduces the risk of stroke and provides effective treatment of peripheral vascular disease (Department of Health, 2004). It helps to improve several risk factors for cardiovascular disease, including raised blood pressure, adverse blood lipid profile and insulin resistance. Activity does not need to be vigorous to confer benefit.

### 12.3 How much is required?

It seems that there is no threshold for the minimal amount of exercise necessary to decrease cardiovascular risk (Anderson et al., 1997; Mayer-Davis et al., 1998) and that any increase in daily energy expenditure is beneficial. However, as indicated in Sections 12.2.1 and 12.2.2, it is also evident that benefit increases in a dose–dependent fashion (Kohl, 2001; Wannamethee & Shaper, 2002), but the debate continues as to whether it is physical activity per se or fitness that is more important for health (although it is noted that regular physical activity improves fitness) (Blair et al., 2001). Furthermore, the optimal intensity of leisure time physical activity is still unclear; some studies indicate that only vigorous intensity activity is associated with decreased risk of premature death (Lee et al., 1995), whereas others also show a role for moderate or light intensity activity (Shaper et al., 1991; Leon et al., 1997).

In support of the former, in a prospective, although small, study (11 year follow-up) of almost 2000 middle-aged men from Caerphilly, South Wales (who had no evidence of CHD at baseline), only leisure activity classified as heavy or vigorous was independently associated with reduced risk of premature death from cardiovascular disease (Yu et al., 2003). Examples of such activities include climbing stairs, jogging, hiking, swimming, badminton, tennis and heavy digging. For many benefits to cardiovascular health, intensity can be traded for duration, provided that total energy expenditure is high. Wannamethee and Shaper (2002) concluded that leisure time physical activity is associated with a 30–50% reduction in risk of CHD. Benefits are evident in both primary and secondary prevention.

In April 2004, a report from the CMO was published (Department of Health, 2004a) which presented the case for increased physical activity. Adults (including older adults) are recommended to take at least 30 minutes of at least moderate intensity activity on five or more days each week. Moderate intensity activity should lead to an increase in breathing rate, an increase in heart rate and to a feeling of increased warmth, possibly accompanied by sweating. The report emphasises that shorter bouts of activity can be accumulated during the day and still count towards the 30 minute minimum. Also, different types of physical activity benefit different body systems, e.g. aerobic activity for heart, or weight bearing activities for bones.

Children and young people should achieve a total of at least 60 minutes of at least moderate intensity physical activity each day. At least twice a week this should include activities to improve bone health (activities that produce high physical stress on the bones), muscle strength and flexibility (Department of Health, 2004a).

The Activity Co-ordination Team (cross-government and led by the Department of Health and the Department of Culture, Media and Sport) plan to target increased mass participation in physical activity (see Section 12.5).

Recommendations are similar in the USA, where it has been proposed that activity leading to an increase in expenditure of about 150 kcal/day (equivalent to 1000 kcal or 4.2 megajoules (MJ) per week) is associated with substantial health benefits and that the activity does not need to be vigorous to achieve benefit (US Department of Health and Human Services, 1996). This could be achieved by walking briskly for 30 minutes per day or by a shorter duration of more vigorous activity (e.g. 15 minutes of running at 10 minutes per mile), or by a longer duration of more vigorous exercise less frequently. Furthermore, because of the dose–response relationship, people who are already achieving moderate levels of activity can expect to derive additional benefit by increasing their activity, and it seems that it is never too late to start. Benefits are apparent even for men over the age of 60 years who become physically active after years of a sedentary lifestyle (Blair et al., 1995). Most importantly, a regular pattern of physical activity must be maintained to sustain the physiological changes (e.g. effects on blood lipids or insulin) that are assumed to be responsible for the health benefits.

The advice for adults to take 30 minutes of moderate intensity activity, preferably on each day of the week, is of importance for limiting health risks for diseases such as CHD and diabetes. But Saris et al.
(2003) advise that this may be insufficient for preventing weight gain or regain for many individuals in the current sedentary environment in which we live. They report that there is compelling evidence that prevention of weight regain in formerly obese individuals requires 60–90 minutes of moderate intensity activity (e.g. walking or cycling) or lesser amounts of vigorous intensity activity. Although definitive data are lacking, it seems likely that activity of at least moderate intensity for 45–60 minutes per day is required to prevent the transition to overweight or obesity (Department of Health, 2004a). People who have been obese and who have lost weight may need to do 60–90 minutes of activity a day in order to maintain weight loss (Department of Health, 2004).

Although the available data is not totally consistent, it has been suggested that intermittent episodes of activity accumulated over the course of a day may have cardiorespiratory fitness benefits comparable to one longer continuous episode (for details of studies, see US Department of Health and Human Services, 1996; Department of Health, 2004a).

To be successful in the long term, physical activity recommendations need to be tailored to an individual’s needs and preferences. The CMO advises that incorporating physical activity into daily routines will be the easiest approach, e.g. walking or cycling for short journeys and taking up active hobbies such as gardening (Department of Health, 2004a).

12.4 Successful interventions

A study among physically inactive middle-aged people has shown that it is cost effective in public health terms to increase activity levels (Stevens et al., 1998). A detailed review of approaches taken to understand and encourage physical activity among adults and children can be found in the report from the US Department of Health and Human Services (1996) and a summary can be found in the report from the Department of Health (2004a). Having confidence in one’s ability to be active (self-efficacy), enjoying physical activity, receiving support from family, friends or peers, and perceiving the benefits to outweigh the barriers or costs seem to be central determinants influencing physical activity levels across the lifespan. Only limited information exists for population subgroups and effective approaches for weight prevention are considered to be few, although advice from health professionals can be an effective means of increasing physical activity levels among adults. Workplace and community initiatives have also been shown to be effective (see Chapter 13, Section 13.9).

Interventions that encourage walking and do not require attendance at a facility (e.g. sports centre or gym) have been shown to be most likely to lead to sustainable increases in overall physical activity (Hillsdon & Thorogood, 1996). Brisk walking seemed to have the greater potential in sedentary adults. Barriers to promotion of physical activity include lack of appropriate training of GPs and other primary care professionals, lack of success in the counseling role and lack of standard protocols (Margetts et al., 1999). GPs are more likely to promote physical activity if they are physically active themselves (McKenna et al., 1998).

In children, interventions to tackle obesity have generally been unsuccessful. It is widely believed that low levels of physical activity and/or increasing sedentary behaviour, such as TV viewing and computer games, is implicated in the etiology of childhood obesity. A recent systematic review indicates that the evidence to date on childhood obesity prevention is not encouraging (Reilly & McDowell, 2003). Evidence from interventions focusing on activity remains limited, and simple, effective and generalisable interventions are lacking. A promising approach is to focus the intervention wholly on inactivity (Reilly & McDowell, 2003). Targeting sedentary behaviour seems to provide a treatment benefit in children who are already overweight that is greater than targeting lifestyle activity, and both strategies add considerably to the effects that can be achieved by dietary approaches alone.

12.5 Recent initiatives in the UK

A number of local and national initiatives are now underway, which aim to encourage greater levels of physical activity. These include:

1. In December 2002, the Strategy Unit report Game Plan set out a new target of 70% of the population to be active (half an hour of exercise five days a week) by 2020. This will be taken forward by the Activity Co-ordination Team (ACT) (Department of Health, 2004a) responsible for establishing a strategic overview and national planning.

2. In April 2003, nine Local Exercise Action Pilots (LEAP) were announced, which aim to test
different Primary Care Trust-led community approaches to increasing access to, and levels of, physical activity in England. These interventions were due to start in September 2003 and will run for two years. They will be fully evaluated.

(3) A report from the CMO (England) was published in April 2004. It sets the case for physical activity and will be the basis for ACT’s delivery plan.


(5) Scotland – An action plan is being developed following consultation on the findings of the Physical Activity Task Force, which reported in June 2002 (www.Scotland.gov.uk).

(6) The Government has distributed 10,000 pedometers to 110 Primary Care Trusts in Neighbourhood Renewal Areas to assess their use as a motivational tool to encourage increased walking.

(7) During 2003–6, more than £450 million is being invested by the Government to transform physical education and schools’ sport, with the aim of trebling the proportion of school children who spend a minimum of 24 hours/week engaged in physical education (PE) and sport within and beyond the curriculum to 75% by 2006.

(8) By September 2005, school sports partnerships (facilitating sports activity) will cover 75% of the schools in England.

12.6 Key points

- Physical inactivity is commonplace in Britain: 6 in 10 men and 7 in 10 women aged 19–64 years do not take enough physical activity to benefit their health.
- Activity levels are also low in children. About 40% of boys and 60% of girls aged 7–14 years spend less than 1 hour per day in activities of at least moderate intensity, and thus fail to meet the recommendation for young people. This is thought to be a cause of the obesity now seen in children: 16% of 2–15 year olds are obese.
- Men and women who are physically active experience a lower risk of cardiovascular disease in general and CHD in particular, and it’s never too late to change the habits of a lifetime. Stroke risk and hypertension also seem to be reduced.
- A physically active lifestyle is linked with improved body weight control and plasma lipid profile (raised HDL-cholesterol and lower triglycerides). There is also benefit for other cardiovascular risk factors, including blood pressure, insulin sensitivity, endothelial function and glucose tolerance.
- There is considerable evidence that regular physical activity protects against type 2 diabetes, the effect being independent of body weight. As with cardiovascular disease, there is some evidence of a dose-dependent response.
- UK adults are advised to accumulate at least 30 minutes of moderate intensity activity (e.g. brisk walking) on at least five days a week. For many benefits to cardiovascular health, intensity can be traded for duration, provided that total energy expenditure remains high. Benefits are evident in both primary and secondary prevention.
- Children and young people should achieve a total of at least 60 minutes of at least moderate intensity physical activity each day. At least twice a week this should include activities to improve bone health, muscle strength and flexibility.
- To prevent obesity, many individuals will need to participate in 45–60 minutes of at least moderate intensity activity each day. In those who have lost weight, 60–90 minutes a day may be needed to prevent weight regain.
- A number of government-funded local and national initiatives are now underway to tackle physical inactivity in the UK.
- A concerted effort is required to encourage physical activity in the UK population. This will require collaboration and a concerted effort by government at all levels, leisure and sport services, schools and colleges, town and regional planners, transport planners and providers, architects, countryside agencies, the NHS and social care, voluntary and consumer groups, employees and workplaces, the media, parents and families. It will be necessary to consider safety measures to encourage cycling or walking.
12.7 Recommendations for future research

- Further research is required to identify ways of promoting physical activity as part of everyday life.
- There is a need for further research on the effect of physical activity on haemostasis and the effect of physical activity on endothelial function.

12.8 Key references


13
A Public Health Approach to Cardiovascular Disease Risk Reduction

13.1 Cardiovascular disease as a public health problem

This chapter starts by considering current approaches to cardiovascular risk reduction in the UK and elsewhere, current dietary recommendations for primary prevention in the context of current dietary trends and trends in physical activity (see Chapter 12). Then, by synthesising the information provided in earlier chapters, it considers whether the current dietary guidelines need revision in the light of new evidence and also the case for reducing inactivity. The final sections address health promotion in children and other subgroups of the population, barriers to change, effective interventions and the potential impact of new knowledge about diet–gene interactions.

The prevention of cardiovascular disease is a major public health challenge that has, for a number of years, been high on the health agendas of governments around the world. Although death rates have been falling in many westernised countries (e.g. USA, Australia, UK), rates are rising rapidly elsewhere. Worldwide, the highest levels are now found in Eastern European countries, such as Latvia, Lithuania and the Russian Federation, and cardiovascular disease is now a leading cause of death in developing as well as developed countries (Pearson, 1999; see Chapter 1, Section 1.3.1).

13.1.1 Approaches in the UK

In the UK, the Department of Health recommendations published in 1994 (Department of Health 1994) were derived from the deliberations of the Committee on Medical Aspects of Food Policy (COMA). These are discussed in Section 13.2 (see also Chapter 1); in Section 13.3 recommendations from other countries are provided for comparison. A target for reduction of cardiovascular disease in England has been published by the UK Government in Saving Lives: Our Healthier Nation (Department of Health, 1999b) and in related documents for Wales, Scotland and Northern Ireland. The target is to decrease the death rate from coronary heart disease (CHD) and stroke in people under the age of 75 years by at least two-fifths by the year 2010. This is in the context of an overall strategy to improve the health of the population as a whole by increasing life expectancy and the number of years people spend free from illness. An associated aim is to improve the health of the worst off in society and to reduce the health gap between sections of society, which has been increasing rather than decreasing in recent years (see Chapter 1, Section 1.3.3). Related targets have been published in other parts of the UK (www.hpw.wales.gov.uk; www.foodstandards.gov.uk; www.scotland.gov.uk).

The risk of heart disease also varies between ethnic groups and between different geographical regions of the UK (see Chapter 1, Section 1.3.3).

A National Service Framework for CHD has been developed (Department of Health, 2000a), the key points of which are summarised in Table 13.1. A National Service Framework for Diabetes has also been published (Department of Health, 2002) (www.diabetes.nsf@doh.gov.uk), the key elements of which can be found in Table 13.2. These cover both prevention and treatment.
Table 13.1  

<table>
<thead>
<tr>
<th>Standard one</th>
<th>Reducing heart disease in the population*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The NHS and partner agencies should develop, implement and monitor policies that reduce the prevalence of coronary risk factors in the population, and reduce inequalities in risks of developing CHD.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard two</th>
<th>Preventing CHD in high-risk patients in primary care</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The NHS and partner agencies should contribute to a reduction in the prevalence of smoking in the local population.</td>
</tr>
</tbody>
</table>

| Standard three | General practitioners and primary care teams should identify all people with established cardiovascular disease and offer them comprehensive advice and appropriate treatment to reduce their risks. |
| Standard four  | General practitioners and primary health care teams should identify all people at significant risk of cardiovascular disease but who have not yet developed symptoms and offer them appropriate advice and treatment to reduce their risks. |
| Standard twelve| NHS Trusts should put in place agreed protocols/systems of care so that, prior to leaving hospital, people admitted to hospital suffering from CHD have been invited to participate in a multidisciplinary programme of secondary prevention and cardiac rehabilitation. The aim of the programme will be to reduce their risk of subsequent cardiac problems and to promote their return to a full and normal life. |

Note: Standards five to eleven relate to symptoms/syndromes of CHD.

*Interventions that should be put into place to improve the cardiac health of the local population were suggested, including the need for a comprehensive local programme of effective policies for reducing smoking, promoting healthy eating, promoting physical activity, and for reducing overweight and obesity, all co-ordinated by the health authority. Approaches for delivering effective prevention policies and programmes were also suggested. Source: Material reproduced from Department of Health (2000a) with permission from The Stationery Office. Crown copyright.

Table 13.2  

<table>
<thead>
<tr>
<th>Standard one</th>
<th>Prevention of type 2 diabetes**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The NHS will develop, implement and monitor strategies to reduce the risk of developing type 2 diabetes in the population as a whole and to reduce the inequalities in the risk of developing type 2 diabetes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard two</th>
<th>Identification of people with diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The NHS will develop, implement and monitor strategies to identify people who do not know they have diabetes.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard three</th>
<th>Empowering people with diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All children, young people and adults with diabetes will receive a service which encourages partnership in decision-making, supports them in managing their diabetes and helps them to adopt and maintain a healthy lifestyle. This will be reflected in an agreed and shared care plan in an appropriate format and language. Where appropriate, parents and carers should be fully engaged in this process.</td>
</tr>
</tbody>
</table>

Note: Standards four to twelve relate to the care of people with diabetes.

**Interventions that promote a balanced diet and physical activity and include support for individuals can reduce overweight and obesity and the prevalence of central obesity in the general population, particularly in subgroups of the population at increased risk of developing diabetes, such as people from minority ethnic communities. Source: Material reproduced from Department of Health (2002) with permission from The Stationery Office. Crown copyright.

13.2  Current dietary recommendations for primary prevention in the UK

Dietary recommendations, which tend to be country-specific, are the product of considerable debate and discussion by expert scientists, and are based on the available evidence. In 1991, the UK Department of Health published Dietary Reference Values (DRVs) which are intended to apply to healthy groups of people. In 1994, COMA published food and nutrient
recommendations to prevent cardiovascular disease (Department of Health, 1994); these are summarised in Table 13.3 and discussed in Chapter 1, Section 1.5.1, and Section 13.3. COMA has been replaced by the Scientific Advisory Committee on Nutrition (SACN), which is a UK-wide advisory committee that advises the UK Health Departments as well as the Food Standards Agency. SACN has recently made additional recommendations on sodium (salt) and long chain n-3 fatty acids, which are reflected in Table 13.3.

The UK Government’s recommendations have been translated into practical food-based advice which is summarised in Table 13.4. It is also important when giving advice to the public to emphasise the need to increase the proportion of unsaturated fats consumed, providing examples of appropriate foods. Table 13.5 illustrates some foods that are rich sources of individual unsaturated fatty acids. Note that because a food is a rich source of a particular fatty acid does not imply it is the most important source quantitatively.

Table 13.3  Recommendations for cardiovascular disease risk reduction in the UK, expressed as population averages.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>Decrease to 35% of food energy (33% total energy* including contribution from alcohol)</td>
</tr>
<tr>
<td>Saturates</td>
<td>Decrease to 11% food energy (10% of total energy*)</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td>No specific recommendations but, if the other guidance is followed, the level would be 13% of food energy (12% total energy*)</td>
</tr>
<tr>
<td>Polyunsaturates</td>
<td>No further increase in n-6 fatty acids (current level about 6.5% of food energy, 6% of total energy*). Concern expressed about intakes above 10% energy. Long chain n-3 fatty acids: increase average intake from 0.1 g/day to 0.45 g/day** (see Chapter 11, Section 11.8.3)</td>
</tr>
<tr>
<td>Trans fats</td>
<td>No more than 2% of energy intake.</td>
</tr>
<tr>
<td>Starch, intrinsic sugars, milk sugars</td>
<td>Increase to 39% of food energy (37% of total energy*)</td>
</tr>
<tr>
<td>Non-milk extrinsic sugars</td>
<td>Restrict to 11% of food energy (10% of total energy*)</td>
</tr>
<tr>
<td>Non-starch polysaccharides (NSP)</td>
<td>Increase to 18 g per day in adults. Proportional, non-specified increase also recommended for children</td>
</tr>
<tr>
<td>Sodium</td>
<td>Reduction in average intake from the current 150 mmol/day (equivalent to 9 g salt/day) to about 100 mmol/day (6 g salt per day). A proportional reduction for children was also recommended, and specific targets have since been published (see Section 13.4.3)</td>
</tr>
<tr>
<td>Potassium</td>
<td>Increase in average intake in adults to 3.5 g/day (90 mmol/day). Proportional, non-specified increase also recommended for children</td>
</tr>
</tbody>
</table>

* Total energy includes contribution from alcohol.
Sources: Department of Health (1991, 1994); Scientific Advisory Committee on Nutrition (2003, 2004**).

Table 13.4  Summary of practical dietary advice to reduce risk of CHD.

- Reduce consumption of all types of fat, for example by selecting lean cuts of meat and lower fat dairy products, by reducing use of oil and full fat spreads (margarine, butter), by eating fewer fried foods, and by moderating consumption of high fat foods such as cakes, biscuits, pastries and savoury snacks.
- Opt for oils/spreads that are higher in monounsaturates and lower in saturates.
- Include more fruit and vegetables in the diet, aiming for at least five portions of a variety of fruits and vegetables each day.
- Include oil-rich fish in the diet at least once per week. (Those with heart disease may benefit from higher intakes. See Chapter 11, Section 11.8.3.)
- Use less salt at the table and in cooking, and look for lower salt alternatives of manufactured foods.
- Include more starchy foods in the diet, e.g. bread, potatoes, yams, rice and pasta, so that at least 50% of energy intake comes from carbohydrate.
- Drink alcohol sensibly, i.e. no more than 2–3 units per day for women and no more than 3–4 units per day for men.

Sources: Material reproduced with modifications from Department of Health (1994) with permission from The Stationery Office. Crown copyright; Scientific Advisory Committee on Nutrition (2004).
Cardiovascular Disease

Though once very different, the dietary advice for people with diabetes is now more or less the same as that for the population at large, with an emphasis on reduced fat intakes and plentiful cereal foods, fruits and vegetables.

13.3 Recommendations in other countries

The revised Report of a Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases has retained its previous emphasis on low fat intakes, with the recommendation of 15–30% of energy from fat for populations around the world (World Health Organization, 2003). This recommendation has been suggested to be inconsistent with other recent national recommendations, for example the USA (see Section 13.3.1) and The Netherlands (Table 13.6), where greater emphasis

Table 13.5 Sources of fatty acids in the UK diet (adults).

<table>
<thead>
<tr>
<th>Type of fat</th>
<th>Sources in the UK diet (% of total intake) in descending order of importance</th>
<th>Rich sources of individual fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturates</td>
<td>Milk and milk products (24%); meat and products (22%); cereal products (18%), e.g. cakes, biscuits, pastries; fat spreads (11%); potatoes and savoury snacks (7%). Mean daily intakes: men 32.5 g (13.4% food energy); women 23.3 g (13.2% food energy).</td>
<td>Myristic acid (C14:0): particularly milk fat, coconut oil, and products made from these. Palmitic acid (C16:0): particularly animal fats, coconut oil, palm oil, and products made from these. Stearic acid (C18:0) particularly meat, cocoa butter (also present in dairy products).</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td>Meat and products (27%); cereal products (17%); potatoes and savoury snacks (12%); fat and spreads (11%); milk and milk products (10%); egg and egg dishes (5%). Mean daily intakes: men 29.1 g (12.1% food energy); women 20.2 g (11.5% food energy).</td>
<td>Oleic acid (C18:1): olive oil, rapeseed oil. (Moderate sources are meat, dairy products, peanut and palm oil.)</td>
</tr>
<tr>
<td>Polyunsaturates</td>
<td>Meats and products (17%); cereal products (17%); potatoes and savoury snacks (17%); fish and fish dishes (oil-rich fish) (14%); vegetables (excluding potatoes) (11%); fat spreads (7%). Mean daily intakes: men 2.3 g (1% food energy); women 1.7 g (1% food energy).</td>
<td>Alpha-linolenic acid (C18:3): rapeseed, walnut, soya and blended vegetable oils, walnuts. (Moderate sources are meat from grass-fed ruminants, vegetables, meat and eggs from animals fed a diet enriched in C18:3.) EPA (C20:5) and DHA (C22:6): oil-rich fish, fish oil. (Moderate sources are foods enriched or fortified with EPA/DHA.)</td>
</tr>
<tr>
<td>n-3 (Omega 3) fatty acids</td>
<td>Cereal products (20%); meat and products (18%); fat spreads (14%); potatoes and savoury snacks (13%); vegetables (excluding potatoes) (9%). Mean daily intakes: men 12.9 g (5.4% food energy); women 9.4 g (5.3% food energy).</td>
<td>Vegetable oils (e.g. sunflower, corn and soya bean) and spreads made from these. (Moderate sources are peanut and rapeseed oils.)</td>
</tr>
<tr>
<td>n-6 (Omega 6) fatty acids</td>
<td>Cereal products (26%); meat and products (21%); fat spreads (18%); milk and products (16%); potatoes and savoury snacks (6%). Mean daily intakes: current intake in men and women is 1.2% energy (DRV = 2%E).</td>
<td>Hydrogenated oils and products made from these can potentially provide large amounts, but generally the levels of these in the UK food supply have been substantially reduced and are now well below the dietary reference value.</td>
</tr>
<tr>
<td>Trans fatty acids</td>
<td>Cereal products (20%); meat and products (18%); fat spreads (14%); potatoes and savoury snacks (13%); vegetables (excluding potatoes) (9%). Mean daily intakes: men 12.9 g (5.4% food energy); women 9.4 g (5.3% food energy).</td>
<td>Vegetable oils (e.g. sunflower, corn and soya bean) and spreads made from these. (Moderate sources are peanut and rapeseed oils.)</td>
</tr>
</tbody>
</table>

DHA: docosahexaenoic acid; DRV: dietary reference value; EPA: eicosapentaenoic acid; %E: percentage of energy intake. Source: Material on sources in the UK diet reproduced from Henderson et al. (2003a) with permission of The Stationery Office. Crown copyright.
### Table 13.6 Summary of recommendations for dietary fat.

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Fat</th>
<th>SFA</th>
<th>MUFA</th>
<th>Total PUFA</th>
<th>Alpha-linolenic acid</th>
<th>n-3 long chain PUFA (EPA/DHA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia (National Heart Foundation, 1999)</td>
<td>No recommen</td>
<td>&lt;8% of energy (SFA and trans)</td>
<td>—</td>
<td>Increase to 10% energy; n-6 PUFA: 8–10% of energy</td>
<td>2 g/day</td>
<td>Long chain PUFA (EPA/DHA).</td>
</tr>
<tr>
<td>Austria, Germany, Switzerland (Deutsche Gesellschaft für Ernährung, 2000)</td>
<td>30% of energy</td>
<td>Max. 10% of energy; trans &lt;1% of energy</td>
<td>&gt;10% of energy</td>
<td>7–10% of energy</td>
<td>Max. 3% of energy</td>
<td>Ratio n-3/n-6 of 1/5</td>
</tr>
<tr>
<td>Belgium</td>
<td>Max. 30% of energy</td>
<td>Max. 10% of energy</td>
<td>—</td>
<td>3–7% of energy; linoleic acid 3–5% of energy</td>
<td>0.5–1% of energy</td>
<td>—</td>
</tr>
<tr>
<td>Finland and Nordic countries (National Nutrition Council, 1998; Sandström et al. 1996)</td>
<td>Max. 30% of energy</td>
<td>&lt;10% of energy (SFA and trans)</td>
<td>10–15% of energy (cis MUFA)</td>
<td>5–10% of energy (n-3 + n-6 ≥ 3% of energy, in which 1% of energy is n-3)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>France (Martin, 2001)</td>
<td>30–35% of energy</td>
<td>8% of energy</td>
<td>20% of energy</td>
<td>5% of energy</td>
<td>0.8% of energy</td>
<td>0.2% of energy</td>
</tr>
<tr>
<td>The Netherlands (Health Council of the Netherlands, 2002)</td>
<td>20–40% of energy for those of optimal body weight; 20–30/35% of energy for those overweight</td>
<td>≤10% of energy; trans ≤1% of energy</td>
<td>8–38% of energy for those of optimal body weight 8–28/33% of energy for those overweight</td>
<td>≤12% of energy (including 2% of n-3)</td>
<td>1% of energy</td>
<td>200 mg/day from oil-rich fish</td>
</tr>
<tr>
<td>United Kingdom (Department of Health, 1991; Scientific Advisory Committee on Nutrition, 2004)</td>
<td>35% of food energy</td>
<td>11% of food energy; trans &lt;2% of energy</td>
<td>(13% of energy by difference; no specific recommendation)</td>
<td>6–10% of energy; linoleic &gt;1% of energy</td>
<td>&gt;0.2% of energy</td>
<td>—</td>
</tr>
<tr>
<td>WHO/FAO report (World Health Organization, 2003)</td>
<td>15–30% energy; up to 35% for highly active groups</td>
<td>SFA &lt;10% of energy (by difference); trans &lt;1% of energy</td>
<td>15–30% of energy (by difference)</td>
<td>Total PUFA 6–10% of energy n-6 PUFA 5–8% of energy</td>
<td>Total n-3: 1–2%</td>
<td>Fish 1–2 times per week. Serving should provide 200–500 mg EPA/DHA</td>
</tr>
</tbody>
</table>

COMA: Committee on Medical Aspects of Food Policy; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; FAO: Food and Agriculture Organization; Max.: maximum; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; WHO: World Health Organization.
has been placed on fat quality. The WHO/FAO recommendations for saturates, n-6 polyunsaturates, n-3 polyunsaturates and trans fatty acids are less than 10%, 5–8%, 1–2% and less than 1% of energy intake, respectively (see Table 13.6). No specific recommendation is given for monounsaturates.

The European Heart Network (2002) has published similar goals to those of the WHO/FAO Expert Consultation: total fat less than 30% of energy, saturates less than 10%, n-6 polyunsaturates 4–8%, n-3 polyunsaturates 2 g/day alpha-linolenic acid and 200 mg/day long chain n-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)), trans fatty acids less than 2%. The stated reason for the recommendation to reduce total fat intake was the perceived need to reduce the energy density (see Section 13.4.1) of European diets for the prevention of obesity.

