LIPIDS IN NUTRITION AND HEALTH: A REAPPRAISAL

MICHAEL I. GURR

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LIPIDS IN NUTRITION AND HEALTH: A REAPPRAISAL

Based upon a compilation of articles on lipids in nutrition written by the author and published in *Lipid Technology* 1989–98

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Foreword

For ten years, Mike Gurr has written his nutrition article in *Lipid Technology*, amounting in all to nearly 60 articles. These have always been well received and have been one of the attractive features of the magazine. He writes clearly and carefully and with a light touch so that even those who might oppose his conclusions recognise his underlying knowledge, his ability, and the power of his arguments. It has now been agreed that all these articles should be organized into a single summarizing volume where they can be presented systematically, revising and updating only where this is necessary. The result is a powerful and critical survey of important aspects of lipid nutrition which will be appreciated by *Lipid Technology* readers and should be compulsory reading for those not familiar with the original articles. It also represents a convenient presentation of material spread through almost 60 issues.

Since 80% of the global production of oils and fats — now exceeding 100 million tonnes — is consumed as food and a further 6% is eaten by animals to produce more human food it is not surprising that nutrition is one of the active areas in lipid science. Older aspects of this field such as the effects of saturated acids continue to be investigated while new aspects like conjugated linoleic acid provide excitement. What are our dietary needs? How do lipids as a whole and lipids as individual compounds affect us in respect of health and disease? These are important questions which attract a lot of attention. Most commentators — whether they be review writers, government health committees, or contributors to newspapers or magazines — are recycling what others have written, generally without careful assessment of the original articles. We are told what has been discovered. All the caveats of the original authors have got lost, suggestions become facts, arguments are simplified, and possibilities become certainties which are repeated and re-presented until they are part of received wisdom and no longer questioned.

Mike Gurr belongs to that small group of writers who is not content with second-hand opinion but goes back to the original papers, reads them

carefully, and re-assesses the conclusions on the basis of his extensive knowledge of nutritional science. His conclusions are sometimes unexpected and do not always accord with the nostrum of the day. They are always thoughtful and merit careful consideration.

There is a growing awareness that some of the early nutrition experiments were not of the best design, that conclusions sometimes went beyond the experimental evidence, that the lipid hypothesis for cardiovascular disease, for example, is far from the whole story, and that early conclusions about cholesterol and saturated acids need to be reconsidered. *Lipid Technology* is pleased to have contributed to the new thinking through Mike Gurr's writing over 10 years and is proud to present this volume of collected revised papers in the belief that they will contribute powerfully to the on-going debate.

Frank Gunstone
Editor, *Lipid Technology*
April 1999, Nether Rumgally

Preface

Between 1989 and 1998, I contributed regular articles to Lipid Technology on nutritional aspects of lipids. Together these provide a clear picture of the development of concepts in lipid nutrition during this time and highlight some important advances in research in six main areas:

•Influence of dietary lipids on blood lipid and lipoprotein concentrations.

•Dietary lipids and cardiovascular diseases.

•Nutritional significance of lipid peroxidation.

•Importance of polyunsaturated fatty acids in nutrition.

•Dietary lipids and weight control.

•Lipids in foods and raw materials.

To provide coherent summaries for those interested in these topics, I have grouped together the *Lipid Technology* articles under these different headings, editing and updating where necessary. Some early articles have been omitted or considerably truncated where the material had become outdated and superseded by later articles.

Many articles did not fall neatly into these topics. These have been edited into the text of different chapters to provide introductory or background material (e.g. digestion and assimilation of lipids, consideration of dietary reference values, lipids and cancer). In the chapter on 'Lipids in foods and raw materials' I have grouped together such topics as lipids in meat, milk, infant foods, palm, or lipids of special interest (e.g. plant sterols, short-chain and medium-chain fatty acids).