### 13.3.1 The United States

The USA has traditionally had more restrictive recommendations on fat than the UK. However, the recent US recommendations suggest a wider range for total fat intake (25–35% of dietary energy compared to the former recommendation of less than 30%), recognising the importance of monounsaturates in reducing blood lipids (Expert Panel on Detection Evaluation and Treatment of High Blood Cholesterol in Adults, 2001). Monounsaturates reduce low-density lipoprotein (LDL) cholesterol but, unlike n-6 polyunsaturates, they do not also reduce protective high-density lipoprotein (HDL) cholesterol. Moreover, low fat, high carbohydrate diets have been shown to increase plasma triglyceride and decrease beneficial HDL-cholesterol levels (see Chapter 1, Section 1.5.2 and Chapter 11, Section 11.7).

The recommendations from the USA shown in Table 13.7 are designed to reduce LDL-cholesterol. The recommendations also emphasise the particular importance of weight reduction (Chapter 11, Section 11.3) and increased physical activity with regard to metabolic syndrome (see Chapter 12), and note that consumption of viscous dietary fibres and plant stanols/sterols can be of help (see Chapter 11, Section 11.9).

### 13.3.2 Finland

One of the most successful examples of initiatives to reduce cardiovascular mortality and morbidity is that of Finland (Puska et al., 1998). As a result of exceptionally high cardiovascular disease mortality rates in the 1960s, a community-based programme known as the North Karelia project was launched in East Finland in 1972 in response to a community petition to demand government funding to tackle the problem. A number of activities were initiated in North Karelia, which served as a basis for a national cardiovascular disease prevention strategy. The programme was devised as a comprehensive intervention involving all sectors of the community with the central aim of reducing the major cardiovascular risk factors (smoking, raised blood cholesterol and hypertension). Diet was recognised as an important aspect of lifestyle to address within the programme, as the North Karelia diet was traditionally very high in dairy fats and salt, and very low in vegetables. The programme involved mass media activities aimed at enhancing knowledge and understanding about healthy lifestyles, the training of health professionals, community-based activities and environmental and policy activities (including collaboration with food manufacturers, caterers and retailers). A wide range of nutrition-related strategies were implemented including dietary guidelines for meals served in schools, hospitals and the workplace, new food labelling rules to highlight the different types of fatty acids and draw attention to ‘heavily salted’ foods, the provision of free salads as an accompaniment to meals in the catering sector, and working with livestock breeders to change the fat content of meat. A collaborative project known as the Berry Project was also initiated to promote the consumption of local berries and to promote berries to farmers as an alternative crop.

**Table 13.7** Recommendations for dietary fat in the USA

<table>
<thead>
<tr>
<th></th>
<th>% of Total Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturates</td>
<td>&lt;7%</td>
</tr>
<tr>
<td>Polyunsaturates</td>
<td>Up to 10%</td>
</tr>
<tr>
<td>Monounsaturates</td>
<td>Up to 20%</td>
</tr>
<tr>
<td>Total fat</td>
<td>25–35%</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>50–60%</td>
</tr>
<tr>
<td>Fibre</td>
<td>20–30 g/day</td>
</tr>
<tr>
<td>Protein</td>
<td>~15%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>&lt;200 mg/day</td>
</tr>
<tr>
<td>Total energy</td>
<td>Balance intake and expenditure</td>
</tr>
</tbody>
</table>

The North Karelia Project had an impressive effect on dietary intake and on the major cardiovascular risk factors. Between 1972 and 1992, intake of saturates fell by a third and blood cholesterol levels fell by 13% in men and 18% in women (Puska et al., 1995). CHD mortality decreased in the 1970s significantly more in North Karelia than elsewhere in Finland. Since 1977, the experiences in North Karelia have been used with considerable success to carry out preventative work at a national level. This has included the production of expert guidelines, the promotion of involvement with industry and the enactment of public policy (e.g. anti-smoking legislation) (Puska, 1996; Puska et al., 1998). Between 1969/71 and 1995, the age standardised CHD mortality rate (per 100 000) fell in North Karelia by 73% and nationwide by 65%.

13.3.3 Norwegian food policy

Nutrition policy has also been successful in reducing CHD mortality in Norway (Johansson et al., 1996; Norum et al., 1997). A food and nutrition policy was initiated with the establishment of a National Nutrition Council in 1946, but evolved over the years to include combined legal, economic, organisational and education measures. For example, the Norwegian government encouraged farmers to increase production of grains for human consumption, potatoes, vegetables and low-fat milk. Measures were introduced to avoid over-production of meat, and the production of grass-fed, rather than grain-fed, beef and less fatty pork was encouraged. There was a considerable reduction in fat intake in Norway between 1975 and 1993, mainly resulting from a reduction in the intake of saturates, and an increase in the consumption of fruit, vegetables and cereals. These changes were reflected in a fall of about 10% in average blood cholesterol levels and a 25–50% reduction in mortality from CHD amongst middle-aged men and women (Norum et al., 1997).

A key feature of the Norwegian approach has been the early recognition of the need for nutrition policy to adopt a multi-sectorial approach and to involve a broad range of sectors. The nutrition policy has been well integrated with health policy and other policy areas. The National Nutrition Council has also played a major role in developing and implementing the policy.

13.4 Dietary trends in the UK and comparison of these with the guidelines

13.4.1 Energy intake

According to annual reports from the National Food Survey, energy intakes in Britain have been falling progressively over recent decades, amounting to a reduction of about 2.3 MJ (540 kcal) per day since 1975 (DEFRA, 2001) (see Chapter 1, Section 1.5.3). These data are supported by information from the National Diet and Nutrition Survey (NDNS) series. During the period 1986/7 to 2000/1, energy intakes have fallen from 10.30 MJ (2450 kcal per day) to 9.72 MJ (2313 kcal) in men and from 7.05 MJ (1680 kcal) to 6.87 (1632 kcal) in women (Gregory et al., 1990; Henderson et al., 2003a). (As the NDNS has calculated energy values in megajoules and kilocalories separately, these values do not correspond precisely with the usual conversion factor used, i.e. 1 kcal = 4.184 kJ.) These reductions have to some extent masked the reductions in fat intake that have occurred over the same period (see Section 13.4.2).

13.4.2 Fat and fatty acids

Trends in fat and saturates in the UK are shown in Fig. 13.1. There has been a considerable reduction in the average amount of fat consumed per person over the past 30 years, falling from 107 g/day in 1975 (Ministry of Agriculture Fisheries and Food, 1977) to 76.2 g/day in 2000 (DEFRA, 2001). The NDNS for adults (Henderson et al., 2003a) has revealed that the target for total fat intake has now been approximately reached in the adult population (18–64 years), but it should be noted that some under-reporting of food intake does occur in such surveys, and it is not possible to determine the exact impact this might have on the achievement of dietary targets.

Average daily fat intake is 35.8% of food energy in men (86.5 g/day) and 34.9% in women (61.4 g/day), compared with 40.4% and 40.3%, respectively, in 1986/7 (Gregory et al., 1990; Henderson et al., 2003a); Table 13.5 shows the dietary sources. Table 13.8 compares intakes of macronutrients among adults in 2001/1 and 1986/7.

A shift in dietary patterns over the past 50 years, coupled with modifications by the food industry to
the fat content of commonly consumed foods such as milk, meat and spreads, has resulted in changes in the nature of fat intakes in the UK (DEFRA, 2001). There has been a shift in the dietary sources of fat over time (Table 13.9). Before the introduction of low fat milk and leaner meat, milk and meat were major contributors to total fat intake, but the proportions (and absolute quantities) provided by these foods have since reduced. Certain cereal-based foods are now making a proportionally larger contribution to the diet than in previous decades.

Also, the increased use of vegetable oils throughout the food industry and the popularity of spreads rich in polyunsaturates has resulted in a change in the ratio of n-6 to n-3 fatty acids. It should be noted that the n-6/n-3 ratio is mainly defined by the intakes of linoleic versus alpha-linolenic acids; the long chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (found in fish oils) make a trivial impact on the ratio. Nevertheless, an important question is whether consumption of large amounts of n-6 fatty acids inhibits conversion of alpha-linolenic acid to EPA and DHA, and thereby further compromises poor EPA and DHA status.

A slight readjustment in overall intakes of n-3 fatty acids has been evident in the last decade, presumably

### Table 13.8  
Comparison of daily intakes of macronutrients by adults, 1986/7 versus 2000/1 and current dietary reference values.

<table>
<thead>
<tr>
<th>Macronutrients</th>
<th>1986/7</th>
<th>2000/1</th>
<th>DRV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Energy (MJ)</td>
<td>10.30</td>
<td>7.05</td>
<td>9.72</td>
</tr>
<tr>
<td>Carbohydrate (% food energy)</td>
<td>44.7</td>
<td>44.2</td>
<td>47.7</td>
</tr>
<tr>
<td>Protein (% food energy)</td>
<td>15.2</td>
<td>15.6</td>
<td>16.5</td>
</tr>
<tr>
<td>Total fat (% food energy)</td>
<td>40.4</td>
<td>40.3</td>
<td>35.8</td>
</tr>
<tr>
<td>Saturates (% food energy)</td>
<td>16.5</td>
<td>17.0</td>
<td>13.4</td>
</tr>
<tr>
<td>Trans fatty acids (% food energy)</td>
<td>2.2</td>
<td>2.2</td>
<td>1.2</td>
</tr>
<tr>
<td>MUFA (% food energy)</td>
<td>12.4</td>
<td>12.2</td>
<td>12.1</td>
</tr>
<tr>
<td>n-3 PUFA (% food energy)</td>
<td>0.8</td>
<td>0.8</td>
<td>1.0</td>
</tr>
<tr>
<td>n-6 PUFA (% food energy)</td>
<td>5.4</td>
<td>5.3</td>
<td>5.4</td>
</tr>
<tr>
<td>Alcohol (% total energy)</td>
<td>6.9</td>
<td>2.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Fibre (g/day)</td>
<td>–</td>
<td>–</td>
<td>15.2</td>
</tr>
</tbody>
</table>

---

DRV: dietary reference value; MUFA: monounsaturates; PUFA: polyunsaturates.
Sources: Department of Health (1991), Gregory et al. (1990) and Henderson et al. (2003a).
due to changes in the fatty acid profile of spreads and oils, and a modest resurgence in the popularity of oil-rich fish. These changes may result from successful communication of the importance of $n$-3 fatty acids in general and of preformed long-chain $n$-3 fatty acids in particular. The increase in the numbers of people eating away from home and also the willingness to try new dishes (prompted by celebrity chefs) may also have had an impact. The Total Diet Survey (which is a model of the national average domestic diet in the UK, based on food consumption data from the National Food Survey) showed daily $n$-3 fatty acid intake rose from 1.6 g in 1991 to 1.8 g in 1995, while total intake of $n$-6 fatty acids fell from 10.7 g to 10.2 g per day over the same period (Ministry of Agriculture Fisheries and Food, 1997b). The National Food Survey series (which includes people of all ages) shows that consumption of oil-rich fish represents a small proportion of total fish intake and has shown a small increase since the early 1990s (Fig. 13.2).

The NDNS of adults (Henderson et al., 2002) showed that average intake of oil-rich fish in this specific group is higher and is now 81 g per day in men and 90 g in women, rising with age. Only 41% of men and 47% of women consumed oil-rich fish during the seven-day survey and, among these, average consumption was 198 g/week for men and 190 g/week for women (Table 13.10). See Section 11.8.3 and Table 13.11 for recent UK recommendations.

Recent efforts to reduce the trans fatty acid concentration in the food supply in the UK have been successful and, unlike in North America, average population intakes (1.2% of energy) are well below the reference value of 2% (Henderson et al., 2003a; see Section 13.5.1).

13.4.3 Sodium and salt

In 1994, COMA recommended a target average intake of salt of 6 g/day (2.4 g sodium) for the adult population (Department of Health, 1994; see Chapter 2). In

<table>
<thead>
<tr>
<th>Table 13.9</th>
<th>Changes in contributions to fat and saturates intakes (g/day) over time.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fat</td>
</tr>
<tr>
<td>Liquid milk</td>
<td>14.7</td>
</tr>
<tr>
<td>(12.2%)</td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>4.8</td>
</tr>
<tr>
<td>(4.0%)</td>
<td></td>
</tr>
<tr>
<td>Other dairy products (e.g. cream and yogurt)</td>
<td>2.0</td>
</tr>
<tr>
<td>(1.6%)</td>
<td></td>
</tr>
<tr>
<td>Carcass meat/poultry</td>
<td>15.9</td>
</tr>
<tr>
<td>(13.5%)</td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td>19.9</td>
</tr>
<tr>
<td>(16.4%)</td>
<td></td>
</tr>
<tr>
<td>Fats and oils</td>
<td>42.2</td>
</tr>
<tr>
<td>(34.9%)</td>
<td></td>
</tr>
<tr>
<td>Cakes, pastries, biscuits</td>
<td>9.2</td>
</tr>
<tr>
<td>(7.6%)</td>
<td></td>
</tr>
<tr>
<td>Other cereals</td>
<td>3.0</td>
</tr>
<tr>
<td>(2.5%)</td>
<td></td>
</tr>
<tr>
<td>Vegetables (e.g. fried)</td>
<td>1.6</td>
</tr>
<tr>
<td>(1.4%)</td>
<td></td>
</tr>
<tr>
<td>Other foods</td>
<td>7.4</td>
</tr>
<tr>
<td>(6.1%)</td>
<td></td>
</tr>
<tr>
<td>TOTAL (g/day)</td>
<td>120.7</td>
</tr>
</tbody>
</table>

Cardiovascular Disease

In 1994, the average salt intake was 9 g/day and has since risen to an average of 9.5 g/day (Henderson et al., 2003b). In the recent NDNS of adults (Henderson et al., 2003).

2003, SACN confirmed that this population goal for adults was appropriate and achievable (Scientific Advisory Committee on Nutrition, 2003). Adjusting for differences in energy intake, this equates to 5 g salt/day for women and 7 g salt/day for men.

Table 13.10  Consumption (g/week) of oily fish among British adults.

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19–24 years</td>
<td>25–34 years</td>
</tr>
<tr>
<td>Consumers, % sample in brackets</td>
<td>* (18%)</td>
<td>195 (33%)</td>
</tr>
<tr>
<td>All, including non-consumers</td>
<td>23</td>
<td>64</td>
</tr>
</tbody>
</table>

*No mean figure given as number of consumers too low.

Table 13.11  New recommendations on oil-rich fish.

<table>
<thead>
<tr>
<th></th>
<th>Oil-rich fish</th>
<th>White fish</th>
<th>Canned tuna**</th>
<th>Marlin, shark, swordfish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls (under 16)</td>
<td>Up to 2 portions* a week</td>
<td>No limit</td>
<td>No limit</td>
<td>Do not eat</td>
</tr>
<tr>
<td>Boys (under 16)</td>
<td>Up to 4 portions per week</td>
<td>No limit</td>
<td>No limit</td>
<td>Do not eat</td>
</tr>
<tr>
<td>Pregnant women and those who may become pregnant</td>
<td>Up to 2 portions per week</td>
<td>No limit</td>
<td>Up to 4 medium-sized cans</td>
<td>Do not eat</td>
</tr>
<tr>
<td>Breastfeeding women</td>
<td>Up to 2 portions per week</td>
<td>No limit</td>
<td>No limit</td>
<td>Up to one portion a week</td>
</tr>
<tr>
<td>Other women, e.g. postmenopausal</td>
<td>Up to 4 portions per week</td>
<td>No limit</td>
<td>No limit</td>
<td>Up to one portion a week</td>
</tr>
<tr>
<td>Men</td>
<td>Up to 4 portions a week</td>
<td>No limit</td>
<td>No limit</td>
<td>Up to one portion a week</td>
</tr>
</tbody>
</table>

*a portion is 140g.
**Fresh tuna counts as oily fish; oils are lost during canning.
total salt intake (estimated by measurement of urinary sodium) was 11.0 g/day in men and 8.1 g/day in women, compared with 10.1 g/day and 7.7 g/day, respectively, in 1986/7 (Gregory et al., 1990). Excluding discretionary salt added in cooking and at the table, the main sources of sodium were cereal products (35%), meat and meat products (26%), milk and milk products (8%), vegetables (excluding potatoes) (7%), and potatoes and savoury snacks (4%). For context, a slice of bread provides about 0.16 g sodium and a 25 g packet of crisps about 0.2 g of sodium.

According to National Food Survey 2000 data, sodium intake from household foods (excluding salt added at the table or during cooking) was then about 2.6 g/day (DEFRA, 2001), equating to about 6.5 g salt/day even without taking food consumed outside the home into consideration. There are no robust data on the amount of salt consumed outside the home, but this is likely to be substantial for some groups (Scientific Advisory Committee on Nutrition, 2003). It is estimated that 15–20% of total sodium intake is in the form of discretionary salt (added in cooking or at the table). Naturally occurring sodium in unprocessed foods contributes approximately 15% (Scientific Advisory Committee on Nutrition, 2003), the remainder coming from processed foods.

In the household food supply, the two main sources of sodium are cereals and cereal products (e.g. bread, breakfast cereals, biscuits, cakes and pastries) at 37%, and meat and meat products at 20.8% (Scientific Advisory Committee on Nutrition, 2003). Foods such as soups, pickles, sauces and baked beans together provide 12.7%, followed by processed vegetables (including snacks & crisps) at 8.5%, milk/cream (5.4%), fats/oiis (4.6%) and fish (2.7%).

In 1994, COMA recommended a reduction in the sodium content of children’s diets but this was not quantified because of the limitations of the available data (Department of Health, 1994). Targets for children (Table 13.12) have now been published (Scientific Advisory Committee on Nutrition, 2003).

The NDNS of young people (4–18 years) found that the average intake of sodium from food in 1997 (excluding that from salt added in cooking or at the table) was 2.6 g/day for boys (equivalent to 6.7 g salt) and 2.2 g/day (5.5 g salt) for girls (Gregory et al., 2000). Among the boys, 1% of the 11–14 year-old group and 8% of the 15–18 year-old group had intakes at 4.5 g sodium/day or more (11.4 g salt). None of the girls or younger boys had such high levels. New targets for these age groups range between 5 and 6 g salt daily.

The UK Government is actively working with food manufacturers, retailers and the catering sector to reduce salt intake, recognising that the scope to do this is influenced by factors such as palatability, consumer acceptance and safety (salt has been used historically as a preservative). Progress has already been made, as evidenced by reductions in the salt levels in bread (www.food.gov.uk) and in a wide variety of processed foods.

### Table 13.12 Intakes of salt and sodium among young people in the UK compared with the new salt targets.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Salt intake (g/day)</th>
<th>Sodium intake g/day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Measured SACN target</td>
<td></td>
</tr>
<tr>
<td>1–3 years</td>
<td>1.4</td>
<td>2</td>
</tr>
<tr>
<td>4–6 years, boys</td>
<td>5.3</td>
<td>3</td>
</tr>
<tr>
<td>4–6 years, girls</td>
<td>4.7</td>
<td>3</td>
</tr>
<tr>
<td>7–10 years, boys</td>
<td>6.5</td>
<td>5</td>
</tr>
<tr>
<td>7–10 years, girls</td>
<td>5.5</td>
<td>5</td>
</tr>
<tr>
<td>11–14 years, boys</td>
<td>5.8</td>
<td>6</td>
</tr>
<tr>
<td>11–14 years, girls</td>
<td>5.8</td>
<td>6</td>
</tr>
<tr>
<td>15–18 years, boys</td>
<td>8.3</td>
<td>6</td>
</tr>
<tr>
<td>15–18 years, girls</td>
<td>5.8</td>
<td>6</td>
</tr>
</tbody>
</table>


et al., 2003b), total salt intake (estimated by measurement of urinary sodium) was 11.0 g/day in men and 8.1 g/day in women, compared with 10.1 g/day and 7.7 g/day, respectively, in 1986/7 (Gregory et al., 1990). Excluding discretionary salt added in cooking and at the table, the main sources of sodium were cereal products (35%), meat and meat products (26%), milk and milk products (8%), vegetables (excluding potatoes) (7%), and potatoes and savoury snacks (4%). For context, a slice of bread provides about 0.16 g sodium and a 25 g packet of crisps about 0.2 g of sodium.

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The UK Government is actively working with food manufacturers, retailers and the catering sector to reduce salt intake, recognising that the scope to do this is influenced by factors such as palatability, consumer acceptance and safety (salt has been used historically as a preservative). Progress has already been made, as evidenced by reductions in the salt levels in bread (www.food.gov.uk) and in a wide variety of processed foods.

### 13.4.4 Fruit and vegetables

At present, men are eating an average of 2.7 (SD 2.0)
portions of fruit and vegetables (excluding potatoes and potato products) per day and women an average of 2.9 (SD 2.0) portions per day (Henderson et al., 2002) rather than the recommended five or more portions (Table 13.13). This equates to an average of 265 g/day for men and 271 g/day for women, compared with an average of about 250 g (approximately three portions) of fruit and vegetables a day in the 1980s when this population was last surveyed (Gregory et al., 1990). Overall, 13% of men and 15% of women consumed five or more portions of fruit and vegetables per day (Henderson et al., 2002). Only 2% of the sample reported eating no vegetables during the seven-day study period, but 21% of men and 15% of women consumed no fruit.

Table 13.13 also shows that the number of portions eaten varies with age, the worst intakes being in young men (19–24 years), all of whom ate less than five portions of fruit and vegetables per day, only managing an average of 0.5 portions of fruit and 0.8 portions of vegetables daily. Young women did not fare much better, averaging 1.8 portions per day (0.9 portions of fruit and 0.9 portions of vegetables). Highest consumptions in this nationally representative sample of British adults were in the oldest age group for both men and women (Henderson et al., 2002).

The NDNS also provides information on regional differences in consumption. There were no statistically significant regional differences: values ranged from 2.6 for men and 2.7 for women in northern England to 3.0 and 3.2, respectively, for south-east England (Henderson et al., 2002). Scotland no longer had the lowest intakes, at 2.9 and 3.0 for men and women, respectively.

Men and women living in households in receipt of state benefits consumed significantly fewer portions than those in non-benefit households (2.1 portions compared with 2.8 for men, and 1.9 compared with 3.1 for women; Henderson et al., 2002). About a third (35% of men and 30% of women) in benefit households had eaten no fruit during the seven-day recording period, and 4% and 6%, respectively, had eaten no vegetables.

Children (4–18 years) are eating on average only two 80 g portions a day (Gregory et al., 2000). This falls short of the recommended 400 g for adults, but is a gradual improvement on intakes since the mid-1970s.

Fresh fruit and vegetables and also fruit juice have increased in popularity over this time-frame (see Table 13.14). These data do not highlight the vast differences in intake between groups, for example the reduced intakes among adults in receipt of benefits referred to above. The NDNS of young people revealed that children in the lowest income groups are around 50% less likely to eat fruit and vegetables than those in the highest income groups (Gregory et al., 2000).

### Table 13.13 Percentage of respondents consuming portions of fruits and vegetables*, including composite dishes, by age and gender.

<table>
<thead>
<tr>
<th>Average daily number of portions fruits and vegetables consumed</th>
<th>Men aged (years)</th>
<th>Women aged (years)</th>
<th>All subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19–24</td>
<td>25–34</td>
<td>35–49</td>
</tr>
<tr>
<td>None</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>&lt;1</td>
<td>38</td>
<td>27</td>
<td>14</td>
</tr>
<tr>
<td>&lt;2</td>
<td>86</td>
<td>54</td>
<td>36</td>
</tr>
<tr>
<td>&lt;3</td>
<td>95</td>
<td>76</td>
<td>59</td>
</tr>
<tr>
<td>&lt;4</td>
<td>95</td>
<td>86</td>
<td>75</td>
</tr>
<tr>
<td>&lt;5</td>
<td>100</td>
<td>93</td>
<td>86</td>
</tr>
<tr>
<td>Mean</td>
<td>1.3</td>
<td>2.2</td>
<td>3.0</td>
</tr>
<tr>
<td>SD</td>
<td>1.03</td>
<td>1.61</td>
<td>1.87</td>
</tr>
</tbody>
</table>

*Excludes potatoes and potato products. All fruit juice consumed in a day counted as only one portion; all beans and other pulses eaten in a day counted as only one portion.


### 13.4.5 Starchy foods and dietary fibre

Table 13.14 shows trends in plant food consumption from 1974 to 1999. Whilst there was little change in overall consumption of vegetables (excluding potatoes and pulses), potato consumption fell progressively,
even when trends in chip consumption were taken into account. Bread consumption also fell, whilst breakfast cereals, rice and more recently pasta began to make a greater impact within the diet. To a large extent, these trends reflect the downward trend in total energy intake that has been a characteristic of dietary patterns for some time, and will have affected dietary fibre intake as it is derived from the food categories listed in Table 13.13.

13.5 Are the recommendations on diet and cardiovascular disease in need of revision?

As is evident from the summary provided in Chapter 11, knowledge about the role of diet in risk factor reduction and the reduction in risk of cardiovascular events themselves continues to expand. It is now recognised that the original diet–heart hypothesis was overly simplistic (see Chapter 11, Section 11.11) and that risk can be mediated through multiple biological pathways other than serum total and LDL-cholesterol. With this in mind, it is timely to consider the need for modification to the dietary advice offered to those with an increased risk of cardiovascular disease.

13.5.1 Dietary fat

There has been much debate about the relevance of a low fat diet versus a diet that is reduced in saturates but still retains a moderate proportion of energy as fat. This is in part because of the difficulty of achieving compliance on low fat diets and partly because of the uncertainty of what should replace the saturates (Clarke & Frost, 2001; see Chapter 11, Section 11.11.1 for a detailed summary).

Advice on dietary fat intake can be summarised as follows (see Chapter 11, Sections 11.7 and 11.8 for details):

- Moderation in fat intake (30–35% of energy), with the partial replacement of saturates by unsaturated fatty acids (monounsaturates, and n-6 and n-3 polyunsaturates).
- Long chain n-3 fatty acids (DHA and EPA), whether via food sources or supplements are likely to be beneficial for those at risk of cardiovascular disease. The richest sources are oil-rich fish; the current recommendation is at least two servings of fish per week, one of which should be oil-rich fish (see Chapter 11, Section 11.8.3). Current average intakes fall well short of this goal. In particular, these fatty acids should be a recommended component of secondary prevention; a dose of approximately 1 g of EPA and DHA combined has recently been recommended by the American Heart Association for people who already have heart disease (Kris-Etherton et al., 2003). The British Dietetic Association in the UK is proposing two or three large servings of oil-rich fish per week for those who have already suffered a cardiovascular event (Hooper, 2001). A key challenge is how to increase intakes of oil-rich fish, which currently fall below targets (see Section 13.4.2 and Chapter 11, Section 11.8.3).
- It remains unclear whether alpha-linolenic acid can replicate the effects on cardiovascular health reported with the longer chain n-3 fatty acids (see Chapter 11, Section 11.8.5).

### Table 13.14 Household consumption trends (g/person/week) in Britain for categories of plant foods, 1974–1999.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total vegetables (all types, excluding potatoes and pulses)</td>
<td>962</td>
<td>1004</td>
<td>937</td>
<td>1041</td>
<td>968</td>
<td>993</td>
</tr>
<tr>
<td>Total fruit (all types)</td>
<td>632</td>
<td>665</td>
<td>628</td>
<td>692</td>
<td>712</td>
<td>766</td>
</tr>
<tr>
<td>Fruit juice</td>
<td>30</td>
<td>63</td>
<td>150</td>
<td>214</td>
<td>240</td>
<td>284</td>
</tr>
<tr>
<td>Pulses</td>
<td>111</td>
<td>125</td>
<td>133</td>
<td>135</td>
<td>117</td>
<td>119</td>
</tr>
<tr>
<td>Nuts and nut products</td>
<td>7</td>
<td>11</td>
<td>12</td>
<td>13</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>Potatoes (fresh and frozen products)</td>
<td>1375</td>
<td>1311</td>
<td>1233</td>
<td>1151</td>
<td>997</td>
<td>872</td>
</tr>
<tr>
<td>Bread</td>
<td>946</td>
<td>890</td>
<td>865</td>
<td>833</td>
<td>758</td>
<td>717</td>
</tr>
<tr>
<td>Rice</td>
<td>16</td>
<td>21</td>
<td>28</td>
<td>30</td>
<td>37</td>
<td>68</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>81</td>
<td>96</td>
<td>117</td>
<td>126</td>
<td>134</td>
<td>134</td>
</tr>
<tr>
<td>Pasta</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>79</td>
</tr>
</tbody>
</table>

Although average *trans* fatty acid intakes have been reduced, some groups of the population may still benefit from a further reduction (see Chapter 11, Section 11.8.6).

### 13.5.2 Other dietary components

A variety of plant foods have been associated with cardiovascular health, including whole-grain cereals, soya, nuts, fruits and vegetables (see Chapter 11, Sections 11.9 and 11.10). In order to capture these potential benefits, emphasis needs to be given to the inclusion of a diverse variety of plant foods in the diet, as depicted in the *Balance of Good Health* (Health Education Authority, 1995). Some of these, *e.g.* soya, have been shown to have modest effects on blood lipids (see Chapter 11, Section 11.9.1), but it is likely that various biological pathways are involved. For the majority of these foods, the active ingredients and the biological mechanisms remain to be elucidated (British Nutrition Foundation, 2003a).