For those who have not been regular readers of my *Lipid Technology* articles, I should point out that my interpretation of the scientific evidence for the significance of dietary lipids in the development of diseases such as heart disease, often differs considerably from those of my nutritionist colleagues. It certainly differs from the 'consensus view' adopted by most public health bodies. Shortly before putting pen to paper for one of my *Lipid Technology* articles, I was present at a meeting on new aspects of fats

processing attended by people from the fats and oils industry. At dinner in the evening, my neighbour, by way of making conversation said "I enjoy your articles in *Lipid Technology*". My face glowed with pleasure; everyone likes praise! He continued: "I particularly like their facetious style". My face fell. My dictionary defines facetious as "characterized by flippant or inopportune humour". It certainly has not been my intention to be flippant or that my articles have anything other than serious scientific intent, although I hope they also make enjoyable reading. It is quite intentional however, that many of these articles are imbued with a certain scepticism (sceptical: "inclined to question the truth or soundness of accepted opinions" — my dictionary again!). The reason for this is that nutrition is a discipline which, as well as being underpinned by several fundamental sciences such as chemistry and physiology, also has distinct sociological attributes since it impinges necessarily on all our lives. It is therefore a subject in which it is all too easy to stray away from strict scientific reasoning, a topic on which I have expounded in another journal (1). It has been my intention in these papers to examine critically the credentials of some popular beliefs about nutrition that have entered or are in danger of entering folklore, rather than to present bland and uncritical regurgitations of the literature. I have taken pains to try to point out why interpretations may differ so widely.

I hope that these 'collected works' will provide a useful source of reference material on important lipid nutrition topics as well as being 'a good read'.

M.I.Gurr
St Mary's, April 1999

Reference

1. Gurr, M.I. (1994) *Biologist*, 41, 191–194.

CONTENTS

Abbreviations

The most frequently-used abbreviations in this book are listed below.

AA	arachidonic acid
ALA	α-linolenic acid
CHD	coronary heart disease
CLA	conjugated linoleic acids
COMA	Committee on Medical Aspects of Food and Nutrition Policy (UK Department of Health)
CRG	Cardiovascular Review Group of COMA
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DRV	Dietary Reference Values (UK Department of Health)
EFA	essential fatty acid(s)
EPA	eicosapentaenoic acid
GLA	γ-linolenic acid
HDL	high-density lipoprotein
LA	linoleic acid
LCPUFA	long-chain polyunsaturated fatty acid(s)
LDL	low-density lipoprotein
MCT	medium-chain triacylglycerols (medium-chain triglycerides)
MUFA	monounsaturated fatty acid(s)
PUFA	polyunsaturated fatty acid(s)
ROS	reactive oxygen species
SFA	saturated fatty acid(s)
TAG	triacylglycerol(s) (formerly triglycerides)
TC	total cholesterol
VLDL	very-low-density lipoprotein

Chapter 1

The Influence of Dietary Fats on the Concentrations of Lipids carried in the Blood and the Significance for Health

The first part of the chapter describes the digestion and absorption of lipids. Biochemical reactions normally occur in an aqueous medium. As lipids are insoluble in water, special strategies are needed for their metabolism, including the processes of digestion, absorption and transport. Thus lipase, the enzyme that digests triacylglycerols by hydrolysing the ester bonds, releasing fatty acids, acts at the oil/water interface of large emulsified lipid droplets. The monoacylglycerol and fatty acid products of digestion then form mixed micelles with phospholipids and bile salts but the details of the way in which the digestion products pass from these micelles to traverse the gut wall are still unclear. After their reassembly in the cells lining the gut, triacylglycerols are coated with phospholipids and a specific protein to stabilize them in the bloodstream. The protein moiety also acts as a means of identification so that the lipoproteins can be recognized by specific cell surface receptors which direct their further metabolism.

The nature of the dietary fat influences the concentration of cholesterol and of the various cholesterol-carrying lipoproteins in the blood. In general, saturated fatty acids tend to raise and polyunsaturated fatty acids to lower the concentration of blood cholesterol. Several decades of research have enabled equations to be devised, which predict changes in blood cholesterol and the lipoproteins from knowledge of the changes in dietary fat composition. The value and shortcomings of these equations are discussed.

Several sections then describe in more detail the influences of different types of saturated and unsaturated fatty acids and of cholesterol itself on blood cholesterol and lipoprotein fractions. For example, chain length has a profound effect on the capacity of saturated fatty acids to raise plasma total cholesterol. Only those with chain lengths C_{12}, C_{14} *and* C_{16} *are effective.*

The extent to which these fatty acids raise cholesterol is dependent on the concentration of linoleic acid in the dietary mix and possibly interactions with other dietary components. As dietary energy from C_{18} trans *monoenoic fatty acids increases in the range 2–11%, there is a tendency for the blood concentrations of LDL-cholesterol and Lp(a) to rise and for HDL-cholesterol to fall. Information on the effects of longer-chain* trans *fatty acids is needed. The influence of the dietary* cis*-monounsaturates is less clear; some experiments indicate a cholesterol-lowering effect, others no effect. It is likely that the level of dietary linoleic acid is a critical factor in determining the relative effects of saturates and* cis *and* trans *monounsaturates. There are clear distinctions between the effects of the* n–*6 and* n–*3 families of polyunsaturated fatty acids on blood lipids. Whereas linoleic acid lowers LDL-cholesterol, the* n–*3 family tend to lower VLDL, the triacylglycerol-rich lipoproteins.*

Advice on healthy eating usually includes the exhortation to reduce dietary fat consumption to 35% of total energy intake or less. This is sound advice for those with a current or potential weight problem but it is usually implied that total fat reduction will of itself also lead to a significant reduction in blood cholesterol. Few studies have addressed the question whether fat reduction alone will decrease blood cholesterol without having also changed fatty acid composition, which confuses the issue. A recent study clearly shows that if dietary fatty acid composition is maintained constant, dietary fat level has little or no influence on the concentration of cholesterol in the blood.

Virtually all the classic research on the influence of dietary fats on blood lipids has examined lipoproteins in fasting blood samples. Recent interest has focused on the immediate effects on blood lipoprotein patterns of single meals and these tend to show few differences between saturated and monounsaturated fatty acids. Much research has suggested that changes in plasma concentrations of triacylglycerol-rich lipoproteins following a meal are more relevant to long-term cardiovascular health than fasting cholesterol values but only recently have these ideas been thoroughly investigated. Studies of postprandial metabolism show that a proportion of people fails to clear triacylglycerol-rich lipoproteins from the plasma immediately after a meal. Partial breakdown of these fat-rich particles yields 'remnant particles' that linger in the bloodstream and stimulate arterial degeneration. So-called 'triacylglycerol intolerance' is part of a more general metabolic syndrome with a strong genetic basis known as the 'atherogenic lipoprotein phenotype'.

Restoration of a more favourable postprandial metabolism may be achieved by increasing the ratio of n–*3 polyunsaturated fatty acids in the diet. The role of exercise in normalizing lipoprotein metabolism needs much more attention from researchers and public health authorities.*

Introduction

Chapter 2 discusses the concept that the concentrations of different lipids in the blood have a major influence on the development of cardiovascular disease. At the core of the so-called 'lipid hypothesis' is the idea that dietary fats are, in turn, major determinants of blood lipid concentrations. This chapter describes research that, over the last half century, has investigated diet–blood lipid relationships and their significance. First, it will be useful to describe the steps preceding the appearance of lipids in the bloodstream, namely the digestion, and absorption of dietary fats.

Fat digestion and assimilation

The trouble with lipids

The trouble with lipids is that they do not have the decency to dissolve in water and Nature has had to devise crafty ways of overcoming this problem (1). Nowhere is this more apparent than in the digestive process and in the transport of lipids in the blood so that they can be delivered to the tissues (**Figure 1.1**). Furthermore, the very fact that lipids are defined in physical terms — solubility in non-polar rather than polar solvents — means that, unlike amino acids or sugars, they are a chemically heterogeneous group and require handling in different ways. Most of what we term 'dietary fat' consists of esters of fatty acids with glycerol (triacylglycerols). Other dietary lipids are also esters (phosphoglycerides, galactosyldiacylglycerols, cholesteryl esters, retinyl esters) but some components are non-saponifiable (cholesterol, plant sterols, cholecalciferol, carotenoids, tocols and many other minor lipid soluble compounds).