An exception to this is the cholesterol-lowering effects of plant stanol and sterol esters, now incorporated into a range of products such as spreads and yogurt-type products (see Chapter 11, Section 11.9.4). Definitive data on the role of antioxidants and folate/folic acid are not yet available (see Chapters 5, 8 and 11, Section 11.10); however, a balanced diet that provides these nutrients is likely to be beneficial for cardiovascular disease and other chronic diseases.

### 13.5.3 Dietary patterns

Evidence is increasing to support the advantages of a dietary pattern approach rather than focusing exclusively on individual dietary components (see Chapter 11, Section 11.11.3). Typically, such diets have been characterised by higher intakes of fruit, vegetables, legumes, whole-grain cereals, poultry and fish.

The whole diet approach has been tested in several interventions: Lyon Diet Heart Study, DASH and the Indian Heart study (see Hu & Willett, 2002). In the Nurses’ Health Study, a diet high in cereal fibre, long chain *n*-3 fatty acids and folate, and low in *trans* fatty acids and glycaemic load (product of glycaemic index and carbohydrate intake) and with a high ratio of polyunsaturates to saturates, strongly predicted CHD risk (RR = 0.4) when highest and lowest quintiles of composite score were compared. An improvement in these dietary factors explained much of the decline in incidence in CHD during the 14 years of follow-up.

Most dietary manipulations result in modest cholesterol reductions of 4–13%, and diet has been considered by some as a relatively ineffective therapy. On the other hand, statins have been shown to reduce LDL-cholesterol by 28–35% in long-term trials, with corresponding reductions in cardiovascular death of 23% in primary and 32% in secondary prevention trials. However, this view is now being challenged. Jenkins *et al.* (2003) report a randomised controlled trial in hyperlipidaemic adults, in which a parallel design was used to compare a ‘control’ lipid lowering diet (low in fat and very low in saturates, 4.5% energy) with a group taking a statin together with the control diet and a third group on a ‘portfolio diet’ that reflected recent advice from the US National Cholesterol Education Program, *i.e.* it included plant sterols (1 g/1000 kcal), soya protein (21.4 g/1000 kcal), viscous (soluble) fibre (9.8 g/1000 kcal), and nuts (14 g almonds/1000 kcal). The diets were calculated to be weight-maintaining. They were all very high in fibre (57 g for the control diet and 78 g for the ‘portfolio’ diet), far exceeding current intakes in the UK which are typically below 20 g/day. Over a one-month period, the three diets reduced LDL-cholesterol by 8%, 30.9% and 28.6%, respectively. C-reactive protein also fell by similar amounts (see Chapter 7). The diets used in this study were very extreme in terms of their fat and fibre content, and excluded meat. They were also provided ready assembled for the subjects, and the efficacy of the approach needs to be tested in free-living subjects. Nevertheless, the results indicate that diet can be effective if a diversity of cholesterol-lowering components are included.

Using an evidence-based approach, organisations such as the British Dietetic Association are now recognising the need to adjust and broaden the emphasis of dietary therapy for those who have already suffered a heart attack, recognising that many of these people will be taking statins and so will already have reduced blood cholesterol levels (Hooper, 2001). Advice on oil-rich fish consumption is particularly prominent.

In conclusion, substantial evidence shows that diets incorporating non-hydrogenated unsaturated fatty acids as the predominant form of fat, whole-grain cereals as the main form of carbohydrate, an abundance of fruits and vegetables, and adequate *n*-3...
fatty acids can offer significant protection against CHD. Such diets, together with regular physical activity, avoidance of smoking and maintenance of a healthy body weight, may prevent the majority of cardiovascular disease in Western populations.

13.5.4 Smoking
Diet has been the major focus of this book, but it should not be forgotten that the advice to stop smoking continues to be at the forefront of government agendas as a means of reducing cancer and heart disease rates. The cessation of smoking will affect many of the emerging risk factors, including a reduction in fibrinogen concentrations and improvement in arterial endothelium-dependent acetylcholine relaxation. It may also have a positive impact on antioxidant status and hence response to oxidative stress.

13.5.5 Physical activity
The importance of increasing habitual levels of physical activity is now in the spotlight and is covered in detail in Chapter 12.

13.6 Health promotion in children
To combat adult cardiovascular disease it is important that positive long-term lifestyle habits are established early. Evidence for this approach is briefly discussed below.

13.6.1 Cardiovascular disease risk factors in childhood
Multifactorial processes are involved in the pathogenesis of atherosclerosis, which develops over time. Autopsy studies indicate that fatty streaks may already be present in the arteries of young people aged between 15 and 34 years (Strong et al., 1999). In the youngest age group (15–19 years), intimal lesions had appeared in all of the aortas and over half of the right coronary arteries, and they increased in prevalence and extent in the older age group. The data do not imply that all streaks progress into advanced lesions; they may, in fact, regress.

There is also evidence to suggest that cholesterol levels in children ‘track’ into adulthood (American Heart Association, 1983), and thus children with raised blood cholesterol levels may be at risk of CHD in later life. In the NDNS, in general, cholesterol concentrations were not high and did not correlate with fat intake (Gregory et al., 2000). Nevertheless, the majority of young people do not consume enough starchy carbohydrates and fibre, and derive a greater proportion (than recommended for adults) of their dietary energy as fat, saturates and/or sugar. If such patterns of dietary intake, which are potential determinants of blood cholesterol, remain unchanged, it is likely that cholesterol levels would eventually rise.

In adults, control of risk factors such as raised lipids, hypertension, smoking, diabetes and obesity is the major strategy for preventing cardiovascular disease. These risk factors were also associated positively with the extent of both fatty streaks and raised lesions (fibrous plaques and other advanced lesions) in the right coronary artery and in the abdominal aorta in young people (McGill et al., 2000). This implies that control of these risk factors should begin in adolescence and young adulthood. Childhood and adolescence are times when preventive measures could be applied, particularly with respect to encouraging physical activity, which has benefits for a number of risk factors (see Chapter 12).

For more details about the health implications of children’s diets, see Buttriss (2002).

13.6.2 Obesity in children
It is evident that children in the UK and elsewhere are getting fatter, both generally (Bundred et al., 2001; Chinn & Rona, 2001) and also in terms of central adiposity (McCarthy et al., 2003). This has been discussed in detail in Chapter 2 and is a major focus of the UK government White Paper on public health (Department of Health, 2004b).

There are a number of studies which suggest that obesity in childhood is associated with adverse risk factors in adulthood, including increased blood insulin and/or insulin resistance (Sinaiko et al., 1999; Freedman et al., 2001; Steinberger et al., 2001), endothelial dysfunction (Celemajer et al., 1992), and that it is a powerful predictor of the metabolic syndrome (Srinivasan et al., 2002; see Chapter 2, Section 2.4.10).

Current evidence suggests that children most at risk from a cardiovascular perspective are those who have a low birthweight but go on to become obese children (see Section 13.7.1 and Chapter 10).
A review of the evidence base concerning physical activity has been commissioned by the Chief Medical Officer (Department of Health, 2004a) and at the beginning of 2004 funding of eight pilot food in schools projects was announced, focusing for example on lunch boxes, vending and tuck shops. The Food Standards Agency has also funded a systematic review of the evidence concerning promotion of foods to children (Hastings et al., 2003; see Buttriss (2004) for a summary).

13.6.3 Type 2 diabetes in children
An increasing incidence of type 2 diabetes in childhood is recognised in international studies and has also been reported in British children (Ehtisham et al., 2000; Fruhbeck, 2000). Children who are obese, insulin resistant and have low physical activity levels are considered at risk (see Chapter 2, Section 2.5). Genetic predisposition is likely to be involved, and the prevention of insulin resistance in childhood may be particularly important for prevention of diabetes and CHD. Whincup et al. (2002) found that British South Asian children (8–11 years) have higher average levels of insulin and insulin resistance than white children, but that these differences are not associated with corresponding differences in fatness; hence, insulin metabolism may be more sensitive to a given degree of adiposity in this population.

13.6.4 High blood pressure during childhood
Blood pressure elevation is a well-established risk factor for cardiovascular morbidity and mortality in adults (see Chapter 2, Section 2.6). More recently, it has been established through autopsy studies and studies using non-invasive imaging techniques that blood pressure elevation is also a risk factor for the development of arteriosclerosis in childhood and adolescence (Daniels, 2002). In particular, systolic blood pressure elevation is considered as important a factor in the morbidity of hypertension in children as in adults (Sorof, 2002). Therefore, children with elevated blood pressure must be identified and appropriately treated.

Hypertension in children is associated with obesity and other risk factors, including a family history of hypertension and an ethnic predisposition to hypertensive disease. Obese children are at approximately a three-fold higher risk for hypertension than non-obese children (Sorof & Daniels, 2002). A health survey in England conducted in 1999 revealed that Pakistani children have significantly higher systolic blood pressure than either children in the general population or children in other ethnic groups (Department of Health, 2001). The survey also found that physical activity patterns were low in these children compared with others of their age.

Early prevention of obesity and the need for increased physical activity are important deterrents of hypertension. The NDNS of young people (Gregory et al., 2000) reported that the least active children tended to have the highest blood pressure. In addition, blood pressure was also higher among those who smoked or drank alcohol. A range of dietary factors, including sodium, potassium and calcium, have been investigated in relation to blood pressure. The contribution of salt to the causation of high blood pressure is controversial, but a reduction in salt intake is prudent and is advised by SACN (Scientific Advisory Committee on Nutrition, 2003), which has set quantified targets for children according to age (see Section 13.4.3).

13.6.5 Summary
Health traits present in childhood, such as blood lipids and insulin, body weight and blood pressure may track into adulthood. Therefore, a prudent health strategy is the adoption of sensible eating habits and an active lifestyle early in childhood. The American Heart Association has published a statement on cardiovascular health in childhood (Williams et al., 2002), which stresses the importance of integrated cardiovascular health promotion in childhood, focused on increasing physical activity and preventing or treating obesity, raised blood pressure, insulin resistance, type 2 diabetes and smoking.

Current nutritional recommendations for the general UK population (Department of Health, 1991, 1994) are shown in Table 13.3. These are applicable for most children over 5 years and can be gradually applied from the age of 2 years. It is also recommended that all children and adolescents participate in physical activity for 1 hour daily which should be of at least moderate intensity (Fox & Riddoch, 2000; Department of Health, 2004a). Prevention of childhood obesity is a major focus in the UK.

The implementation of guidelines and success of health strategies require input from the Government,
health professionals, the food industry and teachers, as well as the children themselves and their parents. Moreover, social and cultural influences must be recognised when designing and implementing initiatives, of which a number are currently underway in the UK (for an overview, see Buttriss, 2002).

13.7 Health promotion for other groups
Some population groups are at particular risk of cardiovascular disease or require specially tailored health promotion approaches.

13.7.1 Pregnant women
Fetal growth restriction resulting in low birthweight, and low weight gain during infancy, are associated with an increased risk of adult cardiovascular disease, hypertension, type 2 diabetes and the insulin resistance syndrome (see Chapter 10). Poor maternal diet is a major cause of low birthweight globally, but its impact on fetal growth in ‘well nourished’ populations remains unclear (see Chapter 10). Animal studies suggest that maternal nutritional status pre- and peri-conceptionally plays a major role in setting the fetal growth trajectory, but there are currently few data in humans. In terms of cardiovascular risk for the offspring, it is not yet possible to provide specific advice for pregnant women, other than the current advice to maintain an appropriate body weight (neither too high nor too low), to eat a healthy, varied diet and to remain moderately active. The prevalence of low birthweight is increased in lower income groups, and is the focus of a number of strategies aimed to improve health of mother and child, as encapsulated in Sure Start initiatives and reform to the welfare food provision known as Healthy Start (www.doh.gov.uk). Recent evidence shows that giving birth to a low birthweight baby is a risk for later cardiovascular disease in the mother (Chapter 10).

There is no strong evidence that the current policy of encouraging catch-up growth during infancy in low birthweight babies is harmful, although more data are needed on the long-term effects of catch-up growth in infancy. There is some evidence that a high weight at the age of one year is associated with a lower risk of adult cardiovascular disease (see Chapter 10, Section 10.1). Current practice in infant feeding in the UK is described in a recent report from the Department of Health (Hamlyn et al., 2002).

However, it is clear that excessive gain in body weight (upward crossing of centiles) in childhood, after infancy, is associated with later obesity and adverse cardiovascular outcome, and should be avoided.

Cardiovascular risk is, however, increased in men and women who experienced an early adiposity rebound in childhood, and in those who gained weight rapidly after infancy (crossing centiles upwards for weight or body mass index in childhood; see Chapter 10, Section 10.3). The adverse effects of rapid childhood weight gain and adult obesity on cardiovascular disease and its risk factors are exaggerated in people who had a low birthweight.

13.7.2 Ethnic minority groups
As demonstrated in Chapter 1, some ethnic groups experience a higher prevalence of cardiovascular disease and related conditions. For example, the prevalences of type 2 diabetes, insulin resistance syndrome and cardiovascular disease itself are considerably higher among people from South Asia (India, Bangladesh, Pakistan, Sri Lanka) living in Britain compared with the national average (see Chapter 1, Section 1.3.3 and Chapter 2, Section 2.5.1).

UK Indian Asians currently face about twice the risk of heart disease compared to the UK average, and type 2 diabetes is four times more likely in the Asian population in Britain. These findings are of particular concern given the relatively young age profile of the South Asian population living in the UK. Hypertension and stroke are common among the African-Caribbean and Bangladeshi populations (see Chapter 2, Section 2.4.2). Obesity is particularly prevalent among African-Caribbean and Pakistani populations (National Audit Office, 2001).

The reasons for these inequalities in health are not clear cut: biological, socioeconomic, cultural and environmental factors are likely to play their part, in conjunction with individual differences in lifestyle and access to health services (Gervais & Rehman, 2003). Furthermore, the three different generations now resident in the UK are likely to have their own distinctive sociocultural experiences. Differences in dietary patterns, even within this ethnic group, can be considerable and need to be taken into account, as do differences in religious customs. Health promotion activities can be further complicated by language barriers.
Programmes and interventions aimed at the general population often fail to reach the ethnic minority groups, and specifically targeted initiatives are required.

13.7.3 Low-income families

Despite the declining incidence of CHD, rates are slower to decline in lower socioeconomic groups, and the prevalence of risk factors tends to be greater (Batty & Leon, 2003). Although linked by a limited income, low-income groups are in fact very heterogeneous, including young families, students, elderly people and a variety of ethnic minority groups. These groups may require quite different health promotion approaches (Buttriss et al., 2004), but a common theme will be the need to be mindful of the cost of recommended dietary patterns. National dietary surveys have typically shown that the diets of low-income groups are poorer in various essential nutrients, often linked to low dietary variety. For example, intakes of riboflavin, folate, vitamins B6, B12, and C, and most minerals were lower in men and women in families living on benefits, compared with those not receiving benefits (Henderson et al., 2003b) and this was reflected in their nutrient status (Ruston et al., 2004). Also, the health expectations of less prosperous groups are typically poorer than the national average, as demonstrated for obesity in Chapter 11, Section 11.3. But poor dietary patterns should not be assumed to be the norm; for example, some ethnic groups typically eat diverse and healthy traditional diets, which include high intakes of fruits, vegetables and other plant foods.

Tackling health inequalities is a major UK Government strategy in all parts of the country, and a number of initiatives have been developed with this in mind (e.g. Sure Start, www.surestart.gov.uk and reform to the Welfare Food Scheme). These initiatives target families with pre-school children and pregnant women in disadvantaged areas. Associated with this is the Department of Health’s recent recommendation that women should now exclusively breastfeed their baby until it is 6 months old. After this time, it recommends breast milk coupled with appropriate weaning foods.

A survey of the diet and nutrition of low-income groups is now underway but is not due to report until 2006 (www.food.gov.uk).

National food and health policies are being developed which include within their focus the challenge of reducing health inequalities. Food and Wellbeing, the nutrition strategy for Wales, prioritises groups most likely to develop diet and health inequalities, and recognises the need to improve physical activity patterns alongside diet. A draft Diet and Nutrition Strategy for Scotland has also been developed. Work continues on a Food and Health Action Plan, led by the Department of Health for England as a key element of the Government’s Strategy for Sustainable Food and Farming.

In July 2003, the Department of Health published a three-year plan Tackling Health Inequalities: A Programme for Action (Department of Health, 2003). In relation to CHD, targeted interventions include reducing smoking in manual groups, and preventing and managing other risk factors such as poor diet, obesity, physical inactivity and hypertension (especially in the over-50s). Smoking is far more prevalent in lower socioeconomic groups. In social class I, about 15% of men and 14% of women smoke; these values are inversely associated with social class and peak in social class V at 45% and 33%, respectively (www.hda.nhs.uk/). There are also targeted interventions to close the socioeconomic gap in infant mortality.

An update on UK Government work in this area can be found at www.doh.gov.uk/healthinequalities/index.htm.

13.8 Barriers to change – the role of the food industry

Increasing the public’s knowledge of the importance of a healthy diet is only one aspect of encouraging a change of dietary habits. Factors such as a person’s age, beliefs, values, life experiences and skills are also very important, and are modified by factors such as socioeconomic status, income and access. These physical and psychosocial constraints to healthy eating need to be considered when designing campaigns to improve diet (Buttriss et al., 2004). There is also a common perception that scientific opinions are conflicting and constantly changing, which has led some consumers to become sceptical about nutrition messages (Patterson et al., 2001). To combat this, it is important that messages are evidence-based, consistent, unambiguous and practical to implement.

Food manufacturers play a significant role in influencing nutrient intakes, particularly with the
increased reliance on ready-made and convenience foods. For example, the positive step taken by industry to make available leaner cuts of meat and reduced fat dairy products has certainly contributed in a major way to the decline in fat intake observed in the UK. Moreover, substantial reductions in the salt content of certain foods (e.g. bread and more recently a wide variety of processed foods) have been made since the 1980s. Ways to further reduce the salt content of processed foods are being sought, which should help consumers to follow the advice to increase the intake of carbohydrates to 50% of energy intake as well as decrease the intake of sodium (see Section 13.2).

Table 13.9 shows the changes in fat intake that have been achieved since 1970, in particular emphasising the changes in proportional contribution made by different food categories. Some of these changes can be linked back to efforts by the food industry to increase dietary choice, e.g. provision of lower fat milk and dairy products and leaner meat.

The food industry has also responded to nutrition research by producing foods with a more beneficial fatty acid profile. Examples include: manipulation of the diets of farm animals resulting in eggs rich in long chain n-3 fatty acids and meat with fatty acid profiles similar to that from grass-fed animals; the use of highly-refined, deodorised and antioxidant-rich fish oils in spreads and fats; and manufactured foods enriched with EPA and DHA via microencapsulation (Kelly & Stanner, 2003). The difficulty with these innovations is that there are cost implications for consumers, not least because such products may be marketed as premium-priced functional foods. Should recommendations for high-monounsaturates diets (as a means to reduce saturates intakes) come into effect, a comprehensive range of foods that are habitually consumed and adapted to be rich in monounsaturates could be made available by industry. Such products have been produced for use in intervention studies with great success in terms of taste and ease of use (Williams et al., 1999). Methods of manipulating the food chain to increase monounsaturates at the expense of saturates have been discussed by UK experts (Sanderson et al., 2002b). Such products would be appropriate for those in whom fat and energy restriction may be unnecessary. Existing and new products are not/should not be considered as a quick fix solution and do not detract from the importance of a balanced diet and regular physical activity.

Examining the potential for manipulating the fatty acid profile of foods is a key objective of a five-year EU-funded project, LIPGENE, which started in February 2004 (www.lipgene.tcd.ie or via BNF website www.nutrition.org.uk).

13.9 Effective intervention

13.9.1 Approaches used for successful lifestyle/dietary modification

A role of the UK’s Health Development Agency (HDA) is to develop an evidence base for effective health promotion in different contexts (www.hda.nhs.uk). It has found that the most effective healthy eating interventions in schools, workplaces, primary care and the community tend to focus on diet only or diet and exercise. Characteristics of effective healthy eating interventions in these settings included use of theories of behavioural change (e.g. goal-setting), a degree of personal contact particularly over time, feedback (e.g. on changes in behaviour and risk factor level) and the promotion of changes in the local environment (e.g. the catering sector). Their findings showed that to be effective, interventions need to be developed around barriers to change in the local community and to work at several levels. Although the magnitude of change was often small and the cost-effectiveness was not always clearly defined, healthy eating interventions in a variety of populations and settings were considered worthwhile and should be implemented (Buttriss et al., 2004).

The HDA has also reviewed the ‘exercise on prescription’ primary care schemes that promote physical activity to the individual, usually via a local leisure centre facility. Although the benefits varied, the interventions were considered effective for a variety of people (Riddoch et al., 1998). Although not in use at the time of the review, the HDA recommended that behavioural change models may prove a useful approach to changing physical activity patterns. In conclusion, a flexible approach to physical activity using non-facility settings, e.g. simply walking more (which tend not to be used in health professional referrals), may be appropriate for a wide range of population groups (see Chapter 12).

A focus away from leisure centres would highlight the need for environmental services to provide the means to support lifestyle changes. In the North
Karelia Study (see Section 13.3.2), cycling lanes were built to support the advice to increase physical activity. Other dietary and lifestyle advice was also communicated to the public, often by the mass media, resulting in a decrease in the CHD death rate by 48% over a 20-year period. The project was based on low-cost intervention activities, where people's participation and community organisations played a key role. Comprehensive interventions in the community were eventually supported by national activities – from expert guidelines and media activities to industry collaboration and policy (Pekka et al., 2002).

With regard to weight management, it is recognised that successful interventions often include the family; parents are important role models, especially for younger children. Experience shows that weight gain is most readily controlled by addressing eating habits, physical inactivity, and psychosocial and family issues. Guidelines on the treatment of childhood obesity have been published by the Royal College of Paediatrics and Child Health (see Gibson et al., 2002).

In March 2003, it was announced that the National Institute of Clinical Excellence (NICE) (www.nice.org.uk), in collaboration with the Health Development Agency, was to develop guidance on the identification, prevention and management of obesity and maintenance of weight reduction. This follows on from the recommendations of the National Audit Office (2001) in its report on obesity.

An overview of the nutrition policies and strategies in Finland, Norway and Sweden concluded that guidelines, recommendations or voluntary labelling standards can be incentives to product development and changes in food production, and that regional demonstration projects may also encourage action and collaboration (Roos et al., 2002).

Developing healthy lifestyle and dietary habits in childhood is a pragmatic approach to preventing cardiovascular disease. There are numerous initiatives in place in the UK to achieve these aims, many of which involve government, health professionals, the food industry and teachers alike. Examples include the Healthy Schools Programme, re-introduction of minimum nutritional standards for school lunches, compulsory teaching of food and nutrition in the programmes of study for Science, Design and Technology, and Personal, Social and Health Education, the Wired for Health website, the School Fruit Scheme and School Travel Plans (encouraging walking and cycling). Not only do these schemes enable children to make informed choices, but they also provide opportunities to put the advice into practice. These initiatives are discussed in greater detail by Buttriss (2002). Clearly, the school is not the only environment where there needs to be a focus on child health. Lister-Sharp et al. (1999), in a review of health promoting initiatives in schools in the UK, concluded that an approach which combines classroom activities with changes to the school environment and/or family, or community involvement, is most likely to be effective. This is consistent with the health promoting schools approach.

The British Nutrition Foundation has recently completed a review to identify the characteristics of effective interactions to change dietary behaviour on behalf of the Food Standards Agency (Buttriss et al., 2004).

13.10 Looking to the future: gene–nutrient interactions

The study of interactions between nutritional and genetic factors is a new and important area of research. Increased knowledge in this area and new techniques for measuring gene expression, gene polymorphisms (genomics), protein expression (proteomics) and metabolic profile (metabolomics) will eventually allow a more refined approach to reducing risk for cardiovascular disease, with diet interventions targeted towards individuals and subgroups that are genetically susceptible and responsive to the effects of nutritional factors. However, the search for candidate genes has proved to be more complex and their identification more elusive than originally thought (Williams, 2003), largely because much of the variation in risk results from interactions between environmental exposures and the genome. Much of the data available currently on gene–nutrient interactions has been derived from retrospective genotyping; prospective studies are now needed. For a detailed discussion of this topic and the societal and scientific barriers, see Mathers (2003) and Williams (2003).
13.11 Key points

- The UK guidelines for cardiovascular disease prevention were last revised in 1994. A major focus of the 1994 recommendations is the reduction of dietary fat, particularly saturates intake, as a proportion of energy. Targets also exist for salt reduction and fruit and vegetable intake.
- Among adults, the population target for total fat intake of 35% of energy has now more or less been met at 35.8% of energy in men and 34.9% of energy in women, although saturates intake remains high at 13.4% of energy (population target is 11% of energy).
- Salt intakes remain above the 6 g/day target for adults and have risen slightly to 9.5 g/day in the period 1986/7 to 2000/1. Progress is being made in reducing salt present in processed foods.
- Current intakes of fruit and vegetables are 2.7 portions per day in men and 2.9 in women, falling short of the recommended minimum of five servings per day. In 2000/1, 13% of men and 15% of women met the target. The lowest intakes are in young adults, particularly young men who currently achieve only 0.5 portions of fruit per day and 0.8 portions of vegetables. Average intake in children (4–18 years) is just two (80 g) portions per day.
- New knowledge about the associations between dietary fatty acid profile and blood lipids and insulin sensitivity now needs to be taken into account, in particular the role of n-3 fatty acids and monounsaturates. Debate continues about the optimal macronutrient composition.
- Furthermore, more is now known about the ability of other dietary components (e.g. whole-grain cereals, soya, plant phytosterols) to lower cholesterol levels. This information also needs to be taken into consideration when providing dietary advice.
- The evidence for benefit of EPA and DHA has strengthened: the major dietary source of these is oil-rich fish. But consumption levels remain low (one-third of a serving per week) and there is also concern about contaminant levels in some types of oil-rich fish (see Chapter 16, Q91). If these issues cannot be resolved, alternative sources may need to be considered, particularly for vulnerable groups such as pregnant women.
- Evidence is increasing to support the advantages of a dietary pattern approach rather than focusing on individual dietary components, as a number of different dietary manipulations can influence plasma cholesterol levels in an additive sense and may also affect other (emerging) risk factors.
- It is crucial that emerging evidence about the impact of physical activity on risk reduction is synchronised with advice for the population on dietary change.
- With the increasing trends in childhood obesity (16% of 2–15 year olds are now obese), cardiovascular disease risk factors are becoming evident in the child population. Because cardiovascular risk factors, including obesity, tend to track from childhood to adulthood, programmes to increase regular physical activity and healthy eating habits in the young hold promise in reducing adult cardiovascular diseases.
- Fetal growth restriction resulting in low birth-weight, and low weight gain during infancy, are associated with an increased risk of adult cardiovascular disease, hypertension, type 2 diabetes and the insulin resistance syndrome. The effects are exaggerated in those with low birth-weight who subsequently become obese. It is not yet possible to provide specific advice for pregnant women, but an appropriate body weight should be maintained along with moderate physical activity. Reform to welfare food provision aims to improve the health of mother and child.
- Despite the declining prevalence of CHD, rates are slower to decline in low-income groups. The challenge of tackling health inequalities is a stated UK Government priority and is likely to require targeted interventions.
- To encourage sustained and positive dietary change, food and health messages need to be evidence-based, consistent, unambiguous and practical to implement.
- Study of the interactions between nutritional and genetic factors is a new and important area of research, and has the potential in due course to enable a more refined approach to reducing the risk for cardiovascular disease.
Cardiovascular disease is the leading cause of death worldwide and its prevention remains a major public health challenge. The causes of cardiovascular disease are multifactorial, involving a combination of non-modifiable and modifiable risk factors. The former include age, sex and genetic predisposition, and the latter include smoking, raised cholesterol, raised blood pressure, physical inactivity and obesity. These risk factors are referred to as the conventional or ‘classical’ risk factors. It is also recognised that people with type 2 diabetes have a two- to fourfold greater risk of cardiovascular disease mortality than non-diabetic individuals. Furthermore, raised blood triglycerides, socioeconomic status and ethnicity contribute to cardiovascular disease risk.

These ‘classical’ risk factors are, however, unable to adequately explain all of the incidence of cardiovascular disease. This implies that some of the variation in heart disease risk between and within populations must be explained by additional factors. Novel risk factors likely to predispose to or protect against the disease have emerged from a greater understanding of the processes involved in atherosclerosis development, plaque rupture and thrombus formation. For example, better information about the roles of oxidative stress, endothelial dysfunction, homocysteine concentration, inflammation, thrombosis and poor maternal nutrition during pregnancy on cardiovascular risk is emerging. It is also becoming clearer how diet might influence these processes and thus may be important in cardiovascular disease prevention.

The conclusions reached by the Task Force are presented below, in chapter order. The recommendations of the Task Force can be found in Chapter 15.

14
Conclusions of the Task Force

14.1 Chapter 1

- Cardiovascular disease refers to disease of the arteries supplying the muscle of the heart (coronary heart disease, CHD), the brain (cerebrovascular disease) and the extremities, especially the legs (peripheral vascular disease). It involves the processes of atherosclerosis (lesions in the arterial wall) and thrombosis (blood clotting), as well as changes to the function of the arterial lining.
- Cardiovascular disease is the leading cause of death worldwide, accounting for around 18 million deaths each year. Around 50% of these deaths are from CHD and a further 25% from stroke.
- The UK has one of the highest mortality rates from cardiovascular disease in the world, with more than one in three people dying from this condition. The disease is also a major cause of illness and disability, including angina and heart attacks.
- Death rates from CHD have been falling in the UK since the early 1970s, while death rates from stroke have declined throughout the latter part of the twentieth century. Both lifestyle modifications and medical therapies have played an important role in this decline.
- Whilst CHD mortality has been falling in the UK, surveys suggest that morbidity from CHD is not falling and, in older age groups, may even be rising. This reflects both ageing of the population and the survival of those who would previously have died.
- There are major social, regional and ethnic inequalities in cardiovascular disease morbidity and mortality in the UK, which appear to be widening.
Conclusions of the Task Force

Conventional risk factors for cardiovascular disease include smoking, raised cholesterol, raised blood pressure, physical inactivity, obesity and diabetes. These ‘classical’ risk factors were reviewed in a previous British Nutrition Foundation report. However, these risk factors cannot fully explain the regional, gender, socioeconomic and ethnic differences in cardiovascular disease, and emerging evidence suggests that other novel risk factors may play an important role.

This Task Force considers some of these novel or emerging risk factors for cardiovascular disease and reviews the potential role of diet in their modification.

14.2 Chapter 2

Insulin resistance, or the relative inability of insulin to facilitate the disposal of glucose in tissues, is a risk marker for diabetes and cardiovascular disease. Insulin resistance can be caused by several genotypes, by several other environmental conditions and by various pathological processes. Insulin resistance can contribute to several different phenotypes. Multiple cardiovascular risk factors co-segregate with insulin resistance to form a ‘metabolic’ or ‘insulin resistance’ syndrome. Obesity is rapidly increasing and is a major factor in the aetiology of type 2 diabetes, insulin resistance, hypertension and cardiovascular disease. Diabetes is increasing as a consequence of increased obesity/low physical activity. Obesity, insulin resistance, low physical activity and diabetes are major contributors to many of the emerging risk factors discussed in later chapters. The main clinical message is that to avoid these risk factors people should stay slim and be physically active throughout life.

14.3 Chapter 3

Conventional lipid-related risk factors include high plasma total and low-density lipoprotein (LDL) cholesterol, low plasma high-density lipoprotein (HDL) cholesterol and elevated plasma triglycerides. Small, dense LDL particles are likely to be the most atherogenic lipid risk factor. These particles are clearly regulated by diet and nutritional factors (e.g. the amount and type of fat in the diet). At present, the methods by which they can be quantified are complicated, expensive and not for routine clinical use. Remnant lipoproteins are formed in the postprandial state and may add to cardiovascular risk. Remnant lipoproteins and small, dense LDL may increase in response to ingestion of low fat diets, but it is uncertain whether the overall effect is detrimental or not. Favourable aspects of low fat diets may balance the potentially negative effects. Fish oils reduce fasting and postprandial triglycerides as well as remnant lipoproteins, and this may partly account for the health benefit of regular fish consumption. The apoE4 gene variant is related to improved responsiveness to cholesterol-lowering by low fat diets but also to susceptibility to cholesterol in the diet.

14.4 Chapter 4

The endothelium is an active tissue and possesses numerous anti-atherogenic functions in a normal healthy state, including regulation of blood flow in response to metabolic demands, inhibition of blood clotting and prevention of adhesion of inflammatory cells to blood vessel lining. Endothelial ‘dysfunction’ can be considered to be present when properties of the endothelium, either in a basal state or after stimulation, have altered in a way that is inappropriate with regard to preservation of normal function. All known established and novel risk factor pathways can contribute to endothelial dysfunction and, as such, estimates of endothelial function may represent an amalgamation of risk. There is no gold standard measurement. Rather, different aspects of endothelial function can be determined by (1) dynamic tests of its vasodilatory capacity, (2) measurement of circulating blood constituents released by endothelial cells, or (3) urinary excretion of specific molecules such as albumin. Some of these measures appear to be independent predictors of vascular disease, at least in studies conducted thus far, and are potential candidates for risk factor stratification. Much more information is needed, however, particularly on
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non-invasive estimates of vasodilatory capacity and with more rigorous assessment of the extent of predictive ability beyond conventional risk parameters.

- Strategies proven to reduce CHD risk improve endothelial function, but all also improve several other risk factors associated with vascular disease. Such observations are not therefore consistent with the notion that improving endothelial function directly lessens vascular risk. Rather, improved endothelial function may be secondary to improvements in risk factor pathways ‘upstream’ in the chain of atherogenic events.

- Numerous dietary factors, such as low fat diets and fish oils, improve endothelial function. The strength of evidence for endothelial function improvement varies for differing nutrients and several have effects on other CHD risk factor pathways. Evidence of nutrient benefits on endothelial function measures cannot be used as evidence of their cardioprotective ability. Rather, randomised trials with clinical endpoints are needed.

14.5 Chapter 5

- Oxidative stress is a process which is not unique to atherosclerosis, but plays an important part in its aetiology and explains many of the cellular processes associated with it.

- The stress is generated from a variety of reactive oxygen and nitrogen species, and possibly from enzymatic processes.

- There is strong evidence from case-control and prospective human studies of the protective nature of antioxidants, certainly in the form of foods rich in these nutrients, against various forms of cardiovascular disease. However, intervention trials with antioxidant supplements appear to offer little protection over the medium term.

- Several methods are available for the measurement of the oxidation of proteins, lipids and nucleic acids, with varying degrees of sophistication.

- Changes in these measures have been associated with cardiovascular disease or in conditions that predispose to it.

- Diets that are rich in antioxidants or supplements of antioxidants can change these indicators, usually in a beneficial manner. However, this cannot be assumed automatically to parallel a decrease in cardiovascular disease, as the major intervention studies have not shown a beneficial effect of antioxidant supplements on cardiovascular events.

- More work is required to determine the extent to which antioxidants can be effective, or whether it is too late to intervene when atherosclerosis is already well established.

14.6 Chapter 6

- Blood coagulation is a complex autocatalytic process involving cellular elements and soluble proteins, self-limiting in time and space, with control through numerous positive and negative feedback loops. Initiation, evolution, maturation and eventual dissolution of the clot or thrombus involves coagulation enzymes (serine proteases) and their cofactors, anticoagulant proteins and cofactors, inhibitory proteins, blood platelets and vascular endothelial cells.

- In addition to prevention of bleeding, the constituent elements have roles in inflammation, angiogenesis and tissue repair.

- The prothrombotic state can be regarded as an imbalance between coagulant and fibrinolytic capacity favouring fibrin deposition and survival. It comprises one or more of hypercoagulability of plasma, unphysiological activation or sensitivity to activation of platelets and/or endothelial cells, and hypofibrinolysis.

- Haemostatic factors positively related to risk of CHD include fibrinogen, von Willebrand factor and (inconsistently) factor VII activity. Plasma tissue plasminogen activator (tPA) and plasminogen activator inhibitor type-1 (PAI-1) antigen and activity are also positively associated with CHD risk. Changes in platelet function in those at high risk of CHD, though strongly suspected, are difficult to substantiate for methodological reasons.

- Emerging haemostatic risk factors for CHD include activated factor XII, prothrombin fragment 1+2, factor IX activation peptide and D-dimer. Markers of platelet activation linked to CHD risk are being actively sought, including surface proteins exposed upon platelet activation.

- Genetic variants of many haemostatic factors are being increasingly recognised, some of which are related to venous thrombosis. Variants associated with risk of CHD have yet to be definitely established.
Conclusions of the Task Force

**14.7 Chapter 7**

- Obesity is associated with several indicators of a prothrombotic state.
- Reports of effects of acute and chronic alcohol consumption on haemostatic mechanisms have been inconsistent. Regular alcohol consumption appears to reduce the level of fibrinogen and some other clotting factors, and also reduce fibrinolytic activity. The impact of alcohol intake on platelet function has not been comprehensively investigated, but may vary with the type of beverage taken and the intensity and duration of consumption.
- Fatty meals cause postprandial activation of factor VII, although the underlying mechanism and its meaning for CHD risk have not been fully elucidated. A high fat diet is accompanied also by an increase in factor VII antigen, together with evidence for suppressed fibrinolysis.
- Low levels of oxidised LDL enhance platelet activity, whereas high levels of HDL tend to suppress platelet activation.
- Dietary saturates increase platelet activity, whereas polyunsaturates have the opposite effect.
- Fish oils attenuate thromboxane A2 synthesis and thereby suppress platelet activation.
- An increasing number of genetic polymorphisms are being described in the haemostatic system, but studies of their potential relevance for responses to diet are in their infancy.

**14.8 Chapter 8**

- Elevated blood homocysteine is a modest independent risk factor for CHD and for stroke. A meta-analysis of the observational studies on this topic showed that among prospective studies after adjustment for other cardiovascular risk factors, a 25% lowering in homocysteine was associated with about 10% lower risk of CHD and 20% lower risk of stroke.
- About 10% of the population have a genetic variant (TT) for the gene that encodes for methylene-tetrahydrofolate reductase (MTHFR) and such individuals have about 25% higher homocysteine levels than those with the CC genotype. A meta-analysis of the observational studies of MTHFR and CHD showed that individuals with 677TT had a 16% higher risk of CHD compared with those with the 677CC genotype.
- The concordance of the risk associations with homocysteine observed in the population studies with that of the genetically determined differences in homocysteine provides support that the vascular risk associations with homocysteine are causal.
- Large trials of folic-acid-based vitamin supplements are currently underway to test whether
lowering homocysteine levels may reduce the risk of CHD and stroke.

14.9 Chapter 9

- The adipose tissue secretes a diverse number of factors, adipokines, that include enzymes, growth factors, cytokines and several other hormones involved in fatty acid and glucose metabolism.
- These adipokines are implicated in chronic inflammation, insulin resistance, dyslipidaemia, hypertension and endothelial dysfunction, perhaps contributing directly to increased cardiovascular risk.
- Weight loss or fasting often correlates with a fall, and weight gain and excess energy intake a rise, in levels of several of the adipokines (leptin, interleukin-6 (IL-6), acylation stimulating protein (ASP)), reflecting changes in adipose tissue mass.
- However, adiponectin is unique as weight loss is associated with an increase, and obesity with a reduction, in circulating levels.
- In humans, detailed macronutrient and micronutrient effects on adipokines are sparse.
- Several of the adipokines also appear to be influenced by both acute and longer duration exercise. However, these data are mainly based on animal studies.

14.10 Chapter 10

- Fetal growth restriction resulting in low birthweight, and low weight gain during infancy, are associated with an increased risk of adult cardiovascular disease, hypertension, type 2 diabetes and the insulin resistance syndrome.
- The fetal origins of adult disease (FOAD) hypothesis proposes that these associations reflect permanent metabolic and structural changes resulting from undernutrition during critical periods of early development. An alternative explanation is that both reduced fetal growth and cardiovascular disease risk factors have common genetic origins.
- Poor fetal and infant growth may contribute to the high rates of cardiovascular disease seen among ethnic minorities in the UK.
- Cardiovascular disease risk is also increased in men and women who had an early adiposity rebound, those who crossed centiles for weight and body mass index (BMI) upwards during childhood, and those who are obese in adult life. The adverse effects of childhood and adult obesity on cardiovascular disease and its risk factors are exaggerated in people of low birthweight, and relatively small in those of high birthweight.
- Fetal growth is related to maternal size and body composition. Poor maternal diet is a major cause of low birthweight globally, but its impact on fetal growth in ‘well-nourished’ Western populations has been inadequately studied and remains unclear.
- In experimental animals, hypertension and insulin resistance can be consistently programmed in the offspring by restricting the mother’s diet in pregnancy. There are currently insufficient data to determine whether maternal nutritional status and diet programme cardiovascular disease risk in humans.
- In addition to low birthweight, fetal ‘macrosomia’ due to maternal gestational diabetes leads to an increased risk of obesity and type 2 diabetes. Maternal obesity is a strong risk factor for gestational diabetes.
- Mothers should be encouraged to attain a healthy BMI (avoiding excessive thinness as well as obesity) and to adopt a varied and balanced diet before and during pregnancy. However, there are currently insufficient data to set exact BMI or weight gain targets or to make specific dietary recommendations to pregnant mothers, with the aim of increasing fetal growth or reducing adult cardiovascular disease outcomes. Moderate physical activity during pregnancy can be recommended.
- There is insufficient evidence to make recommendations about catch-up growth during infancy in low birthweight babies. Accelerated weight and BMI gain in childhood are clear risk factors. Population-wide reduction in childhood BMI should be a public health priority. Current data suggest that the greatest benefit in terms of individual risk-reduction would be in people who had a low birthweight.

14.11 Chapter 11

- Since the UK recommendations for cardiovascular disease were published in 1994, knowledge of the pathophysiology and risk factors for heart disease has progressed, providing the impetus to re-examine current dietary guidelines, the main emphasis of which is reduction in total fat and saturates.
The Task Force’s analysis of the interaction of diet with emerging risk factors suggests that greater emphasis on fat quality (fatty acid profile) is now justified. Furthermore, as well as considering the impact of dietary fat on blood lipids, it is equally important to take into consideration the impact on insulin sensitivity and energy balance. The strengths and weaknesses of different approaches are hotly debated (e.g. low fat versus moderate fat as percentage of energy intake).

It is also now clear that attention needs to be paid to gene–nutrient interactions, as dietary measures that benefit one individual may be detrimental to another, as has now been demonstrated with fish oil in those with a particular genetic variant of apolipoprotein E.

Also, greater attention needs to be paid to the interactive and synergistic effects of diet and activity level, both in a positive and negative sense, and the implications of maternal and fetal nutrition on future health of the offspring.

Overall, the evidence supports the importance of dietary variety. Although the impact of individual foods on risk factors such as blood lipids may be small, if combined these individual influences have the potential to make a substantial impact.

Given people’s reluctance to change dietary patterns, it is important that alongside provision of dietary advice is research that seeks to identify the extent to which the nutrient composition of staple foods, such as meat, milk, grains, fruits and vegetables, can be modified through feeding regimens and breeding programmes, as is being done in the EU-funded Lipgene project, which is focusing on ways of tackling the metabolic syndrome.

Saturates increase and polyunsaturates decrease total and LDL-cholesterol. All three classes of fatty acids (saturates, monounsaturates and polyunsaturates) elevate HDL-cholesterol when they replace carbohydrates in the diet, and this effect is slightly greater with saturated fatty acids. Also, triglyceride levels increase when dietary fatty acids are replaced by carbohydrate. When monounsaturates or polyunsaturates replace saturates, LDL-cholesterol falls and HDL-cholesterol changes only slightly. The benefit of n-3 fatty acids is convincing, largely due to the positive results from secondary prevention trials. The effects of monounsaturates are likely to be more subtle, but are of potential benefit long-term.

With regard to endothelial dysfunction, a habitually high saturates intake increases LDL-cholesterol and, lowers HDL-cholesterol, and by this mechanism impairs endothelial function. Classical risk factors explain only 20% of the variation in endothelial function. Some associations with dietary factors are emerging, but smoking remains a major factor.

With regard to oxidative stress, diets rich in antioxidant nutrients can modify the markers of oxidative damage, usually in a beneficial way, but this does not necessarily mean that cardiovascular disease risk is reduced.

The evidence for an effect of long chain n-3 fatty acids on haemostasis is moderately strong, but most studies have used doses (as fish oil) in excess of 3 g/day. The strongest evidence for oil-rich fish comes from secondary prevention studies in which the incidence of sudden death is reduced. Moderate alcohol intake may be beneficial for haemostasis as is moderate physical activity; smoking is harmful.

Inflammation and adipocyte-related factors are both areas in which associations with diet are beginning to emerge, but research is at an early stage.

Plasma homocysteine is related to CHD and stroke, but there is uncertainty about whether it is a causal relationship. Results of trials with folic acid supplements for prevention of cardiovascular disease are awaited.

For the majority of the population, moderation in fat intake (30–35% of energy) needs to be emphasised, with the partial replacement of saturates by unsaturated fatty acids being implicit within this message. n-3 fatty acids are now recognised as important dietary factors and intakes of 1 g/day (via diet or supplements) are likely to be beneficial for those at risk of a heart attack (secondary prevention). The recommendation for the general UK population has recently been increased to 0.45 g/day of long chain n-3 fatty acids. Coupled with this is the need for a varied diet including a wide range of plant foods, many of which have been associated with heart health. The Balance of Good Health (the UK Government’s plate model depicting a healthy, balanced diet) provides a useful guide for constructing such a diet, and emphasises the importance of lean meat and low fat dairy products as sources of essential nutrients.
14.12 Chapter 12
- Physical inactivity is commonplace in Britain: 6 in 10 men and 7 in 10 women aged 19–64 years do not take enough physical activity to benefit their health.
- Activity levels are also low in children. About 40% of boys and 60% of girls aged 7–14 years spend less than 1 hour per day in activities of at least moderate intensity, and thus fail to meet the recommendation for young people. This is thought to be a cause of the obesity now seen in children: 16% of 2–15 year olds are obese.
- Men and women who are physically active experience a lower risk of cardiovascular disease in general and CHD in particular, and it’s never too late to change the habits of a lifetime. Stroke risk and hypertension also seem to be reduced.
- A physically active lifestyle is linked with improved body weight control and plasma lipid profile (raised HDL-cholesterol and lower triglycerides). There is also benefit for other cardiovascular risk factors, including blood pressure, insulin sensitivity, endothelial function and glucose tolerance.
- There is considerable evidence that regular physical activity protects against type 2 diabetes, the effect being independent of body weight. As with cardiovascular disease, there is some evidence of a dose-dependent response.
- UK adults are advised to accumulate at least 30 minutes of moderate intensity activity (e.g. brisk walking) on at least five days a week. For many benefits to cardiovascular health, intensity can be traded for duration, provided that total energy expenditure remains high. Benefits are evident in both primary and secondary prevention.
- Children and young people should achieve a total of at least 60 minutes of at least moderate intensity physical activity each day. At least twice a week this should include activities to improve bone health, muscle strength and flexibility.
- To prevent obesity, many individuals will need to participate in 45–60 minutes of at least moderate intensity activity each day. In those who have lost weight, 60–90 minutes a day may be needed to prevent weight regain.
- A number of government-funded local and national initiatives are now underway to tackle physical inactivity in the UK.
- A concerted effort is required to encourage physical activity in the UK population. This will require collaboration and co-ordinated efforts by government at all levels, leisure and sport services, schools and colleges, town and regional planners and providers, architects, countryside agencies, the NHS and social care, voluntary and consumer groups, employers and employees, the media, parents and families. It will be necessary to consider safety measures to encourage cycling or walking.

14.13 Chapter 13
- The UK guidelines for cardiovascular disease prevention were last revised in 1994. A major focus of the 1994 recommendations is the reduction of dietary fat, particularly saturates intake, as a proportion of energy. Targets also exist for salt reduction and fruit and vegetable intake.
- Among adults, the population target for total fat intake of 35% of energy has now more or less been met at 36% of energy in men and 35% of energy in women, although saturates intake remains high at 13.4% of energy (population target is 11% of energy).
- Salt intakes remain above the 6 g/day target for adults and have risen slightly to 9.5 g/day in the period 1986/7 to 2000/1. Progress is being made in reducing salt present in processed foods.
- Current intakes of fruit and vegetables are 2.7 portions per day in men and 2.9 in women, falling short of the recommended minimum of five servings per day. In 2000/1, 13% of men and 15% of women met the target. The lowest intakes are in young adults, particularly young men who currently achieve only 0.5 portions of fruit per day and 0.8 portions of vegetables. Average intake in children (4–18 years) is just two (80 g) portions per day.
- New knowledge about the associations between dietary fatty acid profile and blood lipids and insulin sensitivity now needs to be taken into account, in particular the role of n-3 fatty acids and monounsaturates. Debate continues about the optimal macronutrient composition.
- Furthermore, more is now known about the ability of other dietary components (e.g. whole-grain cereals, soya, plant phytosterols) to lower cholesterol levels. This information also needs to be taken into consideration when providing dietary advice.
- The evidence for benefit of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has strengthened: the major dietary source of these is...
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- Oil-rich fish. But consumption levels remain low (one-third of a serving per week) and there is also concern about contaminant levels in some types of oil-rich fish. If these issues cannot be resolved, alternative sources may need to be considered, particularly for vulnerable groups such as pregnant women.

- Evidence is increasing to support the advantages of a dietary pattern approach rather than focusing on individual dietary components, as a number of different dietary manipulations can influence plasma cholesterol levels in an additive sense and may also affect other (emerging) risk factors.

- It is crucial that emerging evidence about the impact of physical activity on risk reduction is synchronised with advice for the population on dietary change.

- With the increasing trends in childhood obesity (16% of 2–15 year olds are now obese), cardiovascular disease risk factors are becoming evident in the child population. Because cardiovascular risk factors, including obesity, tend to track from childhood to adulthood, programmes to increase regular physical activity and healthy eating habits in the young hold promise in reducing adult cardiovascular diseases.

- Fetal growth restriction resulting in low birthweight, and low weight gain during infancy, are associated with an increased risk of adult cardiovascular disease, hypertension, type 2 diabetes and the insulin resistance syndrome. The effects are exaggerated in those with low birthweight who subsequently become obese. It is not yet possible to provide specific advice for pregnant women, but an appropriate body weight should be maintained along with moderate physical activity. Reform to welfare food provision aims to improve the health of mother and child.

- Despite the declining prevalence of CHD, rates are slower to decline in low-income groups. The challenge of tackling health inequalities is a stated UK Government priority and is likely to require targeted interventions.

- To encourage sustained and positive dietary change, food and health messages need to be evidence-based, consistent, unambiguous and practical to implement.

- Study of the interactions between nutritional and genetic factors is a new and important area of research, and has the potential in due course to enable a more refined approach to reducing the risk for cardiovascular disease.
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15

Cardiovascular disease has a multifactorial aetiology. Although there are a number of unmodifiable risk factors, many of the major causes, including hypertension, obesity, diabetes, high blood cholesterol and triglyceride concentrations, are modifiable by diet, lifestyle factors (e.g. physical activity) or drug treatment. Although these ‘classical’ risk factors are discussed in Chapters 1 and 2 and the public health chapters (Chapters 11–13), this report has focused primarily on novel or emerging factors that are currently being suggested as potential risk factors for cardiovascular disease. Evidence to support these novel risk factors is emerging, but further research is needed to assess the strength of the associations with cardiovascular disease, their relationship to diet and the extent to which such associations may be causal. Further work also needs to be done to establish accurate and reproducible assay methods to measure these risk factors, before making recommendations on their future use in clinical practice.

The individual chapters have evaluated the evidence for the relevance of these novel risk factors with cardiovascular disease and highlighted areas that need to be addressed by future research. This chapter provides an overview of these research recommendations (Section 15.1).

In addition, the need for future work to identify the most effective forms of physical activity for cardiovascular disease prevention and ways in which the UK population may be encouraged to improve their physical activity levels have been highlighted (Section 15.2). Research recommendations also include an evaluation of the most effective forms of dietary intervention (Section 15.3).

Using the available evidence on the associations between diet and lifestyle and conventional risk factors for cardiovascular disease, together with more recent evidence for associations of cardiovascular disease with these emerging risk factors, this chapter also includes some general public health recommendations on the need to reduce the burden of cardiovascular disease in the UK (Section 15.4). These recommendations have been formulated for health professionals, as well as policy makers, industry, caterers, employers and the media.

15.1 Priorities for future research: emerging cardiovascular disease risk factors

15.1.1 Relationship between emerging risk factors, diet and cardiovascular disease

Further research is required to establish the strength of the associations between the emerging risk factors described in this report and cardiovascular disease, in order to compare their predictive value with the established (or ‘classical’) risk factors. For example, further work is required to evaluate the independence of many of the novel risk factors for cardiovascular disease and whether these associations are causal. Further investigation of possible synergistic effects of various novel and classical risk factors is also warranted. In addition, more information is needed about how these novel risk factors might be modified by different aspects of the diet.

Specific areas that require further research include the following:

(i) Lipid-related factors

- Long-term prospective studies on the effects of low fat diets (coupled with different sources of carbohydrate) and different fatty acids on body
weight stability, and incidence of diabetes and cardiovascular disease.

- Investigation of the optimum dietary characteristics (fat and carbohydrate content and type) to prevent development of an atherogenic lipoprotein phenotype.
- Investigation of the mechanisms that lead to hypertriglyceridaemia in the setting of a high intake of dietary carbohydrate and of alcohol.
- Investigation of the effects of different dietary patterns such as large, infrequent meals and regular snacking on postprandial lipaemic responses in free-living individuals.

(ii) Endothelial dysfunction

- Prospective studies to assess whether improvements in measures of endothelial function may predict benefit from certain therapies independently of changes in classical risk factors.
- Additional data on the postprandial effects of dietary fat and carbohydrate on endothelial function.
- Additional data on the molecular mechanisms underlying effects of therapies or lifestyle measures on endothelial function.

(iii) Oxidative stress

- Evaluation of the actual effects of oxidative modification of proteins on cellular and tissue function in different cell types and on tissues as a whole.
- A proteomic approach to establish which proteins are actually modified and what the effects on protein function may be.
- Assessment of the permanence of the changes which occur as a result of oxidative stress in arterial tissue and what mechanisms are important in removal of modified lipids and macromolecules.
- A study of the influence of exogenous dietary antioxidants on oxidative processes in pre-existing atherosclerotic plaque.
- A comparison of the effects of a wide range of dietary antioxidants on oxidative modification of lipids and macromolecules in vivo induced by different reactive oxygen and nitrogen species.

(iv) Haemostatic factors

- Prospective studies to assess the importance of emerging haemostatic risk factors for risk of cardiovascular disease.
- Studies to clarify the importance of dietary characteristics (e.g. dietary energy as indicated by body fat, alcohol consumption, saturates, polyunsaturates including fish oils, and various micronutrients) on markers of the haemostatic system.
- Studies of useful markers of pro-coagulant and fibrinolytic activity that together provide an assessment of haemostatic balance (prothrombin F$_1$+2, a marker of the action of FXa on prothrombin; fibrinopeptide A, released by the action of thrombin on fibrinogen; and D-dimer, an end-product of plasmin activity on crosslinked fibrin) could be studied in, for example, childhood and adult obesity, or in relation to acute and habitual alcohol consumption.
- More studies of dietary fat and haemostasis (but studies of acute effects must control the diet in the days beforehand and use an isoenergetic low-fat control meal to avoid confusion of meal-effects with diurnal fluctuations).
- Additional studies to explain the mechanisms involved in the associations between dietary factors and haemostatic factors; for example, is excess body fat associated with an increased plasma fibrinogen concentration because of increased production or decreased clearance of fibrinogen?
- Assessment of the acute effects of specified meals on postprandial levels of haemostatic factors. The possibility of interaction between the characteristics of the habitual diet and the last meal, with respect to the postprandial state of the haemostatic system, is worth bearing in mind in future research.
- Evaluation of the effects of dietary n-3 long chain polyunsaturates on established and emerging haemostatic risk factors. Studies should compare the responses to physiological and supra-physiological doses of these fatty acids.

(v) Inflammation-related factors

- Clarification of the relationships between serum amyloid A, albumin, specific leukocyte subclasses, circulating immune complexes, cytokines, soluble adhesion molecules, heat-shock proteins,
phospholipase A2 and paroxonase-1 and cardiovascular and/or peripheral vascular disease.

- Large, well-controlled trials to determine the influence of dietary components on inflammatory markers of cardiovascular disease.

(vi) Homocysteine

- Large trials (and a meta-analysis of such trials) of folic-acid-based vitamin supplements in people at high risk of cardiovascular disease, to assess whether lowering homocysteine levels can reduce the risk of cardiovascular disease.
- More evidence to determine the minimum effective doses of folic acid and of vitamin B12 associated with the maximum reduction in homocysteine levels.
- More research on the problem of vitamin B12 deficiency in older people and the relevance of this for public health policy on folic acid fortification.
- Clinical trials of homocysteine-lowering vitamin supplements in people with renal disease.