Historically there has been great (and often strident!) debate about the extent of lipolysis of triacylglycerols that is necessary before absorption can occur (2) and about the way in which the lipid digestion products traverse the gut wall. It is now well established that lipid esters are almost entirely hydrolysed before absorption takes place but it is apparent from a recent excellent review (3) that the question of how fat digestion products migrate across the intestinal barrier is still largely unresolved.

Early stages of lipid digestion

Fat digestion may be said to begin in the mouth where a lingual lipase is secreted from glands near the tongue. Most of the hydrolysis probably takes place in the stomach at a pH of about 4.5–5.5 (1,3). This may be relatively more

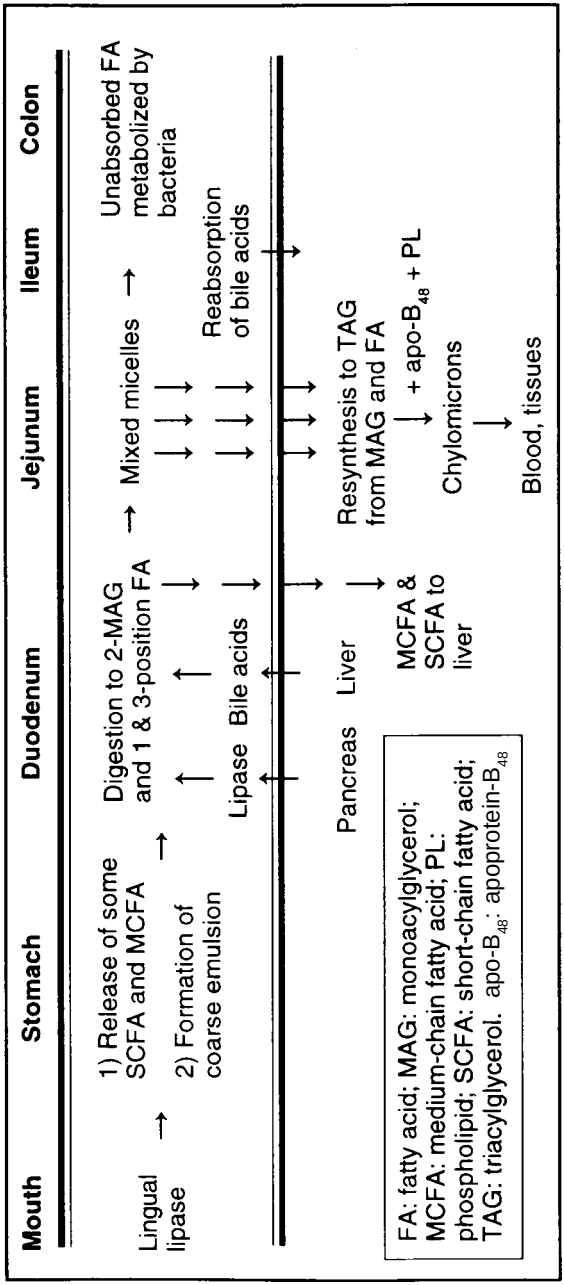

Figure 1.1. Digestion, absorption & transport of lipids.

important in the newborn sucking mother's milk than in children or adults. A lipase identified in human milk reinforces this early digestion and another enzyme, gastric lipase, may provide yet one more opportunity for partial breakdown of lipids before the main digestive site in the small intestine is reached. The main products of gastric digestion are the short and medium chain fatty acids that are esterified predominantly at position *sn*–3 and there is some evidence that these can be absorbed directly into the bloodstream from the stomach.