(vii) Adipokines

- Further investigation into the nutritional regulation of adipose tissue-derived factors in humans.

(viii) Fetal and maternal nutrition

- More information about the determinants of fetal growth and the physiology of the materno-fetal supply line, from maternal food intake to placental function. Studies into the effects of maternal diet should consider micronutrient intakes and status, the balance of macronutrients, and possible paradoxical effects. They should incorporate serial measurements of fetal growth, measurements of neonatal body composition (not just birthweight) and follow-up measurements of cardiovascular risk outcomes in the children. Pre-conceptual nutritional status should be evaluated. It would be helpful to study cardiovascular outcomes in people born during randomised controlled trials of maternal dietary supplementation.
- Consideration of the setting up of a multi-ethnic birth cohort.
- Clarification of the long-term effects of infant ‘catch-up’ growth, aetiology of obesity and the determinants of the age of adiposity rebound. New methods need to be developed to detect, prevent and treat obesity in children and adults. It needs to be established whether targeting efforts to prevent obesity to a ‘high risk’ low birthweight group would be an effective strategy.
- More information about the long-term effects on the offspring of maternal gestational diabetes.

15.1.2 Methods of risk factor assessment

Methods for the assessment of many of the emerging risk factors need to be evaluated and improved for research purposes and, if suitable, for clinical use. Such measures must be reliable and reproducible as well as inexpensive if they are to be offered in a clinical setting or used in studies with large numbers of subjects. For example:

- A method for the quantification of small, dense low-density lipoprotein (LDL) for routine clinical use needs to be devised and evaluated.
- The accuracy and reproducibility of currently available non-invasive methods of endothelial function need to be improved.
- The predictive ability of measures of inflammation for vascular endpoints need to be assessed. In particular, whether such methods improve risk factor stratification beyond that achievable by classical risk factors and C-reactive protein.
- There is currently a lack of tests of platelet behaviour in vivo that are relevant for cardiovascular disease and therefore usable in research studies. There is an urgent requirement for tests that are relatively insensitive to the quality of venipuncture, reproducible and capable of being performed on large numbers of samples inexpensively.

15.1.3 Relationship between emerging cardiovascular disease risk factors and physical activity

Most research into the protective role of physical activity has been limited to the established cardiovascular risk factors, and further investigations of more recently identified risk factors are needed. For example, more data are needed on the assessment of the effects of physical activity on endothelial function, homocysteine and haemostatic factors. In
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addition, the effect of exercise on adipokine expression and secretion needs to be determined.

15.1.4 Genetic variation and gene–nutrient interactions

Further research is needed to identify the genetic determinants of heart disease risk and the nutrient–gene interactions that influence inter-individual variation in response to intakes of specific foods. Much of the data available currently on gene–nutrient interactions has been derived from retrospective genotyping, and prospective studies are now needed.

In relation to the emerging risk factors discussed in this report, areas for future research include:

- A need to identify genetic markers that predict the response of lipids and lipoproteins to differences in dietary intake.
- An examination of the importance of genotype for the responses to diet on emerging haemostatic risk factors, if their effects appear to be important for cardiovascular disease risk (see Section 15.1.1).
- Identification of the genetic determinants of homocysteine levels when examined alone or in combination with each other and with vitamin B_2_, vitamin B_6_, and folate status.
- An investigation of the interactions between genotypic variations of adipose-tissue derived molecules and nutritional regulation.
- Inclusion within epidemiological studies of the fetal origins of adult disease hypothesis of the genes known to influence fetal growth and those associated with cardiovascular disease, and examination of gene–environment interactions.

15.2 Priorities for future research: physical activity and cardiovascular disease

An active lifestyle is considered to have a beneficial effect on body weight and to reduce the risk of CHD and diabetes. Sedentary people have almost double the risk of dying from CHD compared to those who are active and fit (see Chapter 12). Physical activity also has beneficial effects on preventing stroke, treating peripheral vascular disease and on modifying the classical cardiovascular risk factors such as high blood pressure and adverse lipid profiles. The need to promote physical activity amongst adults and young people is therefore well recognised. However, further research needs to help address the obesity/low physical fitness within the UK population and to focus on which elements most directly predispose to cardiovascular disease. There is an urgent need to identify ways of encouraging activity as part of daily life. This has been emphasised by a recent report from the Chief Medical Officer (Department of Health, 2004), which advises that incorporating physical activity into daily routines will be the easiest approach for most people to meet the recommended amount required for health. In addition, there is currently a dearth of data comparing different modalities of exercise of comparable intensity, frequency and duration (such as football training versus rowing versus distance running versus weight training in a gym).

15.3 Priorities for future research: identifying effective dietary interventions

Encouraging people to adopt healthy eating messages is notoriously difficult, not least because individuals are not highly motivated by associations between diet and health and often underestimate their own risk of ill health relative to others. Whilst providing consumers with a knowledge of these messages and the ability to translate them into practical food-based advice is important, the provision of dietary information alone is unlikely to achieve long-term changes in eating behaviour. A greater recognition of the many factors that influence food choice, together with objective evaluation of past interventions (Buttriss et al., 2004) should result in more effective strategies.

There is a need for further research on the barriers to behaviour change, how to overcome these and to explore why some groups are less likely to respond to health messages. There is now a wealth of information from dietary interventions that have attempted to improve fruit and vegetable intake. However, the extent to which successful approaches to increase intakes of these foods can be extrapolated to changing more complex behaviours such as salt and fat intake needs further investigation. More work is also needed on the impact of price on food selection, and the interaction of price with other variables, such as provision of nutrition information.

There is a need to identify life stages or events when individuals may be more amenable to dietary change (e.g. during pregnancy or when identified by healthcare professionals as ‘at risk’) and develop...
strategies to target these subgroups. Given the variation in the prevalence of CHD in the UK population, with highest levels being seen in some ethnic groups and in low-income groups, there is a need to investigate culturally-sensitive and income-sensitive interventions that might be applicable in UK settings.

Young adults in the UK typically have the least varied diets and the greatest likelihood of poor nutrient intakes, and there is a need to identify and evaluate ways of improving the nutrient density of their diets. There are major differences in health behaviour and attitudes among children and young people of different ages. Given the growing concern about childhood obesity, there is an urgent need to understand and address the motivations of young people of different ages and to identify techniques that will encourage them to adopt healthy choices, whether at school or at home, building on work already underway at national level.

15.4 General recommendations

15.4.1 Recommendations to policy makers

Given the new information now available about the influence of diet and cardiovascular disease risk, it is timely to review national dietary guidelines for prevention of CHD and stroke, as has already been done in other countries. To be effective in modifying behaviour, dietary advice needs to be practical and targeted. It may also be timely to review the contemporary applicability of the Government’s Balance of Good Health model (Health Education Authority, 1995), in the context of the emerging science and the findings of the National Diet and Nutrition Survey (NDNS) series, and to consider the opportunities for re-launching the food guide in concert with information on interpretation of nutrition labelling. The recent NDNS of adults could be used as the basis for developing food-based dietary guidelines, targeted at modifying dietary patterns in the context of the emerging science.

Priority should continue to be given to the most disadvantaged to address the widening gap in rates of cardiovascular disease between higher and lower socioeconomic groups, and geographical, gender and ethnic differences.

In order to prevent cardiovascular disease, obesity, diabetes and other chronic lifestyle-related conditions, there is an urgent need for an integrated and UK-wide nutrition and health policy, which incorporates physical activity and other lifestyle risk factors.

To help ensure information is evidence based and up-to-date, there is a need for government and other opinion leaders to champion the work being conducted by professional bodies such as the Nutrition Society and the British Dietetic Association to badge appropriately qualified individuals and high quality training routes. There is a need to evaluate the most effective skills mix and the training needs of the different health professional groups who play a part in providing advice on healthy eating. This information should be fed back into undergraduate and continued professional training.

A concerted effort is required to encourage physical activity amongst all age and gender groups in the UK population. This will require collaboration and a concerted effort by government at all levels, leisure and sport services, schools and colleges, town and regional planners, transport planners and providers, architects, countryside agencies, the NHS and social care, voluntary and consumer groups, employees and workplaces, the media, parents and families.

Strategies to increase physical activity should focus on activity as part of daily life (e.g. walking, cycling), promote a wide range of activities that appeal to different tastes, and address the issue of accessibility of leisure facilities for all social groups. In particular, a public transport system that encourages less car use and greater activity must be a priority. The recent report by the Chief Medical Officer has made some useful suggestions about how to encourage physical activity in the UK (Department of Health, 2004a; see Chapter 12).

Cardiovascular disease prevention strategies must take a long-term approach, which begins in childhood and encourages healthy lifestyles over the life-course. Schools and local authorities should support a whole school approach to food that promotes consistent healthy eating messages in the school dining room, in tuck shops and vending machines and in the classroom. Planning policies should encourage safe routes to school on foot or by bicycle to promote regular physical activity among school children, and facilities should be provided to support physical education in schools. Emphasis should be placed on school nutrition education programmes, including practical cooking skills for all children.
In addition, the Government should continue to work with the food industry to make healthy changes to processed and pre-prepared foods by reducing levels of fat, sugar and salt.

15.4.2 Recommendations to health professionals

Health professionals have a key role in helping their patients and clients to understand the link between diet and cardiovascular disease, and to help them make sense of the plethora of dietary messages reaching them on a daily basis through newspapers, magazines, television and radio. This report is intended to help update those working in this field on new research in this area and in particular to highlight some of the limitations of current knowledge in relation to some of the emerging risk factors, the implications of this for the types of public health messages that can be derived and the research questions that need to be addressed.

15.4.3 Recommendations to industry

The increasing reliance on ready-made and convenience foods highlights the important role of manufacturers in helping to reach some of the dietary targets for cardiovascular disease prevention. Positive steps taken by industry, for example the increase in the availability of leaner meat cuts, reduced fat dairy products and lower fat pre-prepared meals, have contributed substantially to the decline in fat intake observed in the UK since the mid-1970s. The UK food industry has also responded to concerns about levels of trans fatty acids in hydrogenated vegetable oils and has recently provided some alternative strategies to increase intake of n-3 polyunsaturates (e.g. the manipulation of the diets of farm animals resulting in eggs rich in n-3 fatty acids, n-3 enrichment of meat, and the use in fats and spreads of highly refined fish oils that have been deodorised and enriched in antioxidants). The food industry should continue to build on their efforts to provide healthy choices and to modify the composition of existing products as appropriate (e.g. reducing salt, total and saturated fat and sugar content of food, and providing better access to fruit and vegetables and higher fibre products).

Food manufacturers and retailers (including caterers) should also offer a range of portion sizes to consumers and identify viable and sustainable mechanisms to promote healthier food choices. There is also a need to develop a more responsible approach to the promotion of foods to certain target groups (e.g. young children).

Information on packaging still does not enable consumers to choose easily between products. Clear comprehensive and meaningful nutrition labelling and education should be a priority.

In addition, it is crucial that claims about the health promoting properties of foods and drinks are evidence-based, consistent with peer-reviewed scientific evidence and communicated in a responsible way to the public. Industry’s support of the Joint Health Claims Initiative in the UK is an important step in this respect. Progress has been made, but industry needs to take a responsible attitude to promoting foods with implied health claims where the evidence is equivocal. Industry sectors should undertake research to demonstrate the potential of their manufactured foods to contribute to healthy eating by studying the effects of different ingredients on cardiovascular disease risk factors.

15.4.4 Recommendations to caterers

As the frequency of eating meals away from home increases, the nutritional content of food in eating establishments is a factor of growing importance. Caterers should increase the availability of healthier choices, with reduced levels of salt, fat or added sugar, and provide increased access to fruit and vegetables. Access to practical training about healthier catering practices should be provided for caterers based on the Food Standards Agency and Department of Health (2002) guide Catering for Health.

15.4.5 Recommendations to workplaces/employers

Since many employees eat at least one meal and often several snacks at the workplace, potential for influencing employee health is evident. Making changes to the working environment (e.g. changing the food available in the cafeteria or vending machines, point-of-choice information and policies about foods served at company events) can stimulate healthy choices and help to provide a positive work culture that may aid the initiation and maintenance of dietary change.
15.4.6 Recommendations to the media

The relationship between diet and health currently enjoys widespread media coverage, providing both entertainment and health information. However, failure to communicate messages simply, accurately and consistently can result in public confusion, leading to apathy among some and unnecessary anxiety in others. In particular, it is essential that media reporting of new studies linking diet and emerging risk factors for cardiovascular disease are placed in context of the research literature and that any claims do not jump ahead of the evidence base.

15.5 Key references


16
Cardiovascular Disease: Answers to Common Questions from Medical Journalists

The purpose of this chapter is to summarise the key points of this report in simple terms using a question and answer format. Many of the questions used are commonly asked by medical journalists. The questions are grouped under headings, and generally follow the same order as the chapters in the report.

16.1 Definition of cardiovascular disease

Q1. What is cardiovascular disease?
Cardiovascular disease refers to disease of the arteries supplying the muscle of the heart (coronary heart disease, CHD), the brain (cerebrovascular and carotid artery disease) and the extremities, especially the legs (peripheral vascular disease). It involves the processes of atherosclerosis (lesions in the arterial wall) and thrombosis (blood clotting), as well as changes to the function of the arterial lining.

16.2 Epidemiology of cardiovascular disease

Q2. Does the UK have a high death rate from cardiovascular disease compared to other countries?
Cardiovascular disease is a major cause of death, particularly in the Western world, but increasingly so elsewhere. There are wide variations in death rates from cardiovascular disease around the world and, despite recent improvements, those in the UK are amongst the highest. Countries in Eastern and Central Europe, where mortality from cardiovascular disease has been rising rapidly recently, have higher rates than the UK. The lowest rates of cardiovascular disease amongst developed countries are found in Japan and the Mediterranean countries, such as France, Spain and Italy.

Q3. Are cardiovascular disease rates falling around the world?
On a global scale, the burden of cardiovascular disease is growing at an alarming rate. By 2020, it is projected that it will surpass infectious disease as the world’s leading cause of death and disability. Mortality from cardiovascular disease is rising rapidly in developing countries as they become increasingly industrialised and urbanised, and their large populations adopt more westernised diets and lifestyles. This trend is particularly serious for low- and middle-income countries that already have to tackle the dual problem of infectious disease and undernutrition. Many countries of Central and Eastern Europe (most notably countries of the former Soviet Union) have also experienced an increase in cardiovascular disease mortality since the 1990s. This increase has been more pronounced in men than in women. Whilst the reasons for this rise in cardiovascular disease mortality are not clear, it is thought that alcohol, and binge drinking in particular, may be a major determinant in some countries (e.g. Russia).

In contrast, USA, Canada, Israel, Australia and New Zealand have seen large falls in premature cardiovascular disease mortality rates since the 1970s, while Western European countries, such as the UK, Belgium and Norway have seen smaller reductions.
For example, amongst men aged 35–74 years cardiovascular disease death rates fell by 39% between 1988 and 1998 in the UK. However, the number of people suffering from cardiovascular disease in the UK (for example, those with angina or having a heart attack) is not falling and may be rising in older age groups. This is probably associated with increased life expectancy and with improvements in treatment procedures.

Q4. Are there specific groups within the UK that are at high risk of cardiovascular disease?

Heart disease is more common in men than in women, and the prevalence increases with age in both sexes. Within the UK, mortality rates from heart disease are higher in Scotland and the north of England than they are in the south of England. They are also higher in manual than in non-manual social classes.

Certain ethnic groups seem to be particularly prone to cardiovascular disease. People from the Indian subcontinent are more likely to suffer from CHD than the white British population, while people of African and Caribbean descent living in the UK appear to be at greater risk of stroke.

Q5. Why are men at greater risk of heart disease than women?

Young men are more likely to die from heart disease than young women in most industrialised countries. Although female oestrogens are allegedly protective against heart disease, recent data go against this as a major explanatory factor. Rather, men’s elevated risk may arise predominantly because they store more fat in central abdominal regions than do women. They have more to gain therefore from improving lifestyles, in particular by stopping smoking and increasing levels of physical activity, to prevent heart disease and diabetes. However, they are often less likely than women to seek medical advice or respond to health promotion activities.

Q6. So, is heart disease a man’s disease?

Heart disease is far from being a man’s disease. Although women do not seem to be as aware of the risk of heart disease as their risk of breast cancer (British Heart Foundation, 2003b), it is the single biggest killer of women in the UK. One in six women currently die from the condition, and in 2001 heart disease claimed the lives of over 54,000 women (British Heart Foundation, 2003a). This is more than four times the number of deaths from breast cancer.

Q7. How can individuals reduce their risk of cardiovascular disease?

The likelihood of an individual developing cardiovascular disease is influenced by some factors that are outside their control, such as genetic make-up, gender, race (see Chapter 1) and perhaps their early growth pattern (see Chapter 10). However, some of the detrimental effects can be counteracted by changes in behaviour, such as taking more exercise, stopping smoking, maintaining a healthy body weight and eating a varied and well-balanced diet.

Q8. Why has advice to reduce the risk of cardiovascular disease focused so much on fat in the diet?

Dietary fats have been regarded as having an important influence on cardiovascular disease because of their effects on blood cholesterol levels. Populations with a high intake of saturates (saturated fatty acids) have raised blood cholesterol levels and have a high prevalence of heart disease. Laboratory studies have also shown that blood cholesterol can be influenced by the balance of different types of fatty acids in the diet; blood levels of low-density lipoprotein cholesterol are lowered when some saturates (e.g., myristic and palmitic acid) are replaced by monounsaturates, polyunsaturates or carbohydrate. Fatty acids may also affect cardiovascular disease risk via other mechanisms (e.g., by influencing the tendency of blood to clot). Current recommendations recognise this by giving advice to cut down on the amount of fat in the diet, particularly saturates (principally found in spreads, full fat dairy products, fatty meat products and foods such as biscuits and cakes).

While blood cholesterol is an important risk factor for heart disease, a recent estimate suggested that even if the whole UK population managed to reduce their cholesterol levels below 6.5 mmol/l, there would only be around a 10% reduction in CHD deaths because CHD is a multifactorial disease. Thus advice to reduce risk of cardiovascular disease must also include advice to change other aspects of the diet
(e.g. to eat more fruit and vegetables, to reduce salt intake), as well as stopping smoking, taking more exercise and maintaining a healthy weight.

Q9. What is the most important risk factor for cardiovascular disease?

The risk of cardiovascular disease cannot be predicted from a single risk factor. Cardiovascular disease is a multifactorial disease, which arises out of interactive effects of different combinations of risk factors. The effect of different risk factors is also, to a greater or lesser extent, dependent upon individual susceptibility.

The ‘classical’ risk factors for cardiovascular disease are smoking, raised blood cholesterol, raised blood pressure, physical inactivity, obesity and diabetes; these are discussed in more detail in Chapters 1, 2, 11, 12 and 13. However, these cannot explain all cases of heart disease and this has led to a search for other risk factors, many of which have been discussed in detail in this report.

Q10. What might the novel risk factors described in this report add to what we know about cardiovascular disease?

Although cigarette smoking, elevated blood pressure and cholesterol account for many cases of cardiovascular disease, there is reason to believe that other risk factors may account for some of the differences in cardiovascular disease rates within and between populations. A better understanding of the role of these novel risk factors may help to identify other ways of establishing those at risk and additional approaches to tackle the condition.

Q11. Are changes in diet and lifestyle becoming less important as treatment for cardiovascular disease and its risk factors improves?

A number of highly effective drugs (such as lipid lowering agents, ACE-inhibitors, aspirin and beta blockers) are now available in developed countries, such as the UK, and have had a substantial impact on reducing cardiovascular disease mortality. However, the treatment of cardiovascular disease by drugs or medical intervention has important cost implications. In 2001, the cost of prescriptions for lipid lowering drugs alone (including statins) in the UK was just under £440 million (British Heart Foundation, 2003a). Encouraging changes to diet and lifestyle can prevent heart disease without the side effects associated with surgery and drug therapy. Being physically active and eating a balanced diet can also tackle several cardiovascular risk factors simultaneously. Thus lifestyle changes remain critical to reducing the prevalence of heart disease and stroke.

Q12. Why do the French have low rates of heart disease compared to the UK?

Around the Mediterranean, CHD rates are uniformly low, but in many of these countries this can be explained by lower blood cholesterol levels. The French, however, have a high intake of saturates, and their cholesterol levels, blood pressure and the proportion of those who smoke are similar to those in other Western industrialised nations. Yet, the risk of dying from a heart attack for a man in France is only around a third of that of a man in the UK, and a French woman’s risk is one-fourth of that for a woman in the UK. This has been termed ‘the French Paradox’. By contrast, life expectancy is not better in France than in the UK, owing to higher rates of cancer and violent deaths.

Although there may be some under-reporting of heart disease mortality in France, this is unlikely to be the whole explanation. The relative immunity of the French to heart disease has been attributed mainly to their red wine consumption. Regular consumption of moderate amounts of alcohol (around 2 units per day) can reduce risk of heart disease, and this has been attributed in part to increased blood levels of high-density lipoprotein (HDL) cholesterol (see Chapters 1, 3), an inhibition of platelet aggregation (see Chapter 6) and improved endothelial function (see Chapter 4). Many investigators have claimed that red wine is particularly beneficial due to the presence of flavonoids which may act as antioxidants in the body, but this remains to be substantiated. Other potential contributors suggested to explain the French Paradox have included the cardioprotective effects of a Mediterranean diet (that emphasises olive oil, fibre, fruits, vegetables and fish) and various measures implemented in the early 1900s to improve the nutrition and health of mothers and children in France (see Chapter 10).
16.3 Definitions and prevalence of diabetes, the insulin resistance syndrome and obesity

Q13. What is insulin?
Insulin is a hormone produced by the pancreas that allows glucose (obtained from foods containing carbohydrate) to be absorbed from the bloodstream into the cells. This is very important because glucose is the main source of energy that the body needs in order to function properly.

Q14. What is diabetes?
Diabetes, properly known as diabetes mellitus, is a chronic disorder in which the body’s sugar (glucose) level is too high. There are two main types of the disease. Type 1 diabetes (also known as insulin dependent diabetes) occurs when the pancreas does not produce the hormone insulin, which is needed to control blood sugar levels. Type 1 sufferers usually develop the disease during childhood or adolescence. Type 2 diabetes (or non-insulin dependent diabetes) is the most common form of the disease and occurs mostly in adults who are overweight. It arises when the body does not produce enough insulin or the body’s tissues become resistant to insulin, which causes the blood glucose levels to rise (see Chapter 2).

Q15. How many people suffer from type 2 diabetes?
About 1.4 million people in the UK have been diagnosed with diabetes, but experts estimate that about the same number have the condition without knowing it.

Q16. What are the possible complications?
People with diabetes have a higher chance of developing heart disease, strokes, high blood pressure, circulation problems, nerve damage, and damage to the kidneys and eyes. The risk is particularly high for sufferers who are obese, who smoke or who are not physically active.

Q17. How is diabetes linked to cardiovascular disease?
People with diabetes are at greater risk of cardiovascular disease, although the reasons for this are only partly understood. It is thought that if blood glucose levels are higher than normal and not controlled, this may affect the lining of the body’s arterial walls, increasing susceptibility to atherosclerosis (furring up of the arteries).

As well as insulin resistance and high glucose levels, people with type 2 diabetes tend to have central obesity, high blood pressure and abnormal lipid concentrations. All of these are risk factors for cardiovascular disease and when they occur together they are often called the metabolic syndrome (see Chapter 2).

Q18. What are the main risk factors for type 2 diabetes?
Risk factors include increasing age, family history and being overweight, particularly with central obesity (see Q75). People of Asian or African-Caribbean origin and women who have developed diabetes during pregnancy are also at greater risk. Low birthweight and diabetes in the mother during pregnancy have recently been identified as risk factors for type 2 diabetes (see Chapter 10).

Q19. Can lifestyle changes reduce the risk of developing diabetes?
Being physically active, maintaining a healthy body weight and eating a healthy diet that is low in fat, particularly saturates, and high in fibre-rich foods, such as whole-grain cereals and fruit and vegetables, can lower the risk of developing the condition.

Q20. What is insulin resistance?
A person who is insulin resistant has cells that respond sluggishly to the action of insulin. This means that the body’s cells cannot take up enough glucose and the level of glucose in the bloodstream remains high. This signals yet more insulin to be released from the pancreas until the glucose is taken up by the cells. If the pancreas fails to sustain this increase in insulin secretion, type 2 diabetes develops.

Q21. How common is insulin resistance?
Experts suggest that 10–25% of the adult population may be resistant to insulin to some degree. People
who are overweight, those who have a parent or sibling with type 2 diabetes, women who developed diabetes during pregnancy and some ethnic groups (e.g. South Asians) are at increased risk of insulin resistance and the insulin resistance syndrome.

Q22. What causes it?
Insulin resistance is thought to be caused by both genetic and lifestyle factors. Physical inactivity, a high fat diet, excess alcohol consumption and increased body weight, high blood pressure and raised cholesterol are linked to the incidence of insulin resistance, and may trigger the condition in people who are genetically prone to it. All these factors are also linked to an increased risk of cardiovascular disease, and this may be one reason why there is a high incidence of heart disease and stroke among people with type 2 diabetes.

Q23. What do we mean by the insulin resistance syndrome?
The insulin resistance syndrome (also referred to as the metabolic syndrome or syndrome X) refers to a combination of health problems, including insulin resistance, abnormal levels of blood fats (high triglycerides and low HDL or ‘good’ cholesterol), central obesity and high blood pressure. These are all risk factors for type 2 diabetes and heart disease. It is estimated that people with this syndrome are around three times more likely to die from cardiovascular disease, even after controlling for other risk factors (see Chapter 2, Section 2.3.2).

Q24. How common is the insulin resistance syndrome?
To some extent this will vary depending on the definition used (see Chapter 2, Section 2.3.2), but it is likely that as the population ages and the problem of overweight and obesity continues to rise, the number of people with the syndrome will increase.

Q25. What can be done to prevent it?
Adopting a healthy lifestyle, which means eating a healthy diet, maintaining a healthy body weight, not smoking, being physically active and drinking alcohol in moderation, can help to avoid all of the features of the syndrome. Because the conditions occur in a cluster, steps taken to bring one of them into the healthy range will probably improve the others. For example, if you’re overweight, losing up to 10–15% of your body weight will bring down blood pressure and increase cells’ sensitivity to insulin. Similarly, being more physically active can promote weight loss and raise HDL-cholesterol levels.

Q26. How is obesity usually defined?
Obesity is generally defined as a body mass index (BMI) of more than 30 kg/m² (see Chapter 2, Section 2.4.1). Waist circumference (which indicates central or abdominal obesity) is also thought to be important in determining risk of cardiovascular disease (see Q75). An increased risk to health is associated with a waist circumference of over 94 cm (34 in) in men and over 80 cm (32 in) in women. A substantial risk to health is associated with a waist circumference of over 102 cm (40 in) in men and over 88 cm (35 in) in women (see Chapter 2, Section 2.4.2).

Q27. Why is the worldwide increase in obesity of concern in relation to cardiovascular disease?
People who are obese are two to three times more likely to suffer from cardiovascular disease. Obesity is associated with other features of the insulin resistance syndrome (e.g. it increases the risk of high blood pressure, insulin resistance and abnormal levels of blood fats). There is also a strong link between obesity and type 2 diabetes, even with modest degrees of overweight. For example, women with a BMI of just 25 kg/m² have a more than five-fold increased risk, while those with a BMI of more than 35 kg/m² have more than fifty-fold increased risk compared to women with an ideal body weight (BMI 20–25 kg/m²).

Q28. How many adults in the UK are currently overweight or obese?
The prevalence of obesity in Britain has trebled since the mid-1980s. Figures from the National Diet and Nutrition Survey suggest that 25% of men and 20% of women are obese (BMI > 30.0 kg/m²) and a further 42% of men and 32% of women are overweight (BMI 25–30 kg/m²). If these trends continue, it is estimated that 30% of the adult population will be obese by 2010.
Q29. What are the health implications of being overweight during childhood?

Apart from the social and psychological problems experienced by overweight children, there are also long-term risks. Compared with normal weight children, obese children have higher blood pressure and insulin levels, and a lipid pattern that is associated with heart disease. Children who are overweight in their teens are more likely to be overweight as adults and this ‘tracking’ effect is accompanied by an increase in cardiovascular risk factors during adulthood. There is also some evidence that adolescents, regardless of whether they remain obese as adults, are more likely to develop heart disease (see Chapter 2, Section 2.4.10).

Q30. How much weight loss can reduce cardiovascular risk?