Digestion in the small intestine

As the baby is weaned onto solid food, the major site of digestion shifts to the upper part of the small intestine, the duodenum, and this remains the primary site of fat digestion into adulthood (1,3). The stomach still has a role, since its churning action creates a course oil-in-water emulsion, stabilized by phospholipids. Furthermore, proteolytic digestion in the stomach serves to release lipids from food particles where they are generally associated with proteins as lipoprotein complexes. The fat emulsion entering the duodenum from the stomach mixes with bile, which supplies bile salts and phospholipids, and pancreatic juice, which supplies lipases.

The enzyme known as pancreatic lipase catalyses the hydrolysis of fatty acids from positions 1 and 3 of triacylglycerols to yield 2-monoacylglycerols. Very little hydrolysis occurs at position *sn*–2 and there is limited isomerization to 1-monoacylglycerols, which may become substrates for further lipase action. The enzyme attacks triacylglycerol molecules at the oil-water interface of large emulsion particles but hydrolysis can only occur after both enzyme and surface have been modified to allow interaction. Bile salt molecules accumulate on the surface of the lipid particles displacing other surface-active constituents. One side of the rigid planar structure of the bile salt steroid nucleus is hydrophobic and interacts with the oil surface; the other contains hydrophilic groups that interact with the aqueous phase. Bile salts confer a negative charge on the oil droplets, which attracts a small protein, colipase, to the surface. Thus, bile salts, colipase and pancreatic lipase interact on the surface to form a ternary complex, which also incorporates the calcium ions that are necessary for enzyme activity.

Phospholipase A_2, present in pancreatic juice as an inactive 'proenzyme' is activated by the release of a terminal peptide by trypsin and catalyses the hydrolysis of the fatty acid in position *sn*–2. A pancreatic cholesteryl hydrolase also cleaves the fatty acid from any ingested cholesteryl esters, and retinyl esters are also hydrolysed.

The rate at which pancreatic lipase hydrolyses fatty acids from positions 1 and 3 of triacylglycerols depends on a number of physical and chemical characteristics of the fatty acids. In general, the longer the chain length, the slower the release. Thus, the rate of hydrolysis of the C_{20} and C_{22} fatty acids found in fish oils, and the erucic acid found in older varieties of rape, for example, is significantly slower than that of the more common C_{16} and C_{18} acids. The degree of unsaturation itself seems to have minimal influence. Fatty acids of short and medium chain length are rapidly released. Whether short-term slower rates of hydrolysis of long-chain fatty acids are nutritionally significant is a moot point since in the longer term the digestion of almost all dietary triacylglycerols is complete. However, maldigestion can occur when, in malnutrition or disease, the pancreas fails to secrete enough lipase, the liver fails to supply sufficient bile acids, or emulsification of food fats in the stomach is inefficient.

Undigestible fats

Chemical bonds other than O-acyl in simple acylglycerols are not attacked by lipase. Examples are the bonds between fatty alcohols and citric acid in so-called 'retrofats', the ester links between fatty acids and glycerol hydroxyls in glycerol polyesters, and the ester links between fatty acids and sucrose hydroxyls in sucrose polyesters. A discussion of the resistance of these bonds to lipase digestion in regard to the exploitation of non-digestible `fats' in low energy foods can be found in Chapter 5.

Absorption of fat digestion products

Short-chain and medium-chain fatty acids (generally those with chain lengths shorter than C_{12}) are absorbed across the gut wall as individual fatty acids. When they enter the bloodstream they are bound to plasma albumin in which form they are transported to the liver, where they are rapidly oxidized (1). Other fat digestion products (monoacylglycerols, long-chain fatty acids, lysophospholipids, cholesterol) mixed with bile acids form mixed micelles with a core of non-polar components and an outer shell of amphiphilic constituents. Minor non-saponifiable lipids (e.g. fat-soluble vitamins) partition into the hydrophobic core. It has always been stated that during absorption components leave the mixed micelles, migrate across a so-called 'unstirred water layer' adjacent to the brush border membranes and then traverse the membrane into the enterocytes (the absorbing cells that line the gut), although no clear description of either part of this two step migration has ever been given. Recently this whole concept has been challenged by the discovery that

pancreatic lipase actually attaches to the brush border membrane and releases digestion products to binding proteins present in the membrane (3). Whereas this neatly accounts for the ordered traverse of lipolysis products, the transport of non-saponifiable compounds from the hydrophobic core still has to be explained.