Long-term studies have shown that for obese people a sustained modest weight loss of 5% to 10% of body weight can improve a number of risk factors for heart disease (e.g. lower blood pressure, blood glucose concentrations and cholesterol levels). Decreases of just 4 kg over a 4-year period have been shown to cut the risk of diabetes by more than a half.

Q31. Why is being physically active important in reducing cardiovascular disease risk?

Regular physical activity can reduce the risk of diabetes and cardiovascular disease by improving lipid levels, aiding weight loss and lowering blood pressure. Physical activity also has multiple beneficial effects on the emerging risk factors discussed in this Report (see Chapter 2, Section 2.8 and Chapter 12, Section 12.2).

Q32. How much can genes influence the risk of insulin resistance and cardiovascular disease?

There is much ongoing research into this very question. In some rare families, there are powerful and dangerous genes which make it almost certain that an individual will get insulin resistance or cardiovascular disease. However, such families and genes are uncommon and in most people there are only weaker genetic effects. Our present estimate is that about 30–50% of an average individual’s chance of getting insulin resistance and cardiovascular disease will come from his or her genes, whilst the rest will depend upon their lifestyle.

16.4 Lipid-related factors

Q33. What is cholesterol?

Cholesterol is a fatty substance (a lipid) that is found in the bloodstream and in all cells. It has an important role as part of the walls or membranes of each cell. It is also a key component in the manufacture of hormones (chemical messengers in the body) and bile acids (that promote the absorption of fat from the diet).

Some foods, such as meat, poultry, shellfish and dairy products, contain dietary cholesterol. Organ meats, such as liver, are especially high in cholesterol, while it is not found at all in foods of plant origin. However, only a small proportion of cholesterol in the body comes from the cholesterol in food. It is made mostly in the liver and this process is stimulated by saturates (see Chapter 13, Table 13.5 for the main sources of saturates in the UK diet).

Q34. What are ‘good’ and ‘bad’ cholesterol?

There are two main types of blood cholesterol, which are often referred to as ‘good’ and ‘bad’ cholesterol.

Low-density lipoprotein (LDL) is the main cholesterol carrier in the blood. If too much LDL-cholesterol circulates in the blood, it can build up in the lining of the arteries and form atheromas or fatty deposits. These can cause the arteries to narrow in a process called atherosclerosis. An elevated level of LDL-cholesterol is associated with increased risk of heart disease, and is therefore referred to as ‘bad’ cholesterol.

High-density lipoprotein (HDL) is called ‘good’ cholesterol as it carries cholesterol away from the arteries and back to the liver. High levels of HDL-cholesterol help to protect against the development of atheroma in the arteries, while a low level of HDL-cholesterol may increase risk of atherosclerosis.

Thus, a high level of LDL- and low level of HDL-cholesterol (known as a high LDL/HDL ratio) increases the risk of atherosclerosis, while a low level of LDL- and high level of HDL-cholesterol (a low LDL/HDL ratio) is desirable.
Q35. What is Lp(a) cholesterol?
Lipoprotein(a) or Lp(a) is a lipoprotein particle found in the bloodstream. Lp(a) levels appear to be largely genetically determined. High levels are associated with an increased risk of heart disease (see Chapter 3).

Q36. What are triglycerides and how are they linked with heart disease?
Fat in food and fat stored in the body is in the form of triglycerides. A triglyceride is a substance that is composed of three fatty acids attached to a single glycerol molecule. Triglycerides are carried in the blood bound to proteins forming high- and low-density lipoproteins. Like cholesterol, triglyceride in the blood comes either from the diet or from its synthesis in the liver. High triglyceride levels are associated with increased risk of heart disease and stroke.

Q37. What are apolipoproteins?
Apolipoproteins are particles of protein that are mostly formed in the liver and intestine. They play an important role in the production and transport of cholesterol around the body. There are at least nine types of apolipoprotein, including apoA-I, apoB and apoE. Each type bonds with cholesterol in the blood to form either the protective cholesterol, HDL, or the more harmful cholesterol, LDL (see Q38 and Chapter 3).

Q38. What part do they play in coronary heart disease?
High levels of certain apolipoproteins may increase risk of heart disease, diabetes or stroke. For example, high levels of apoB are associated with higher risk of heart disease as it is the main protein in LDL-cholesterol. In contrast, high levels of other apolipoproteins can protect against coronary disease. For example, ApoA-1 is the major protein in the more protective HDL-cholesterol (see Chapter 3).

High levels of apoE can affect the breakdown of cholesterol in the blood, and also influence the progression of heart disease, depending on the subtype of the apolipoprotein. ApoE2 appears to play a protective role as it goes hand in hand with lower LDL levels, while apoE4 slows the removal of LDL from the circulation, and may increase the risk of heart disease.

Q39. What causes high cholesterol?
There are several factors that may contribute to high blood LDL-cholesterol and/or low HDL-cholesterol levels. These include a diet that is high in saturates, lack of physical activity, family history, being overweight, drinking alcohol excessively and smoking. Rarely, high blood cholesterol can also be caused by a condition that runs in the family called familial hypercholesterolaemia.

Q40. Do low fat diets have beneficial effects on blood lipids?
Consuming a diet that is low in fat can lower blood levels of total and LDL (‘bad’) cholesterol. As diets that are low in fat, particularly those that are high in complex carbohydrate, are usually lower in energy (calories) than high fat diets, they can also improve body weight (see Chapter 3, Section 3.2.1 and Chapter 11, Section 11.3.2). However, there is accumulating evidence that the type of fat is of even greater importance for cardiovascular disease than the total amount of fat in the diet. In particular, lowering the amount of saturates in the diet is important in reducing blood cholesterol levels (see Q37 and Chapter 11, Section 11.8).

Q41. What sorts of fats are in foods?
Fat in food is composed of two main types of fatty acids, saturates and unsaturates. Unsaturated fatty acids can be either polyunsaturates or monounsaturates (see Chapter 1). There are two main types of polyunsaturates: n-6 fatty acids obtained predominantly from the seeds of plants, such as sunflower oil or soya oil, and n-3 fatty acids, some of which are predominantly from seeds (e.g. rapeseed and linseed), and others that are present in large amounts in fish oils and have attracted particular attention. All foods contain a combination of saturates, polyunsaturates or monounsaturates, but the proportion of each varies greatly with different foods (see Chapter 13, Table 13.5).
Q42. What effects do these fatty acids have on blood lipids?

Saturates raise blood cholesterol levels more than anything else in the diet. n-6 polyunsaturates lower LDL-cholesterol levels associated with CHD, but also appear to lower HDL-cholesterol protective to the heart. Monounsaturates have been found to help lower the amount of LDL-cholesterol in the blood but maintain HDL-cholesterol levels (see Chapter 13, Table 13.5 for dietary sources). This is likely to be a factor contributing to the ability of Mediterranean-style diets, which are rich in monounsaturates, to protect against cardiovascular disease.

One of the ways in which the long-chain n-3 polyunsaturates found in oil-rich fish may protect against heart disease is via their ability to lower blood triglyceride levels. There is also some evidence that high intakes of these fatty acids may additinally protect against heart disease by positive effects on other blood lipids (e.g. by lowering small, dense LDLs and reducing remnant lipoproteins; see Chapter 3). Their effect on blood cholesterol levels, however, is less clear, and it may be that individuals react differently to these fatty acids. The extent to which the n-3 fatty acids present in seeds can simulate the effects of those from oil-rich fish remains to be clarified.

Q43. What about trans fatty acids?

Trans fatty acids are produced during the process of hydrogenation of unsaturated fats and are principally found in manufactured confectionery products (e.g. biscuits, cakes and chocolates) and some margarines. They have a particularly adverse effect on lipoproteins (they increase LDL-cholesterol and Lp(a) and decrease HDL-cholesterol), and have been shown to increase risk of heart disease. In the UK, however, they contribute a relatively small proportion of total energy compared with saturates, and current average intakes are well below the dietary reference value (see Chapter 3 and Chapter 11, Section 11.8.6).

Q44. Is eating between meals a problem?

While there is little evidence to date that snacking has negative metabolic consequences or any effect on body weight after controlling for total energy intake, more research is needed to clarify the effects of meal frequency on blood lipids and body weight (see Chapter 3). Nevertheless, some highly palatable snack foods are high in fat and energy-dense, which may encourage over-eating. Irregular eating habits can also create difficulties for those trying to control their weight.

Q45. How does exercise affect blood lipids?

Exercise has a number of beneficial effects on the blood fats cholesterol and triglycerides. Regular exercise is associated with an increased ability to clear fat particles from the bloodstream after meals. This is because the exercised muscles need more energy from fat and thus utilise the fat quickly so that it is cleared from the bloodstream. Exercise also affects blood cholesterol levels by increasing HDL-cholesterol (‘good’ cholesterol) levels. Long-term exercise programmes may also reduce LDL-cholesterol (‘bad’ cholesterol) concentrations. In addition, being physically active can help to control weight, diabetes and high blood pressure, all of which would reduce the risk of heart disease (see Chapter 12).

Q46. Can the effect of different diets on blood lipid levels vary between individuals?

Yes, genetic make-up can have a large effect on blood lipid levels and the way in which the body deals with different diets. For example, two healthy individuals eating the same food and with similar lifestyles can have quite different blood cholesterol levels. Also, some individuals may experience greater benefit from changing their diet and lifestyles than others (see Chapter 3).

16.5 The role of the endothelium

Q47. What is the endothelium and what is its main role?

The endothelium is the layer of cells lining various blood vessels of the body. It regulates the normal functioning of blood vessels and plays a role in functions as diverse as: expansion and contraction of blood vessels, repair of damage, formation of new vessels, and immune responses to infection. Due to its key position, the endothelium also forms a selective
barrier between the blood and the underlying tissue, allowing oxygen and nutrients to cross, but not dangerous substances. The range of functions that the endothelium is involved in means that its damage can have serious consequences to the vascular system. These include the formation of atherosclerotic plaques, the build-up of fatty materials within the walls of the arteries, and potentially angina, stroke and heart attacks.

Q48. What is endothelial dysfunction?
Any abnormality in the function of the blood vessel lining which, when functioning properly, helps prevent the process of blood vessel narrowing and fat accumulation.

Q49. How does endothelial dysfunction affect cardiovascular disease risk?
In this circumstance, rather than preventing the process of blood vessel narrowing and thus heart disease, the lining of the blood vessels can actually contribute to this process by allowing more cholesterol to enter blood vessels and more harmful inflammatory processes to occur at the lining of blood vessels. In other words, when the endothelium is ‘dysfunctional’ it can actually accelerate many of the processes leading to blood vessel narrowing and thus increase risk of cardiovascular disease.

Q50. Does diet influence endothelial function?
Some dietary factors do appear to alter the function of the blood vessel lining. For example, long chain $n$-3 fatty acids (found predominantly in oil-rich fish), Mediterranean-style diets (rich in monounsaturates) and some vitamins (e.g. folic acid) appear to enhance the ability of the blood vessel lining to prevent heart disease. In contrast, other agents, either acutely or chronically (e.g. high fat levels), can harm blood vessel function.

Q51. Can other lifestyle factors influence endothelial function?
Yes, other lifestyle factors are important. Physical activity (see Chapter 12), moderate weight loss and smoking cessation can all improve endothelial function. There is also some evidence to suggest that a moderate alcohol intake may have beneficial effects on the endothelium (see Chapter 4).

16.6 Diet and oxidative stress

Q52. Why do nutritionists and dietitians promote the ‘five-a-day’ message?
There is now a considerable body of evidence that has shown diets rich in fruit, vegetables and other plant foods to be associated with a reduced risk of suffering or dying from a number of diseases, in particular cardiovascular disease and some cancers, but also age-related eye conditions such as cataract and macular degeneration, and chronic lung disorders. This has led to the recommendation to eat at least 400 g (five 80 g portions) of fruit and vegetables every day.

Q53. What constitutes a portion?
The recommended ‘five portions a day’ excludes potatoes and nuts, but includes pulses (e.g. baked or red kidney beans and lentils), although they collectively count only as one portion per day no matter how often they are eaten. Similarly, unsweetened fruit juice can also constitute a portion, but only one per day however much is drunk. Fruit and vegetables do not have to be raw – frozen, canned, dried and cooked versions all count towards the five-a-day target. Fruit- and vegetable-based dishes (e.g. fruit crumble, vegetable soup, curry, pizza) will also count as long as they contain at least one portion of fruit and vegetables per serving.

A portion is generally considered to be around 80 g. This corresponds to:
- a piece of a large fruit (e.g. half an avocado or grapefruit, a large slice of melon, a couple of rings of pineapple)
- one medium sized vegetable or fruit (e.g. apple, orange, banana)
- a couple of small fruits (e.g. two plums)
- one cup of very small fruit (e.g. grapes)
- 1/2–1 tbsp dried fruits (e.g. dates, sultanas)
- 2–3 tbsp cooked or canned fruit
- two tbsp raw, cooked, frozen or canned vegetables
- a bowl of salad
- three heaped tbsp beans or lentils (but only counts once per day)
- a glass of fruit juice (but only counts once per day).
Q54. What is so good about fruit and vegetables?
Fruit and vegetables collectively contain a wide range of nutrients, including vitamins with antioxidant properties (e.g. vitamins C and E, and carotenoids such as beta-carotene), folate and other B vitamins, a wide range of minerals including potassium and iron, essential fatty acids and dietary fibre. In addition, there are known to be tens of thousands of bioactive compounds (or phytochemicals), including flavonoids, glucosinolates and phyto-oestrogens, which have been suggested to have beneficial properties with respect to human health. The range of nutrients and phytochemicals present varies considerably between different types of fruit and vegetables. So, the advice with regard to consumption of these foods should focus on variety, to ensure that a wide array of nutrients and bioactive substances are consumed.

There is also likely to be a displacement effect – that is, by eating more fruit and vegetables we tend to eat less of the high fat and energy-dense foods that may increase the risk of obesity, heart disease and diabetes.

Q55. Do antioxidant nutrients reduce the risk of heart disease?
The search for the specific constituents of fruit and vegetables that might protect against heart disease has led to considerable interest in the role of the antioxidant nutrients found in these foods, e.g. vitamins E and C, and beta-carotene. Free radical damage has been implicated as a factor in the development of heart disease and stroke, and a number of antioxidant nutrients are important in the body’s defence systems (see Chapter 5). However, whilst promising and consistent results have been reported in animal and in vitro studies, e.g. tissue culture, human intervention trials have not generally supported a role for these nutrients in heart disease prevention. The contribution of other plant constituents with antioxidant properties, such as flavonoids and sulphur-containing compounds, remains to be established.

Q56. Can supplements be as effective as fruit and vegetables in reducing your risk of cardiovascular disease?
Intervention trials have not supported the notion that supplements provide the same protection against chronic diseases as increasing fruit and vegetable intake. This might be because it is the cocktail effect of the many substances present in whole fruit and vegetables that confer the health properties or that the substances tested are not those responsible.

Q57. Is it true that chocolate contains substances that might be good for the heart?
There is evidence that chocolate, which contains a range of bioactive substances including flavonoids, can increase the antioxidant capacity of the blood and reduce the oxidation of LDL-cholesterol, which is a pre-requisite step for cholesterol being deposited in the arterial wall. The consumption of cocoa-rich products has also been shown to have beneficial effects on other processes associated with cardiovascular disease, e.g. helping to prevent blood platelets from clumping together and forming a clot, and increasing blood vessel flexibility. However, much of this work has been done in a test tube and needs to be supported by human studies. It should also be remembered that chocolate and products containing it tend to be energy-dense and contain relatively high amounts of fat and sugar, and thus should be eaten in moderation.

16.7 Diet and blood clotting
Q58. How and why does blood clot?
Blood is usually a liquid as it is being pumped around the body by the heart, but under some circumstances (e.g. following a cut) it needs to solidify and form a clot. Blood clotting (also known as coagulation) is triggered by a protein called tissue factor, found on cells in the deeper layers of the blood vessels and not normally exposed to blood until there is an injury. Tissue factor reacts with a blood protein called factor VII, and with the aid of small blood cells called platelets initiates a series of complex chemical reactions to produce a substance called thrombin. Thrombin converts a blood protein fibrinogen to fibrin at the site of the wound. These strands of fibrin trap platelets and other blood cells, forming a blood clot (see Chapter 6). This process protects the body from excessive bleeding, ensuring that a clot forms at the site of a wound or injury – either internally or on the body surface. As part of the body’s natural healing mechanism, clots are usually dissolved and reabsorbed by the body.
Q59. What happens when the clotting mechanism goes wrong?

The clotting process (also known as haemostasis) functions as a careful balance between blood flowing and stopping, and between clotting and dissolving/reabsorbing clots. The consistency of the blood is regulated and maintained by a large number of different proteins, some of which are involved in clot formation (coagulation), whilst others are involved in the prevention of clot formation (anticoagulation) and dissolving of formed clots (fibrinolysis). Abnormalities in the amounts of either these coagulation, anticoagulation or fibrinolytic proteins can cause problems. If the blood is prone to clot too little, then there is a risk of haemorrhage; too much and there is a risk of clots forming where they are not wanted. For example, clots that develop in an artery supplying blood to the heart or brain can cause a heart attack or stroke.

Q60. What markers suggest that the blood might be prone to clotting?

Raised concentrations of some of the substances involved in blood clotting or the removal of blood clots from the circulation seem to predict cardiovascular disease risk. For example, high blood levels of the blood clotting protein fibrinogen seem to be associated with increased risk. Emerging research is also identifying a number of other markers (e.g. PAI-1, tPA, von Willebrand factor) that may help in the assessment of patients at risk for CHD (see Chapter 6).

Q61. What can be done to prevent clots forming in the arteries?

There are a number of widely used drugs that act as anticoagulants (which help to prevent blood clots) or clot busters (that help to dissolve blood clots), which can be prescribed to people who are at high risk of cardiovascular disease. One of the most commonly used antithrombotics is aspirin.

However, there are several diet and lifestyle factors that may increase the risk of a blood clot forming. Being overweight, inactive, smoking and consuming a diet that is high in fat (particularly saturates) increases the risk. In contrast, a moderate amount of alcohol may reduce the tendency of blood cells to stick together and form a clot. Studies have also demonstrated longer bleeding times and a reduced tendency for platelets to stick together and form a clot in subjects consuming high doses of n-3 fatty acids, found primarily in oil-rich fish (see Chapter 6).

Q62. Are some people genetically more prone to blood clots?

A number of changes (mutations) in the genes that code for some of the factors involved in coagulation or anticoagulation have been identified, and some of these have been shown to increase the risk of blood clots forming in veins inappropriately. Individuals who inherit these mutations may have an increased risk or ‘predisposition’ to developing blood clots compared to the rest of the population.

16.8 Inflammation

Q63. What is inflammation and what effect does it have on the risk of heart disease?

There are two types of inflammation: acute and chronic. Acute inflammation is a normal process whereby white blood cells are recruited into tissues in response to an injury, infection or an allergen. This is accompanied by the common signs of acute inflammation – pain, swelling, redness and heat. These symptoms are caused by blood vessels dilating around the affected area, bringing substances involved in the inflammatory process to dispense or disperse the damaged tissue or bacteria.

In contrast, chronic inflammation is a longer-term problem. This occurs when the body’s inflammatory response is prolonged beyond normal and can occur because the immune system becomes overstimulated, overactive or fails to switch off its response (or any combination of the three). This type of inflammation can be detrimental as it may injure body tissues; it has been implicated in the aetiology of inflammatory diseases, such as rheumatoid arthritis, psoriasis and inflammatory bowel disease. It has also been suggested that prolonged inflammation of the arteries may be one of several factors that contribute to atherosclerosis (narrowing and hardening of the arteries). The result is that the arteries become constricted, their elasticity and flexibility disappears and the volume of blood able to travel through them at any given time is reduced. This ‘hardening’ can lead
to elevated blood pressure and increase the risk of a heart attack or stroke (see Chapter 1).

Q64. What causes the type of inflammation associated with increased risk of heart disease?

It is not yet known exactly what causes the inflammation that might increase risk of cardiovascular disease. Some researchers suggest that inflammatory cells are recruited to the sites of developing atherosclerotic plaques in response to deposits of cholesterol or oxidised lipid products on the artery wall. Other possible factors that may cause an inflammatory response in the body are smoking, uncontrolled diabetes mellitus and high blood pressure. Others suggest that infection, possibly caused by a bacteria or a virus, might contribute or even start the narrowing process. Possible infectious bacteria include Chlamydia pneumoniae and Helicobacter pylori (see Chapter 7).

Q65. Can markers of inflammation be used to identify people at risk of heart disease?

Levels of some markers of inflammation (e.g. C-reactive protein, fibrinogen and lipoprotein-associated phospholipase A2) have been linked with risk of cardiovascular disease. For example, researchers have found that people with high blood levels of C-reactive protein, which indicates underlying levels of inflammation, are more likely to develop heart disease and stroke than those with lower levels of this protein. This increased risk has been shown to be independent of other risk factors, such as high cholesterol levels, smoking, high blood pressure and obesity. As sensitive tests for C-reactive protein are now available, the protein could be measured to identify those at high risk of developing a first heart attack or stroke (it is likely to be of limited value in secondary prevention as a heart attack increases C-reactive protein levels). However, it is not yet known whether C-reactive protein is a causative agent or a consequence of heart disease, or whether reducing circulating levels of this protein can actually lower cardiovascular disease risk (see Chapter 7).

Whether markers of inflammation, such as C-reactive protein, add anything of value over conventional markers of heart disease as yet remains unclear. The best marker of inflammation to measure is also still being debated.

Q66. Can inflammation be reduced by drugs or diet?

There are a number of pharmacological agents available to reduce inflammation, but we need further research to predict their ability to reduce cardiovascular risk. Some aspects of the diet also seem to influence the inflammatory response. For example, the long chain n-3 fatty acids (found primarily in oil-rich fish) and some antioxidant nutrients (e.g. vitamin C and beta-carotene) seem to have anti-inflammatory effects, but we need more research to determine whether these nutrients influence cardiovascular disease risk in this way.

16.9 Homocysteine

Q67. What is homocysteine?

Homocysteine is an amino acid in the blood that is produced as the body digests and breaks down protein. It is normal to have a certain level of homocysteine in the blood. However, during the past few years, researchers have suspected that high levels of homocysteine increase the risk of cardiovascular disease (see Chapter 8).

Q68. Should people at high risk of cardiovascular disease be screened for elevated homocysteine levels?

The link between high levels of low-density lipoprotein (LDL) cholesterol (the ‘bad’ form of this fatty substance) and cardiovascular disease is well known, and doctors routinely measure cholesterol to gauge a person’s risk. In the past few years, several studies have suggested that elevated levels of homocysteine may also increase the risk of heart disease and stroke. However, this link has not yet been proved, and recent data suggest that homocysteine levels may be a less important risk factor for heart disease than expected. Therefore, unlike cholesterol, homocysteine is not routinely measured and most people who suffer from heart disease will not have their levels measured at routine check-ups. For most people, measuring homocysteine or screening for a genetic mutation that predisposes to high homocysteine is not warranted right now. However, targeted testing is recommended, such as for young people who develop unexpected heart disease or stroke.
Q69. What aspects of the diet and lifestyle can alter homocysteine levels?

Several of the B vitamins are involved in homocysteine metabolism (folic acid, vitamin B6, vitamin B12 and riboflavin) and supplementation with these vitamins, particularly folic acid, can lower homocysteine levels. Homocysteine levels are higher in current smokers than in non-smokers and in people who consume very high amounts of coffee, but are lower in individuals who are moderate alcohol drinkers.

Q70. Should people take vitamin pills containing folic acid to reduce risk of heart disease?

No, taking folic acid to prevent risk of heart disease or stroke is not currently recommended. Whether having extra folic acid lowers the risk of heart attack and stroke must first be put to the test in clinical trials, which are currently underway.

Q71. Why don’t we fortify foods with folic acid in the UK when it is mandatory in the USA?

There is concern that fortification of flour with folic acid for the prevention of birth defects (neural tube defects such as spina bifida) could be hazardous for older people due to delay in the diagnosis of vitamin B12 deficiency or exacerbation of either peripheral neuropathy (a disease affecting the nerves) or other neuropsychiatric complications associated with vitamin B12 deficiency. Experts believe that fortification of flour with folic acid should be accompanied by screening of people aged 75 years or over for vitamin B12 deficiency, or there should be combined fortification of flour with folic acid and vitamin B12.

Q72. Why is being obese a risk factor for cardiovascular disease?

Being overweight increases the chance of having a heart attack. This is partly due to the strain placed upon the heart by excess weight, but obese individuals are also more likely to experience a number of cardiovascular risk factors, including diabetes, high blood pressure (hypertension), an adverse blood lipid profile and abnormalities of blood clotting factors. Emerging research has also shown that fat (adipose) tissue secretes a number of substances (collectively referred to as adipokines) that may themselves increase the risk of cardiovascular disease. The good news is that all of these risk factors are reduced when obese people lose weight.

Q73. What are adipokines?

Adipokines are a group of substances, including enzymes, cytokines and hormones, which are produced in, or released into the blood from, fat tissue. Circulating levels of many of these substances are affected by obesity and have been shown to regulate, directly or indirectly, a number of the processes that contribute to the development of heart disease, including hypertension, endothelial dysfunction, chronic inflammation and insulin resistance. Whilst a lot more research is needed, it is hoped that a greater understanding of the role of these substances and how their levels are regulated might help to combat obesity and cardiovascular disease in the future (see Chapter 9).

Q74. Does diet influence levels of these adipokines?

As weight loss leads to a reduction in the amount of fat in the body, it is associated with a fall in levels of several of the adipokines. Similarly, weight gain and excess energy intake is associated with a rise in many of these substances. At the moment there is insufficient evidence of the effect on adipokines of individual nutrients, but this remains an active area of research.

Q75. Does where you carry excess fat influence your risk of heart disease?

The medical risks from being overweight are increased if excess fat is distributed abdominally (i.e. around the stomach). Men and post-menopausal women tend to lay down fat around the abdomen and have an ‘apple-shaped’ distribution of fat, while pre-menopausal women tend to have a ‘pear-shaped’ distribution, with fat on the thighs and buttocks. ‘Apples’ generally carry a higher risk of developing heart disease and diabetes than ‘pears’. Measuring your waist circumference is the quickest and simplest way to estimate whether abdominal fat is increasing
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your risk of cardiovascular disease (see Chapter 2, Section 2.4.2 and Q26).

Q76. What is leptin?

Leptin is a hormone produced by the fat cells, that is believed to control appetite and regulate body weight via receptors in the brain. A decrease in body fat leads to a decrease in the hormone, which in turn stimulates food intake by increasing appetite. Increased body fat leads to increased levels of the hormone, which act to reduce food intake by turning off the feeling of hunger in the brain. By this mechanism, weight is usually maintained within a relatively narrow range.

Q77. Can leptin be used to treat obesity?

Around 10 years ago, researchers gave leptin to obese mice that were genetically leptin deficient and found that they lost a substantial amount of weight. This led to great excitement both in the media and the scientific community about the possible role of leptin as a treatment for human obesity. However, researchers quickly discovered that most obese humans are, in fact, very unlikely to be deficient in leptin. Whilst rare cases of leptin deficiency have been identified in a small number of obese children, most obese people appear to have much higher levels of the hormone than non-obese people, so giving them more would have little effect. This is not completely unexpected since obese people have more fat and so will produce more leptin, but it seems that they may be resistant to high levels of the hormone so that it does not signal effectively to the brain to eat less. We need to discover more about the functions of leptin and the role it plays in weight gain before we can estimate its potential role in helping to treat obesity.

16.11 Nutrition during pregnancy and fetal growth

Q78. Are small babies at greater risk of developing cardiovascular disease in later life?

Babies who are born small for gestational age (not those born small because of premature delivery) are at increased risk of cardiovascular disease, hypertension and type 2 diabetes in later life (see Chapter 10). Babies born excessively large because of maternal diabetes during pregnancy also have an increased risk of adult type 2 diabetes.

Q79. Are small babies more likely to become obese in adult life?

There is some evidence that they have more central obesity in adult life (fat around the upper body and in the abdomen, which is a risk factor for cardiovascular disease) (see Chapter 2, Section 2.4.10, Q26 and Q75). There is no evidence that small babies develop increased total body fat. However, other effects of low birthweight on the body’s metabolism, for example insulin resistance, may lead to increased risk of obesity-related disease at relatively low levels of total body fat. Lower birthweight babies have reduced adult lean body mass.

Q80. What is the ‘fetal origins of adult disease’ (or ‘thrifty phenotype’) hypothesis?

This hypothesis, put forward by Professor David Barker, proposes that undernutrition during critical periods of development in fetal life and infancy has permanent effects on body build, on the structure of individual organs and tissues, and on hormone systems and metabolism, which lead to an increased susceptibility to adult diseases, including cardiovascular disease, type 2 diabetes and osteoporosis.

Q81. Is growth during early childhood important in terms of cardiovascular disease risk?

The effects of linear (height) growth in childhood are unclear, although shorter adult height is a risk factor for cardiovascular disease. Accumulation of body fat during childhood has a strong effect on later disease risk. Children who cross centile lines upwards for body mass index are more likely to become fatter adults and to develop cardiovascular disease. This effect appears to be strongest in people who were born small.

Q82. Do we know what aspects of a mother’s diet are associated with low birthweight?

Mothers of low past or current nutritional status, as measured by height and body mass index, or blood
levels of micronutrients, have lower birthweight babies. However, intervention trials of nutritional supplements in Western countries have shown little or no increase in birthweight. Most trials have used energy and/or protein, or single micronutrients, and started in mid-pregnancy. There is a need for more research in this area, especially of pre-conceptual effects.

Q83. Are early influences on susceptibility to later disease irreversible or is lifestyle during adulthood still important?

We do not know if adverse metabolic programming in early life is reversible or not. We do know that low birthweight interacts with childhood adiposity and with adult lifestyle risk factors for cardiovascular disease, including obesity and smoking; thus adult lifestyle is very important.

16.12 The effect of different diets on heart disease risk

Q84. Should low carbohydrate, high fat diets be encouraged as a method of weight loss?

For those who need to lose weight, the key aspect is reducing energy intake below energy expenditure. This is best done through a combination of increased physical activity and reduced energy intake. The macronutrient source of the energy (i.e. fat versus carbohydrate) is generally of secondary importance. The low carbohydrate approach to dieting has attracted considerable publicity, but a systematic review of the evidence has demonstrated that weight loss whilst on such diets is primarily the result of a decrease in energy intake, rather than being associated with reduced carbohydrate per se. The review also advises that such diets have been popularised in the absence of detailed information on the possible adverse effects of very low carbohydrate intakes, and in particular their effect in people with cardiovascular disease, dyslipidaemia, type 2 diabetes or hypertension (see Chapter 11, Section 11.3.3). Concern has been expressed about the limited information available on the effects of carbohydrate intakes below 20 g per day, about use of the diets for periods longer than 90 days, about use in people over the age of 50 years, and about the suitability of the dietary approach for people with diabetes who need to lose weight.

Q85. What does glycaemic index mean?

Glycaemic index (GI) is a measure of the rate at which sugar is absorbed into the bloodstream after eating a specific food. It is determined by comparing the blood glucose response after ingestion of a portion of the test food providing 50 g of carbohydrate with the response to a reference food proving an identical amount of carbohydrate (usually either glucose or white bread). Whole-grain foods, pasta, apples and pulses are examples of low GI foods, whereas potatoes, white bread, rice and bananas all have a higher GI. The GI of a food or food ingredient is moderated by how it is prepared, by its degree of ripeness (e.g. fruit) and by the other foods consumed with it (see Chapter 11, Section 11.3.2).

Q86. Are low glycaemic index diets useful for people with diabetes or those trying to lose weight?

The effect of low GI foods is to regulate the rate at which sugars are absorbed; hence inclusion of such foods is often recommended as part of the dietary regimen for diabetics. There is also evidence of beneficial effects on blood lipids (particularly in the context of the insulin resistance syndrome, e.g. triglycerides) in diabetic patients. However, whether the GI of foods has a role to play in appetite and body weight regulation in the general population is much more controversial. There is currently no evidence that low GI diets are superior to high GI diets with regard to long-term body weight regulation (see Chapter 11, Section 11.3.2). Also, use of GI may be confusing for the public, as many of the starchy foods recommended as important components of a balanced diet (e.g. potatoes and rice) have a high GI, and conversely common dietary carbohydrates that are primarily sugars may have a low GI, because about half the carbohydrate is present as fructose (e.g. in the case of ordinary sugar, sucrose) or as galactose (as in the case of the carbohydrate source, lactose, in milk).

Q87. Is a low fat diet the best approach to reduce heart disease risk?

Current dietary recommendations in the UK advise that the population average fat intake should be reduced to 35% of energy, a level that has more-
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or-less been achieved, and that intake of saturates should be reduced to 11% of energy, and n-6 polyunsaturates increased to 6.5% of energy. In the past, other countries have adopted even lower targets for total fat (e.g. 30% of energy) and for saturates. This strategy is primarily aimed at reducing LDL-cholesterol levels. In recent years, however, evidence has accumulated about the potential of low fat/high carbohydrate diets to adversely affect other blood lipid markers (e.g. triglycerides and small, dense LDL particles), and on the potential benefits of other families of fatty acids on other cardiovascular risk factors, particularly insulin resistance. This evidence points to the need for more attention to be paid to the positive attributes of the long chain n-3 fatty acid found in oil-rich fish and to monounsaturates (as found in olive oil and rapeseed oil, for example).

In order to include such fatty acids in adequate amounts, some experts have argued strongly for a more flexible approach to total fat recommendations, especially for subjects with the insulin resistance syndrome (syndrome X). But the answer is not yet clear cut and more evidence is needed before conclusions can be drawn (see Chapter 11, Sections 11.6–11.8). What is known, however, is that regular moderate intensity physical activity can counteract the impact of a low fat diet on increased triglycerides levels, as can inclusion in the diet of long chain n-3 fatty acids present in oil-rich fish (see Chapter 13).

16.13 Physical activity

Q88. How much physical activity should we be doing?

It seems that there is no threshold for the minimal amount of exercise necessary to decrease cardiovascular risk, and that any increase in daily energy expenditure is beneficial. However, to gain real benefit, adults (including older adults) should take at least 30 minutes of at least moderate intensity activity on five or more days each week. Moderate intensity activity should lead to an increase in breathing rate, an increase in heart rate and to a feeling of increased warmth, possibly accompanied by sweating. The good news is that shorter bouts of activity can be accumulated during the day and still count towards the 30-minute minimum.

Children and young people should achieve a total of at least 60 minutes of at least moderate intensity physical activity each day. At least twice a week this should include activities to improve bone health (activities that produce high physical stress on the bones), muscle strength and flexibility.

Taking 30 minutes of moderate intensity activity on at least five days a week will limit health risks for diseases such as heart disease and diabetes, but 45–60 minutes per day is required to prevent the transition to overweight or obesity, and people who have been obese and who have lost weight may need to do 60–90 minutes of activity a day to maintain their weight loss.

Q89. What types of activities count?

All types of activity, provided they are of at least moderate intensity, contribute towards the 30 minute minimum, and different types of physical activity benefit different body systems, e.g. aerobic activity for heart, or weight bearing activities for bones. For many people, the easiest way of increasing their daily activity levels may be to incorporate more brisk walking, stair climbing or cycling into their daily routine, or to take up active hobbies such as gardening. Others will find it easier to join a gym or to make use of local sports centre facilities.

16.14 Public health recommendations to reduce cardiovascular disease risk

Q90. Are we meeting the current dietary targets for cardiovascular disease prevention in the UK?

The good news is that the target to adjust the population average fat intake down to 35% of energy has more-or-less been met in both men and women, and good progress has been made towards the 11% of energy target for saturates, although there is still room for improvement; average intake of trans fatty acids is now well below the 2% of energy target at 1.2% of energy in both men and women. In contrast, since the targets were last revised in 1994, salt intakes have, if anything, risen slightly and so are still well above the 6 g/day target, at 9.5 g/day, and the majority of the public still fail to meet the five-a-day fruit and vegetable target and the ‘at least five-a-week’ physical activity target (30 minutes of at least moderate intensity activity on at least five days per week). In the light of the new evidence summarised in this Task Force Report, an important question to be
addressed is whether the targets now need some adjustment, especially in terms of fatty acid profile, paying more attention to intakes of monounsaturates and $n$-3 polyunsaturates.

**Q91. It is recommended that people increase their intake of oil-rich fish, but what about the risk from contaminants such as dioxins?**

A weekly serving of oil-rich fish is recommended because, for most people, the impact of the $n$-3 fatty acids (also known as omega 3) provided will far outweigh any risk from contaminants such as dioxins and mercury. A particular role of oil-rich fish has been identified for preventing death from heart disease in those who have already suffered a heart attack (see Chapter 11, Section 11.8.3), although average intakes in the UK population remain low at a third of a portion per week.

The Scientific Committee on Nutrition has recently reviewed the evidence and made recommendations on the maximum level at which the health benefits of preventing heart disease clearly outweigh the possible risks from dioxins. Men, boys and women past child-bearing age are advised to eat up to four portions of oily fish per week, while women of child-bearing age, including pregnant and breast-feeding women, and girls, can eat up to two portions a week (www.food.gov.uk). In addition, the Food Standards Agency (FSA) has advised pregnant and breast-feeding women, and those intending to become pregnant, to avoid eating shark, swordfish and marlin because the mercury present in these fish can potentially harm an unborn child’s nervous system. They also advise pregnant women to limit the amount of tuna eaten to no more than four medium-sized cans of tuna or two fresh tuna steaks a week.

**Q92. Do the dietary recommendations need to be revised in light of new research into the links between diet and heart disease?**

It is a recommendation of this Task Force that current dietary recommendations for the prevention of cardiovascular disease and related risk factors need to be reviewed in the light of new research now available, particularly that concerning the influence of the fatty acid profile of the diet, but also new information about other dietary factors that influence blood lipids and other risk factors (see Chapter 13).

**16.15 Key references**


Absolute risk (or absolute risk reduction) The rate of a defined attribute (such as the prevalence or incidence of a disease) among the group exposed minus that among the non-exposed.

Acetylcholine One of a group of chemicals known as neurotransmitters. Found throughout the brain, acetylcholine enables nerve cells to communicate with each other.

Acquired immunity An immune reaction involving lymphocytes that is specific to any given antigen that gives rise to immunological memory.

Acute coronary syndromes Denotes a hospitalisation for unstable angina, or thrombolysis for suspected myocardial infarction, or an emergency revascularisation procedure for relief of ischaemic chest pain at rest.

Acute phase proteins Serum proteins produced by the liver during the early phase of an immune response.

Acute phase response A term used to describe the physiological events that occur following tissue damage. This includes the systemic effects of inflammation, e.g. fever, endocrine changes, changes in fluid and electrolyte balance, and the synthesis of specific proteins (acute phase proteins).

Adenosine monophosphate (AMP) An intermediary substance formed during the body’s process of creating energy in the form of adenosine triphosphate (ATP) from food.

Adenosine triphosphate (ATP) The energy store for all cell processes. The breakdown of ATP to adenosine diphosphate (ADP) or adenosine monophosphate (AMP), releases phosphate groups and usable energy in the cell.

Adhesion molecules Molecules expressed on the cell surface that are involved in the direct binding of one cell to another cell or to specific substrates (e.g. glycoproteins).

Adipocyte A fat cell.

Adipokines Substances (e.g. cytokines, growth factors, enzymes, hormones) that emanate from, or are expressed in, adipose tissue.

Adiponectin A hormone produced and secreted exclusively by adipocytes (fat cells) that regulates the metabolism of lipids and glucose. Adiponectin influences the body’s response to insulin and has anti-inflammatory effects on the cells lining the walls of blood vessels.

Adipose cell A fat storing cell.

Adipose tissue Tissue found under the skin and around the body organs that is composed of fat storing cells.

Adiposity rebound The age at which young children begin to increase their body fat stores. Research has suggested that this may be a useful predictor of the development of obesity, with those experiencing the rebound at a younger age running an increased risk of subsequent obesity.

Adiposity An indicator of the extent of fat in the body.

Adipsin A protein secreted by adipose tissue.

Adrenaline A hormone released in response to stress.

Alleles Alternative forms of a gene. A single allele is inherited separately from each parent.

Aneurysm A localised, pathological, blood-filled dilatation of a blood vessel caused by a disease or weakening of the vessel’s wall.

Angina A tight pain (typically across the chest) with or without breathlessness caused by an inadequate supply of oxygen to the heart muscle. Angina is often triggered by exercise, stress or cold weather.
Angiogenesis  The process of developing new blood vessels.

Angiotensin-converting enzyme (ACE)  An enzyme that converts angiotensin I to a biologically active form, angiotensin II. ACE inhibitors are used to combat hypertension.

Angiotensin-converting enzyme (ACE) inhibitor  A drug that blocks the action of angiotensin-converting enzymes. It reduces the constriction of the blood vessels, veins, and arteries, resulting in the lowering of blood pressure.

Angiotensinogen  A protein produced by the liver involved in vasoconstriction (narrowing of blood vessels). Excess production is often implicated in high blood pressure.

Antigen  A substance (usually a protein that is foreign to the body) that causes the formation of antibodies.

Antioxidant  A compound that prevents or protects against the damage which could be caused by the oxidation of fatty acids and proteins.

Apolipoprotein  A protein that transports fat-soluble substances in the blood.

Apolipoprotein A-I (apoA-I)  The primary protein constituent of high-density (HDL) lipoprotein. It is also found in chylomicrons. Increased levels of apoA-I are associated with reduced risk of coronary heart disease.

Apolipoprotein B (apoB)  One of the proteins of the low-density lipoprotein which transports lipid and cholesterol to the tissues. Also present in very-low-density lipoprotein.

Apolipoprotein E (apoE)  Occurs in all types of lipoproteins and is thought to be involved in the conversion of very low-density lipoproteins to intermediate-density lipoproteins and the removal of low-density lipoproteins from the circulation.

Apoptosis  Programmed cell death (‘suicide’).

Arachidonic acid  An unsaturated 20 carbon fatty acid obtained in the diet from animal fats or synthesised in the body from a dietary source of linoleic acid. Used to synthesise molecules such as prostaglandins and thromboxanes.

Arrhythmia  Any form of irregular electrical activity in the heart leading to irregular heart beats. It can be intermittent or continuous.

Atherogenic  Having the capacity to start or accelerate the process of atherogenesis or the formation of lipid deposits in the arteries.

Atherogenic lipoprotein phenotype (ALP)  A heritable trait characterised by a predominance of small, dense low-density lipoprotein particles, increased levels of triglyceride-rich lipoproteins, reductions in high-density lipoproteins and an increased risk of myocardial infarction (see Chapter 3, Section 3.2).

Atherosclerosis  The process in which fatty and fibrous deposits cause thickening and hardening of the arterial walls.

Atrial natriuretic peptide/factor (ANP)  A hormone released from the heart in response to elevated pressure in the heart. ANP causes vasodilation (relaxation of the vessel wall).

Attributable risk  This provides a measure of the excess risk between the exposed and the non-exposed in a population.

Autocrine  Pertaining to cell messengers that act at or near the site where they are produced (cf. endocrine).

Beta oxidation  A biological procedure in which two carbon atoms are cleaved from a fatty acid chain.

Betaine (trimethylglycine)  Found in several tissues in humans. It is involved in homocysteine metabolism as an alternative methyl donor and is used in the treatment of homocystinuria in humans.

Bias  Systematic deviation of study results from the true result, due to the way(s) in which the study is conducted.

Blood pressure  A measure of the force that the circulating blood exerts on the walls of the main arteries. The pressure wave transmitted along the arteries with each heartbeat is felt as the pulse – the highest (systolic) pressure is created by the heart contracting, and the lowest (diastolic) pressure is measured as the heart fills.

Body mass index (BMI)  An index of obesity calculated as weight in kilogrammes divided by the square of height in metres ($w / h^2$) (see Chapter 2, Section 2.4.1).

Caffeic acid  A type of phenol found in various fruits, vegetables and citrus fruits which has antioxidant-like activities.

Cardiovascular disease  A disease of the heart or circulation. This broad term encompasses coronary heart disease, peripheral vascular disease and stroke.

Carotenoids  A group of red, orange and yellow pigments found in plant foods and in the tissues of organisms that consume plants. Carotenoids have
antioxidant activity. Some, but not all, can act as precursors of vitamin A.

Case-control study  A study that compares people with a disease or condition ('cases') to another group of people from the same population who do not have that disease or condition ('controls').

Catecholamines  Amines derived from the amino acid tyrosine, including adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine, which act as hormones or neurotransmitters.

Central obesity  Accumulation of fat around the abdominal (stomach) region. Also known as abdominal obesity or upper-body obesity or 'apple' body shape. This is associated with an elevated risk of cardiovascular disease and is more common in some ethnic groups, e.g. South Asians (see Chapter 1, Section 1.3.3, Chapter 2, Section 2.4.2 and Chapter 9, Section 9.1.2; cf. lower-body obesity).

Chemokine  A family of cytokines involved in a wide variety of processes, including acute and chronic types of inflammation, infectious diseases and cancer.

Chemotaxis  Directed movement of cells in response to a concentration gradient of a chemical substance such as chemokines or cytokines.

Cholecystokinin  A hormone that stimulates the contraction of the gallbladder with release of bile and the secretion of pancreatic enzymes into the small intestine.

Cholesterol  A fat-like substance found in the bloodstream as well as in bodily organs and nerve fibres. Most cholesterol in the body is made by the liver. It is an essential constituent of cells, but when present in excess becomes a key component in the development of atherosclerosis.

Chronic infection  Infection that persists without effective resolution.

Chylomicron  Circulating lipid particles defined by size, density and apolipoprotein content. Large particles composed mainly of triglycerides and cholesterol recently absorbed from a meal, which are delivered to muscle, adipose tissue and the liver.

Cis fatty acid  The form of most naturally occurring unsaturated fatty acids, where the hydrogen bonds are on adjacent sides of double bonds, resulting in a bend in the hydrocarbon chain at that point (cf. trans fatty acids).

Coagulation of the blood  The process whereby bleeding (or haemorrhage) is normally arrested in the body. Coagulation is part of the process of haemostasis, which is the arrest of bleeding from an injured or diseased blood vessel (see Chapter 6).

Coagulopathy  Abnormality of the blood that interferes with its normal clotting.

Complement  System of serum proteins and a group of membrane proteins that interact in a complex cascade reaction sequence. Involved in the control of inflammation, the destruction of cell membranes and the activation of phagocytes.

Confidence interval  This is the range of values within which we can be 95% sure that the true answer lies when we estimate something with some uncertainty, though this description is only approximate. Wide confidence intervals reflect a lot of uncertainty about the value and arise from small sample sizes and/or large variability.

Confounding  Occurs when an observed association (or lack of one) is, in fact, due to a mixing of effects with a third factor that is associated with both the exposure and the disease (for further description and examples, see Chapter 1).

Conjugated linoleic acid (CLA)  A series of isomers of linoleic acid found predominantly in the meat and milk of ruminant animals.

Coronary arteries  The arteries supplying blood to the muscle of the heart.

Coronary heart disease (CHD) (or ischaemic heart disease)  Heart disease resulting from the build-up of fatty deposits in the lining of the coronary arteries. It may cause angina, a heart attack or sudden death.

Correlation  Two variables are said to correlate if variation in one is systematically accompanied by variation in the other. If one increases as the other decreases, this is a negative correlation. If one increases as the other decreases, this is a negative correlation.

Correlation coefficient  A number between −1 and 1 that measures the degree to which two variables are linearly related. Values approaching +1 indicate that there is a strong positive relationship between the variables. Values approaching −1 indicate that there is a strong inverse relationship. Values around zero indicate there is no relationship at all.

Corticotrophin  A hormone produced by the pituitary gland (also known as ACTH) which stimulates the adrenal gland to produce cortisol.
Glossary

Cortisol  The body’s primary stress hormone.
C-reactive protein  A plasma protein that rises in the blood with inflammation (see Chapter 7, Section 7.4.1).
Cystathionine beta-synthase  The enzyme that converts homocysteine to cystathionine and then cysteine, using vitamin B6 as a cofactor (see Chapter 8, Fig. 8.2).
Cytokines  Small, hormone-like proteins released by leukocytes, endothelial cells and other cells to promote an inflammatory immune response to an injury.
Cytotoxic  A substance that is harmful to cell structure and function, and ultimately causes cell death.
D-dimer  A fragment produced during the degradation of a clot.
Desquamation  The detachment of cells from the surface of an epithelium.
Dimer  A molecule that consists of two similar (but not necessarily identical) subunits.
Dizygotic twins  Twins born after fertilisation of two separate oocytes. They may be of different sexes and are no more likely to resemble each other than any other sibling pairs (cf. monozygotic twins).
Docosahexaenoic acid (DHA)  A long chain \( n-3 \) (omega-3) fatty acid that is abundant in oil-rich fish (see also eicosapentaenoic acid).
Eicosapentaenoic acid (EPA)  A long chain \( n-3 \) (omega-3) fatty acid that is abundant in oil-rich fish (see also docosahexaenoic acid).
Endocrine  Pertaining to hormones and the glands that make and secrete them into the bloodstream through which they travel to affect distant organs (cf. autocrine).
Endogenous  Originating from within the body (cf. exogenous).
Endothelin  A substance produced by the body that plays an important role in regulating blood flow.
Endothelium  The membrane lining various vessels and cavities of the body, including the heart and blood vessels. It consists of a fibrous layer covered with thin flat cells, which render the surface perfectly smooth and secrete the fluid for its lubrication.
Enzyme  Protein that speeds up (catalyses) a metabolic reaction \textit{in vivo} or \textit{in vitro}.
Eosinophil  White blood cells with coarse granules within the cytoplasm that can modulate an inflammatory reaction. The numbers of eosinophils in blood often rise when there is an allergic reaction in progress.
Epididymis  A long, tightly coiled tube that lies behind each testicle.
Epigenetic  Describes a factor or mechanism that changes the expression of a gene or genes without changing their DNA sequence. In more general terms, an epigenetic factor is something that changes the \textit{phenotype} without changing the \textit{genotype}.
Euglycaemic clamp  This provides steady-state measures of insulin action (see Chapter 2, Table 2.2).
Exogenous  Originating from outside the organism. For example, insulin taken by a diabetic is exogenous insulin (cf. endogenous).
Factor V Leiden  A mutation of the (coagulation) factor V gene that results in increased risk of a thrombosis in veins (blood clot).
Factor VII  A circulating protein made by the liver that is one of (about 40) of the factors involved with blood clotting (see Chapter 6).
Familial hypercholesterolaemia  An inherited condition characterised by abnormally high cholesterol levels in the blood. Affected individuals are unable to process low-density lipoprotein (LDL) cholesterol properly, and they are at increased risk for coronary heart disease.
Fatty streaks  Fatty deposits in the lining of artery walls that can lead to atherosclerosis.
**Fetal macrosomia**  A condition in which a baby is considerably larger than normal, usually due to maternal diabetes during pregnancy.

**Fetal origins of adult disease (FOAD) or thrifty phenotype hypothesis**  A hypothesis that suggests that the association between small size at birth and subsequent development of cardiovascular disease and its risk factors (*e.g.*, glucose intolerance, increased blood pressure, dyslipidaemia) reflects permanent effects of fetal under-nutrition.

**Fibrin**  The protein formed during normal blood clotting that is the essence of a clot.

**Fibrinogen**  The soluble, circulating precursor of the insoluble blood clotting protein, fibrin (see Chapter 6).

**Fibrinolysis**  The way in which blood clots are removed from the circulation.

**Fibrinopeptide A**  Released as part of the clotting process. An elevated level may indicate an abnormal clotting process.

**Fibroblast**  A cell from which connective tissue is developed. It makes and secretes collagen.

**Fibronectin**  An adhesive glycoprotein that is found in connective tissue, where it crosslinks to collagen, and is also involved in blood clotting.

**Flavonoid**  A brightly pigmented compound found in fruits and vegetables that gives these foods their colour. They have been shown to have antioxidant properties.

**Foam cells**  Lipid-laden cells, often derived from white blood cells, named for their foamy appearance under the microscope, which may contribute to the development of atherosclerotic lesions.

**Folate/folic acid**  Folate is a B vitamin found in green leafy vegetables (especially sprouts, spinach, green beans, peas), potatoes, fruit (especially oranges), milk and dairy products. A synthetic form (folic acid) is also found in some fortified foods (*e.g.* breakfast cereals, bread, yeast extract) and in vitamin supplements.

**Free radical**  Molecules that contain an unpaired electron and are therefore highly reactive.

**Gene**  Unit of heredity in a chromosome controlling a particular inherited characteristic of an individual.

**Genetic**  Inherited; a genetic disease is one that is inherited via a faulty gene.

**Genotype**  The genetic constitution (the genome) of a cell, an individual or an organism. The genotype is distinct from its expressed features, or phenotype (*cf.* phenotype).

**Genotoxic**  Describes a substance that is able to cause harmful changes to DNA.

**Glucagon**  A hormone produced by the pancreas to raise the level of glucose (sugar) in the blood.

**Glucocorticoid**  A hormone that predominantly affects the metabolism of carbohydrates and, to a lesser extent, fats and proteins (and has other effects). Cortisol is the major natural glucocorticoid in humans.

**Glucose tolerance test**  A test of the body’s ability to metabolise carbohydrate by administering a standard dose of glucose under controlled conditions and measuring the blood and urine for glucose at regular intervals thereafter. The glucose tolerance test is usually used to assist in the diagnosis of diabetes.

**Glycated haemoglobin**  Haemoglobin that is bound with glucose. Measurements of glycated haemoglobin are used to assess how well-controlled diabetes has been in the preceding 3–4 months.

**Glycaemic index (GI)**  A scale for evaluating foods, based on the rate at which sugar is absorbed into the bloodstream after eating a specific food.

**Glycogen**  A polymer of glucose, the form in which carbohydrate is stored, predominantly in the liver and skeletal muscle.

**Granulocyte**  A type of white blood cell filled with microscopic granules containing enzymes that digest micro-organisms. Neutrophils, eosinophils and basophils are all types of granulocytes.

**Growth factors**  Secreted regulatory proteins that control the survival, growth, differentiation and effector function of cells.

**Hard endpoint**  Measurement of a clinical outcome (*e.g.* heart attack, stroke or death) (*cf.* surrogate marker).

**Heterogeneous (heterogeneity)**  Denotes dissimilarity. This term can be used to denote the discrepant results obtained within or between epidemiological studies or randomised trials that can be either statistical (meaning that studies used different statistical methods) or clinical (meaning that studies evaluated different types of subjects, treatments or outcomes) (*cf.* homogeneous).

**High-density lipoprotein (HDL)**  Circulating lipid particles defined by size, density and apolipoprotein content. HDLs transport cholesterol from cells to the liver, where they are degraded or repackaged. HDLs are responsible for removing excess cholesterol from the blood, preventing a
build-up of cholesterol on the artery walls. This is why HDL-cholesterol is referred to as ‘good’ cholesterol. High levels of HDL-cholesterol are associated with low risk of CHD.

**Homocysteine** A sulphhydril amino acid derived from the metabolic conversion of methionine, which is dependent on vitamins (folate, B₁₂, and B₆) as cofactors or cosubstrates (see Chapter 8).

**Homocystinuria** A congenital disease in which an affected individual is unable to metabolise or utilise methionine properly. The main features of the condition are abnormality of the lens of the eye, mental retardation, fair complexion, fair hair and a high cheek colour.

**Homogeneous (homogeneity)** Denotes similarity. For example, if results are similar and consistent from one study to another, then the results are said to be homogeneous (cf. heterogeneous).

**Hormone** A circulating substance secreted by a gland that influences another organ.

**Hypercholesterolaemia** Concentrations of cholesterol in the blood higher than normal (or reference) values. Causes include dietary and genetic.

**Hypercoagulability** The concept of a hypercoagulable state should refer specifically to the coagulability of plasma. Hypercoagulation does not include the state of play of the fibrinolytic system (see Chapter 6, Section 6.3).

**Hyperglycaemia** A greater than normal concentration of glucose in the blood, most frequently associated with diabetes mellitus.

**Hyperhomocysteinaemia** Abnormally high blood concentrations of homocysteine. This is an independent risk factor for cardiovascular disease (see Chapter 8).

**Hyperinsulinaemia** High blood insulin levels.

**Hyperlipidaemia** High levels of fats (lipids) in the blood.

**Hyperphagia** Abnormally increased appetite for food, frequently associated with injury to the hypothalamus.

**Hyperplasia** An increase in the number of cells in a tissue.

**Hypertension** Elevated blood pressure (usually defined as a blood pressure of 140/90 mmHg or above).

**Hypertriglyceridaemia** Concentrations of triglycerides in the blood higher than normal (or reference) values.

**Hypertrophy** Enlarging of cells or an organ. May be used in distinction to hyperplasia.

**Hypoglycaemia** The level of glucose in the blood being too low, usually under 2.5 mmol/l. Left untreated, hypoglycaemia will eventually result in a person becoming unconscious.

**Hypothalamo-pituitary-adrenal (HPA) axis** Part of the central nervous system. The HPA axis or stress axis is a chain from the hypothalamus in the brain over the pituitary to the adrenal gland. It plays an important role in adaptation of organisms to stressful situations. Long-term functional alteration of the HPA axis has been linked with pathophysiological states such as diabetes and hypertension.

**Impaired glucose tolerance (IGT)** A state that is borderline to diabetes and associated with elevated risk of cardiovascular disease (see Chapter 2, Table 2.13). IGT often progresses to diabetes in later life.

**In vitro** From the Latin meaning ‘in glass’. The term is applied to biological processes studied experimentally in isolation from the organism, as distinct from in vivo, which refers to the study of processes in the living organism (cf. in vitro).

**In vivo** Observations carried out inside the living body of animals, including man (cf. in vitro).

**Incidence** The rate at which new events occur in a population, i.e. the number of new cases of a disease in a specific period of time, divided by the total population at risk of getting the disease during that period (cf. prevalence).

**Infant mortality rate** Deaths during the first year of life per 1000 live births.

**Inflammation** The reaction of the body to any injury, which may be the result of trauma, infection or chemicals. In response, local blood vessels dilate, increasing blood flow to the injured site, and white blood cells invade the affected tissue engulfing bacteria or other foreign bodies.

**Innate (or natural) immunity** Natural non-specific host defences (cf. specific immunity).

**Insulin** A hormone secreted by the pancreas in response to food intake. It circulates in the blood and assists in the movement of glucose into cells where it is used as a source of energy.

**Insulin resistance** A condition in which the body’s cells are less responsive (or sensitive) to the action of insulin. This causes more insulin to be released by the pancreas, resulting in an excess amount of insulin circulating in the blood. This metabolic abnormality underlies type 2 diabetes (see Chapter 2, Section 2.5).
Insulin resistance syndrome  Refers to a set of heart disease risk factors which have been found to occur together causing a substantial increase in the risk of a heart attack. This is also known by other names like syndrome X, Reaven’s syndrome and metabolic syndrome (see Chapter 2, Table 2.5).

Insulin sensitivity  A measure of how much insulin is required for cells to import glucose. Patients with type 2 diabetes typically have insulin resistance (high insulin levels with high blood glucose).

Insulin-like growth factor (IGF)  A hormone that promotes growth whose chemical structure is similar to insulin. While insulin primarily affects the body’s metabolic system (energy delivery and use), IGF helps regulate cell growth.

Interleukins  Molecules made by leukocytes that are involved in signalling between cells of the immune system.

Intermediate-density lipoprotein (IDL)  Short-lived lipoproteins containing about 30% cholesterol that are converted in the circulation to low-density lipoproteins.

Intermittent claudication  A symptom of poor circulation in which muscle pain is experienced during exercise and relieved by rest.

Intima  The layer of the arterial wall that is nearest to the lumen and includes the endothelial cells.

Intrinsic factor  A chemical substance produced by the gastric mucosa (stomach), which is necessary for intestinal absorption of vitamin B₁₂. A deficiency in intrinsic factor can cause pernicious anaemia (a condition characterised by a fall in the number of red blood cells).

Ionomycin  An ionophore for calcium commonly used to determine the different roles of calcium within cells.

Ionophore  A compound that can carry specific ions through membranes of cells or organelles.

Ischaemia  Reduced or inadequate blood, and thus oxygen, supply to a part of the body. Cardiac ischaemia (restricted blood and oxygen supply to the heart) could result from a heart attack.

Isoforms  Alternative forms of a protein/enzyme.

Isoprostanes  A marker of lipid peroxidation in the body.

Leptin  A hormone produced by the ob (obese) gene and secreted by fat tissue that acts on the brain to regulate appetite and has a central role in fat metabolism (see Chapter 9, Section 9.2).

Leukocyte  A white blood cell found in blood and lymph nodes. During the inflammation process, the body uses leukocytes to fight infections and help repair damaged tissues. However, the body can improperly trigger the inflammatory-response process, causing leukocytes to attack healthy tissue. These situations can result in a large range of conditions, including heart disease (see Chapter 7).

Leukotrienes  A type of eicosanoid. These are a group of hormones that are derived from arachidonic acid and have a role in allergic or inflammatory reactions in the body.

Levodopa (L-dopa)  The naturally occurring form of the amino acid dihydroxyphenylalanine (DOPA), which is a precursor of the neurotransmitter dopamine.

Lipaemia  The presence of a conspicuous amount of fat in the blood, for example, after a fat-rich meal.

Lipase  An enzyme that breaks down fat.

Lipid  A collective name for fats, oils, cholesterol and other fatty substances.

Lipoatrophy  Loss of body fat.

Lipogenesis  Synthesis of fatty acids and lipids.

Lipolysis  The breakdown of fat.

Lipolytic  Breaking down fat (lipolysis).

Lipoprotein  Fat particles with associated proteins that facilitate cholesterol and triglyceride transport throughout the body. The four basic classes are high-density, low-density and very-low-density lipoproteins, and chylomicrons.

Lipoprotein(a) or Lp(a)  A lipoprotein particle similar to low-density lipoprotein cholesterol with an attached protein. Studies have suggested an association between elevated blood levels of Lp(a) and an increased risk of heart disease.

Lipoprotein lipase  The enzyme that catalyses the breakdown of lipids through the hydrolysis of linkages between fatty acids and glycerol in triglycerides and phospholipids.

Logistic regression  When the distribution of a dependent variable depends upon the value of some other variable(s), the expected value of the dependent variable is given by a mathematical relationship called the regression of the dependent variable (e.g. rate of disease) on the independent variable(s). In logistic regression, the dependent variable is an expression of the odds of the occurrence of a disease or other attribute of interest.

Low birthweight  Usually defined as infant birthweight below 2500 g.
Low-density lipoprotein (LDL) The particles that deliver cholesterol to tissues where it is needed for membrane structure or to manufacture steroid hormones and bile acids. Too much LDL-cholesterol in the blood leads to a build-up of cholesterol (referred to as plaques) in the artery walls. These build-ups can eventually lead to ischaemia and thrombosis, by impeding adequate perfusion of the tissues with blood carrying oxygen. If this blockage is to the muscle of the heart, this may result in a heart attack. This is why LDL-cholesterol is referred to as ‘bad’ cholesterol. A high fat diet can result in raised LDL-cholesterol levels in the blood.

Lower-body obesity Accumulation of fat around the hips, thighs and bottom (also called a ‘pear’ body shape; cf. central obesity).

Lymphocyte A small white blood cell (leukocyte) that plays an important role in defending the body against disease. There are two main types of lymphocytes: B cells and T cells. B lymphocytes produce antibodies, while T lymphocytes attack and destroy antigens directly.

Macrocytic anaemia Anaemia in which the average size of red blood cells is larger than normal. This usually occurs as a result of a deficiency in either vitamin B₁₂ or folate. This may be because of inadequate intake or as a consequence of impaired absorption of these vitamins (e.g. the ineffective production of intrinsic factor impairs vitamin B₁₂ absorption).

Macrophage A large white blood cell that has scavenger properties and normally collects at infection sites to remove foreign bodies. It is also involved in the development of atherosclerotic lesions.

Media The middle layer in the artery wall. It is composed of smooth muscle cells that control the diameter of the artery.

Meta-analysis A discipline that reviews critically and combines statistically the results of previous research in an attempt to summarise the totality of the evidence relating to a particular medical issue.

Metabolic syndrome See insulin resistance syndrome.

Metalloproteinases A group of enzymes responsible for breaking down and reforming body tissues, and breaking down and removing dead matter.

Methionine An amino acid found in protein-rich foods, such as meat, fish and dairy products.

Methyl-tetrahydrofolate reductase (MTHFR) An enzyme that is required to convert homocysteine to methionine (see Chapter 8, Fig. 8.2). Impaired activity of this enzyme results in increased blood levels of homocysteine.

Microalbuminuria Excretion of small amounts of the protein albumin, found on urine tests.

Monoclonal antibodies A class of antibodies that are all identical and all bind in the same way to the same target substance. This makes them a highly selective and sensitive way of detecting the presence of substances they have been made to detect.

Monocyte A type of white blood cell that circulates in the blood. They are transformed into macrophages in the artery wall.

Monomer Individual unit from which polymers are made.

Monounsaturates (monounsaturated fatty acids) Fatty acids containing one double bond.

Monozygotic twins Identical twins who develop from a single ovum fertilised by a single spermatozoan (cf. dizygotic twins).

Multivariate analysis Statistical analysis containing one dependent variable and several independent variables (cf. univariate analysis).

Mutation A heritable change in genetic material (i.e. a change which can potentially be passed from parent to child). This change may occur in a gene or in a chromosome, and may take the form of a chemical rearrangement, or a partial loss or gain of genetic material.

Myocardial infarction A heart attack which occurs when an artery to the heart muscle is blocked.

Myocardium The muscular substance of the heart.

n-3 (or omega-3) polyunsaturates Fatty acids with their first double bond at the third carbon atom from the methyl end (–CH₃) of the molecule. These include alpha-linolenic acid (C₁₈:₃) (sources of which include rapeseed, walnut, soya and blended vegetable oils), eicosapentaenoic acid (C₂₀:₅) and docosahexaenoic acid (C₂₂:₆) (the main sources of which are oil-rich fish) (see also eicosapentaenoic acid and docosahexaenoic acid).

n-6 (or omega-6) polyunsaturates Fatty acids with their first double bond at the sixth carbon atom from the methyl end (–CH₃) of the molecule. These are the typical fatty acids of vegetables oils (e.g. sunflower, corn and soybean) and spreads made from these.

Natural killer cell A lymphocyte that kills targets such as certain tumour cells.
NCEP-1 National Cholesterol Education Program step one diet (20% fat).
Necrosis Uncontrolled cell death.
Neonatal During the first 28 days after birth.
Nested case-control study A case-control study that is nested (or embedded) within a cohort study. The cases are usually all of the cases in the cohort, while the controls are selected at random from the non-cases. Since the cohort is well-defined, it is appropriate to compare the rates of exposure among the cases and controls.
Neural tube defects Birth defects that occur when the neural tube does not form correctly. Neural tube defects usually occur during the first month of pregnancy, before many women know they are pregnant.
Neuropathy A disorder of the nerves that affects their signalling functions. Neuropathy is a complication of diabetes.
Neurotransmitter Chemical messenger whose function is to transmit nerve signals (impulses) from one nerve cell to another.
Neutrophil A type of white blood cell that can act as a phagocyte. Neutrophils are the most numerous cell type in the bloodstream and the major cell type found in acute inflammatory lesions.
Nimesulide A non-steroidal anti-inflammatory drug with a weak action against prostaglandin synthesis and a potent anti-inflammatory action.
Nitric oxide Perhaps more correctly known as nitrogen monoxide (NO), this is a mediator of many physiological responses (see Chapter 5).
Non-Q wave infarction A small heart attack not detectable by routine ECG examination, but which may be detectable by clinical symptoms and biochemical findings.
Non-starch polysaccharide Another term for dietary fibre.
Non-alcoholic steatohepatitis (NASH) A liver disease that closely resembles alcoholic liver disease, but occurs in individuals consuming little or no alcohol. The initial step in its development seems to be the deposition of excess fat within the liver, which is associated with inflammation and scarring. In a few cases it may ultimately progress to cirrhosis (irreversible, potentially fatal, liver damage).
Non-esterified fatty acids (free fatty acids) Fatty acids that are free in the bloodstream rather than esterified to glycerol.

Noradrenaline (norepinephrine) A neurotransmitter released by nerve terminals, particularly known to be concerned with states of arousal.
Norepinephrine See noradrenaline.
Ob gene Gene that produces leptin, a protein secreted by fat cells associated with satiety. Variation in the ob gene may be a rare genetically determined cause of obesity in humans.
Ob/ob gene A defect in the ob gene that results in a failure of leptin production.
Obesity An excessive accumulation of body fat, defined by the World Health Organization as a body mass index (BMI) greater than 30 kg/m² for Caucasians (see Chapter 2, Section 2.5.1).
Odds ratio A ratio used in epidemiological studies (in particular case-control studies) to compare the odds of an event in an exposed versus non-exposed group, or in an intervention versus control group.
Osteoblast A cell that forms new bone.
Osteopaenia Mild thinning of the bone mass that results when the formation of bone is not enough to offset normal bone loss. Osteopaenia is generally considered the first step along the road to osteoporosis, a condition in which bone density is extremely low and bones are porous and prone to fracture.
Oxidation A chemical reaction that involves the loss of electrons; it usually, but not always, involves direct participation of oxygen.
Oxidative stress A condition in which the production of oxidants and free radicals exceeds the body’s ability to inactivate them.
Paracrine Pertaining to cell messengers (cytokines) which act locally. They are produced by neighbouring cells or the extracellular matrix, as distinct from endocrine or hormonal messengers (cf. endocrine).
Peripheral neuropathy A condition resulting from injury to the peripheral nerves in the body that carry signals between the central nervous system (the brain and spinal column) and the muscles, skin and internal organs. When peripheral neuropathy first develops, people often report a tingling or prickling in the toes, although it may also start in the fingers. Over time, this tingling may gradually spread up the feet or hands and worsens into a burning, shooting, and/or throbbing pain. People who have severe peripheral neuropathy may experience extreme pain and may have difficulty walking.
Peripheral vascular disease (PVD)  This often co-exists with CHD and causes pain in leg muscles following exertion.

Peroxides  Free radicals that result from the reaction between fatty acids and oxygen.

Peroxisome proliferator-activated receptors (PPARs)  Members of the nuclear hormone receptor family. Three subtypes of PPAR have been described, α, δ (also called β) and γ, encoded by different genes (for further information see Chapter 9).

Peroxynitrite  A nitrogen reactive species (ONOOH) formed by the reaction between nitric oxide and superoxide under inflammatory conditions.

Phagocytosis  A process whereby cells engulf and destroy foreign material, e.g. bacteria, cells, cell debris and other small particles. Cells that act in this way are called phagocytes.

Phenotype  The physical characteristics of an individual that result from the combination of genetic and environmental factors. By contrast, the genotype is merely the genetic constitution (genome) of an individual (cf. genotype).

Physical activity  Any bodily movement produced by contraction of skeletal muscle that substantially increases energy expenditure (see Chapter 2, Table 2.17 for definitions of related terms).

Physical activity ratio (PAR) (also called metabolic equivalent, MET)  The energy cost of an activity per minute as a multiple of the basal metabolic rate (BMR). For example, the energy cost for sitting is $1.2 \times BMR$, for jogging $7 \times BMR$. By combining all activities over a 24-hour period the physical activity level (PAL) can be obtained.

Physical fitness  A set of attributes (e.g. cardiorespiratory endurance, skeletal muscle endurance, skeletal muscle strength and power, flexibility, agility and balance) that people have or acquire that relate to the ability to perform physical activity (see Chapter 2, Table 2.17).

Plaque  Area within an artery wall that has been affected by atherosclerosis.

Plasminogen activator inhibitor-1  An inhibitor of the fibrinolytic system. It is biologically plausible that elevated levels could suppress fibrinolysis and result in an increased risk of thrombosis (blood clots).

Plasminogen  A precursor of plasmin, an enzyme that digests the protein fibrin, the main constituent of blood clots.

Platelet aggregation  The process by which platelets are induced to clump together and form aggregates. These become enmeshed in the fibrin network and form a blood clot.

Platelets  Small blood cells that are involved in blood clotting.

Pleiotropic  Having multiple effects. For example, for statins there would be effects other than cholesterol lowering, such as improving or restoring endothelial function, enhancing the stability of atherosclerotic plaques, decreasing oxidative stress and inflammation, and inhibiting the thrombogenic response in the vascular wall.

Polycystic ovary syndrome (PCOS)  A hormonal problem of women that causes irregular or no menstrual periods, acne, obesity and excess hair growth. PCOS is a disorder of chronically abnormal ovarian function and hyperandrogenism (abnormally elevated androgen levels).

Polygenic disease  Genetic disorders (e.g. heart disease, diabetes and some cancers) resulting from the combined action of alleles of more than one gene. Although such disorders are inherited, they depend on the simultaneous presence of several alleles; thus the hereditary patterns are usually more complex than those of single-gene disorders.

Polymorphism  The existence of variation of a genetic characteristic in a population that is too common to be due merely to new mutation. A polymorphism must have a frequency of at least 1% in the population.

Polyunsaturates (polyunsaturated fatty acids)  Fatty acids containing two or more double bonds; common in vegetable oils (see also $n$-3 and $n$-6 polyunsaturates).

Ponderal index  An expression of weight for height used mainly for infants. In newborns the index is usually expressed as birthweight in kilograms divided by birth length in centimetres cubed ($\text{kg/cm}^3$).

Population attributable risk (or attributable fraction)  A measure of the impact an exposure or risk factor has in a given population, in terms of excess risk of disease. It depends not only on how strongly the exposure in question is associated with a particular disease but also on the prevalence of the exposure/risk factor (see Chapter 1, Section 1.4.3).

Postprandial  After a meal.

Precocious  Unusually early development.

Premature heart disease  Heart disease before the age of 75 years.
Preterm Birth occurring after a gestation of less than 37 weeks.

Prevalence This is a measure of the total number of existing cases of a disease or condition at a particular point in time (or during some specified time period). Prevalence is usually expressed as a percentage of the total population, or per 1000, 10 000, or 100 000 people (cf. incidence).

Primary prevention Measures taken to prevent someone from developing a disease (e.g. modifying the diet to reduce coronary risk before there are signs of heart disease).

Probucol A drug used to lower LDL- and HDL-cholesterol.

Prodrome A premonitory symptom or sign, indicating the onset of a disease.

Prospective (follow-up, cohort or longitudinal) study Data on exposure is first collected and subjects are followed up for the development of a given condition or outcome. A randomised controlled trial, for example, is always prospective.

Prostacyclin The prostaglandin hormone PG12, a natural hormone made by normal artery wall lining cells to inhibit the formation of abnormal blood clots.

Prostaglandins A type of eicosanoid. These hormone-like substances participate in a wide range of body functions. They have several actions as inflammatory mediators, particularly those derived from the n-6 fatty acid, arachidonic acid, such as prostaglandin E2.

Proteoglycans Proteins conjugated with carbohydrate groups.

Prothrombin A protein blood coagulation factor; also known as factor II; the precursor of the clotting enzyme thrombin.

Prothrombotic state Implies an imbalance between coagulation and fibrinolysis that favours fibrin deposition and clot formation (see Chapter 6, Section 6.3).

Randomised controlled trial (RCT) In a randomised controlled trial, participants are assigned by chance to receive either an experimental or control treatment. Both groups are followed up for a specified time and the effects of the intervention on a specific outcome (e.g. serum cholesterol level, death rates) are analysed. The idea behind the randomised controlled trial is that when it is done properly, the effect of a treatment can be studied in groups of people who are the same at the outset and treated the same way except for the intervention being studied. Any differences then seen in the groups at the end can be attributed to the difference in treatment alone, and not to bias or chance.

Reactive oxygen species (ROS) A collective term that includes free radicals of oxygen and non-radical derivatives of oxygen, such as hydrogen peroxide and singlet oxygen.

Regression dilution A term that describes the dilution/attenuation of the estimated relationship between two variables when a single measured value is used instead of the usual or average value over a period of time.

Relative risk The rate of a defined attribute among the group exposed, divided by that among the non-exposed.

Remnant-like particles (RLP) Remnant-like particles include all apoB48-containing lipoproteins plus a subfraction of apoB100-containing lipoproteins, the latter of which are enriched in apoE and cholesterol. Increased blood concentrations of RLP are observed in coronary heart disease, diabetes mellitus, hypertriglyceridaemia and fatty liver disease.

Renin An enzyme produced by the kidney and released into the blood in response to stress. It reacts with a compound in the liver to produce angiotensin which causes blood vessels to constrict and raises blood pressure.

Resistin A protein secreted by adipose tissue (see Chapter 9, Section 9.7).

Reticular endothelial system A term for the network of phagocytes and endothelial cells throughout the body.

Retrospective study In a retrospective study (e.g. a case-control study), the outcomes of a group of people are examined in hindsight, using existing records or recalling past exposure. Retrospective studies are generally limited in the data available for analysis, as the data have rarely been collected with the needs of that particular study in mind. This kind of limitation means that a retrospective study is usually less reliable than a prospective study (cf. prospective study).

Reverse causality Arises when what was assumed to be the cause is in fact the effect, and vice-versa. This concept is relevant in case-control studies when a disease or illness can cause individuals to change their behaviour, alter biochemical parameters, or have other effects.
Reverse cholesterol transport  The removal of cholesterol from cells and from atherosclerotic lesions. This process involves high-density lipoprotein cholesterol, which is often referred to as ‘good’ cholesterol.

Risk factor/risk marker  Characteristic found to be related to the subsequent occurrence of disease.

Risk ratio  The ratio of risk in the treated group to the risk in the control group – used in randomised trials and cohort studies.

Salicylate  A type of phenol found naturally in some foods, including fruits, vegetables and nuts, and in many herbs and spices.

Saturates (saturated fatty acids)  A fatty acid containing no carbon–carbon double bonds, typical of ‘hard’ fats and animal fats.

Scavenger receptors  Receptors on macrophages that recognise the modified forms of LDL particles.

Secondary prevention  Measures taken to limit the effects or progression of disease once it has occurred. For example, the use of a statin drug in individuals with heart disease to reduce the risk of a subsequent heart attack.

Sensitivity  The quality of being sensitive. In screening for a disease, this refers to the proportion of persons with the disease who are correctly identified by a screening test.

Signal transduction  A basic process in molecular cell biology involving the conversion of a signal from outside the cell to a functional change within the cell.

Sleep apnoea  A condition in which a person stops breathing while asleep, which may be due to frustrated efforts to breathe against blocked upper airways (so combined with snoring).

Small, dense low-density lipoprotein (LDL) particles  LDL particles vary with respect to their size, density, composition and physiochemical properties. An increased proportion of small, dense LDL is associated with increased cardiovascular risk. This has been attributed to several factors, including greater susceptibility to oxidative modification and efficient infiltration into the arterial vessel wall (see Chapter 3).

Specific (or acquired) immunity  An immune reaction involving lymphocytes that is specific to any given antigen and gives rise to immunological memory (cf. innate immunity).

Specificity  The quality of being specific. In screening for a disease, this refers to the proportion of persons without a disease who are correctly identified by a test.

Stable angina  The pain is present only during exertion or extreme emotional distress and disappears with rest (cf. unstable angina).

Statins  A group of drugs that reduce the concentration of low-density lipoprotein (LDL) cholesterol in the blood.

Stenosis  Narrowing of a vessel making flow of blood difficult.

Stroke (cerebrovascular disease)  Damage to part of the brain resulting from a breakdown in the blood supply (ischaemia) or haemorrhage. In the UK, around 85% of strokes are due to ischaemia, of which the major cause is thrombosis in the arteries supplying the brain.

Subcutaneous  Beneath the skin.

Superoxide dismutase  The enzyme that converts superoxide radicals (O$_2^•−$) into hydrogen peroxide (H$_2$O$_2$).

Surrogate marker  A laboratory measurement or physical sign that is used in intervention trials as a substitute for a clinically meaningful endpoint (e.g. elevated blood cholesterol levels as a marker of heart disease risk) (cf. hard endpoint).

Sympathetic nervous system  Part of the nervous system that prepares the body for activity by raising blood pressure and speeding up the heart rate.

Syndrome X  See insulin resistance syndrome.

Systemic  Relating to, or affecting, the whole body.

Systemic infection  Infection spreading throughout the body.

Thiazolidinedione  A class of drugs used to treat type 2 diabetes that lower the blood sugar by increasing the sensitivity of cells to insulin. These drugs also increase HDL (‘good’) cholesterol.

Thiol  A sulphhydryl (–SH) containing compound.

Thrifty genotype  The basic premise of the ‘thrifty gene’ hypothesis is that certain populations may have genes that determine increased fat storage, which in times of famine represent a survival advantage, but in a modern environment (with a high energy diet and physical inactivity) result in obesity, type 2 diabetes and cardiovascular disease.

Thrombomodulin  An endothelial cell receptor with anticoagulant and anti-inflammatory effects.

Thrombosis  The pathological condition in which a blood clot blocks an artery or vein and stops the blood flow through it.
Thrombophilic states  A disorder of the haemostatic system in which there is a tendency to the occurrence of thrombosis.

Thromboxane  A type of eicosanoid that causes blood to clot and increases the stickiness of platelets.

Thrombus  A pathological blood clot.

Tissue plasminogen activator (tPA)  An enzyme found in many tissues which converts plasminogen to plasmin, and therefore promotes the breakdown of a thrombus or blood clot in a blood vessel. tPA is used in the treatment of patients with a heart attack as a clot-dissolving drug.

T-lymphocytes  A type of white blood cell produced in the bone marrow that mature in the thymus gland.

Tocopherols  Forms of vitamin E.

Total homocysteine  Blood total homocysteine is the sum of all protein-bound and free forms of homocysteine.

Trans fatty acids  Unsaturated fatty acids have some of their carbon atoms in their ‘backbone’ joined by double bonds, and can exist in two different geometric forms. These are called the cis and trans forms. In trans fatty acids the two hydrogen atoms are on opposite sides of the double bond. Trans fatty acids occur naturally in small amounts in foods produced from ruminant animals, such as meat and dairy products, but most of the trans fatty acids in the diet are produced during the hydrogenation (hardening) process that converts vegetable oils into solid fats. They are therefore found in hard margarines, processed foods, cakes and biscuits (see Chapter 13, Table 13.5 for sources).

Transcription factor  A protein that controls when genes are switched on or off and whether genes are transcribed or not.

Triglyceride (or triacylglycerol)  A type of fat – the major type of fat in the diet, also present in the bloodstream (see Chapter 3).

Tumour necrosis factor-alpha (TNFα)  A cytokine produced by many types of leukocyte, named after its ability to kill tumour cells in tissue culture. It modifies the response of many cells and causes inflammation; it has been implicated in the pathology of a wide range of chronic inflammatory conditions including heart disease (see Chapters 7 and 9).

Tunica adventitia  Connective tissue layer on the outside of arteries and veins.

Type 1 diabetes mellitus  Also known as insulin-dependent diabetes mellitus, this is a disease in which an autoimmune process in the pancreas leads to destruction of the insulin-producing islet cells, producing a form of diabetes characterised by insulin deficiency.

Type 2 diabetes mellitus  Previously known as adult-onset diabetes and non-insulin-dependent diabetes mellitus. This is a disease in which there is both a failure of the body to respond normally to insulin (insulin resistance) and the body does not make enough insulin or is unable to make proper use of it, causing glucose concentrations to increase in the bloodstream (see Chapter 2, Section 2.5).

Tyrosine kinase  An enzyme that can transfer a phosphate group to a tyrosine residue in a protein. This is an important function in signal transduction to regulate enzyme activity.

Uncoupling protein (UCP)  Substances found inside cells which, when activated, burn up energy, giving off small amounts of heat, rather than storing the excess as fat. Originally UCP-1 was found in the brown fat cells of hibernating bears, rodents and human newborn babies. Similar proteins have now been identified in the cells of adult humans as well (UCP-2 is found in white fat and UCP-3 in human skeletal muscle).

Univariate analysis  Statistical analysis containing one dependent variable and one independent variable (cf. multivariate analysis).

Unsaturates (unsaturated fatty acids)  Fatty acids containing one or more carbon–carbon double bonds (see also monounsaturates and polyunsaturates).

Unstable angina  This is considered an acute coronary syndrome and may signal an impending heart attack. It may be a new symptom or a change from stable angina. The angina may occur more frequently, more easily at rest, feel more severe, or last longer (cf. stable angina).

Vasculitis  Inflammation affecting blood vessels.

Vasoconstriction  Narrowing of the blood vessels resulting from contracting of the muscular wall of the vessels. The opposite of vasodilation.

Vasodilation  Widening of blood vessels resulting from relaxation of the muscular wall of the vessels. The opposite of vasoconstriction.

Ventricles  Pumping chambers of the heart. The left ventricle pumps blood around the body and the right ventricle pumps blood to the lungs.

Very-low-density lipoprotein (VLDL)  A class of
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lipoproteins that transports triglycerides from the liver to the adipose and muscle tissues. They are produced mainly in the liver and primarily contain triglycerides in their lipid cores.

**Visceral obesity** Excess of body fat around the internal organs of the body, specifically those within the abdomen (*e.g.* liver or intestines).

**VO₂max** The maximum amount of oxygen that the body can utilise per minute of work. This is often used as an evaluation of a person’s cardiovascular efficiency.

**von Willebrand factor (vWF)** A protein involved in blood clotting that is produced by the vascular endothelium (see Chapter 6).

**Zymogen** An inactive biomolecule that is a precursor to an enzyme.
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Note: CHD: coronary heart disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; RLP: remnant-like particles; PVD: peripheral vascular disease. Page numbers in **bold** refer to answers to journalists’ questions. Page numbers in *italics* refer to tables or figures.

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