Malabsorption can occur, even when digestion is functioning normally, due to defects in the small intestine affecting the absorbing surfaces. This may occur during severe bacterial infection of the gut or sensitization of the gut to dietary components such as gluten in coeliac disease, or allergens. The excretion of fat in the faeces is then massively increased (steatorrhoea) and 10-hydroxystearic acid, formed by bacteria, is present in high concentration in stools. A major problem in severe fat malabsorption is essential fatty acid deficiency (1).

Resynthesis of absorbed digestion products

Once the digestion products are inside the enterocyte, the cell has to take steps to protect itself from their highly disruptive detergent properties. This is accomplished by their attachment to a small molecular mass fatty acid binding protein immediately they enter the cell (3). It has been presumed that these proteins also have a carrier function in taking monoacylglycerols and fatty acids across the cytoplasm to internal cellular membranes where they will be reconstituted to triacylglycerols but as yet there is no direct evidence of such a function. Indeed the way in which lipids travel around cells as well as in and out of them is still very much a mystery.

Lipid digestion products are reconverted into triacylglycerols in the enterocyte by sequential esterification of the 2-monoacylglycerols to form first a diacylglycerol and then a triacylglycerol (1,3). There are also enzymes for re-esterifying cholesterol and lysophosphoglycerides.

Transport of fats in the blood

The biological problem of how to transport water-immiscible lipids in the predominantly aqueous environment of the blood has been solved by stabilizing the lipid particles with a coat of amphiphilic compounds — phospholipids and proteins (1,4) — to form lipoproteins. The protein moieties are known as apolipoproteins and have much more than a stabilizing role. They also confer specificity on the particles, allowing them to be recognized by specific receptors on the surfaces of cells in different body tissues and organs, thereby enabling them to be taken up from the blood and regulating their metabolism.

The lipoprotein particles that are assembled in the enterocytes during fat absorption are called chylomicrons. Essentially all the components have been synthesized or resynthesized *in situ* (4). The apoprotein, named apo-B_{48}, is unique to the enterocyte and identifies particles carrying lipids of exogenous (dietary) origin. From the enterocytes, they enter the lymphatic system before draining into the bloodstream (4). In well-nourished people who eat fairly frequently, the first port of call is the adipose tissue, where the chylomicrons, identified by their apo-B_{48} tag, are depleted of some of their triacylglycerols by the enzyme lipoprotein lipase that lurks in the blood capillaries at the surface of the tissue. Fatty acids are taken up into the tissue and undegraded 'remnant particles' are then picked up by the liver for further processing.

Another type of lipoprotein (very-low-density lipoprotein, VLDL) carries mainly triacylglycerols that have been synthesized in the liver, as distinct from those that are absorbed from the diet. In man, most cholesterol is carried in smaller particles called low density lipoproteins (LDL), so-called because the preponderance of lipid to protein gives them a rather low density. Another kind of lipoprotein important in cholesterol transport has more protein than lipid and is called a high-density lipoprotein (HDL). All lipoproteins contain combinations of cholesterol, cholesteryl esters, triacylglycerols, phospholipids and proteins but the proportions differ. The different ratios of lipid to protein confer different densities that allow separations to be made by centrifugation.

Prediction of lipid and lipoprotein responses to dietary fat and cholesterol

Background

Detailed scientific study of the influences of different dietary fats and fatty acids on the types and concentrations of lipids in the blood dates from the 1950s with the work of the US physiologist, Ancel Keys and others. They fed human subjects diets that contained different amounts of fat and cholesterol and differed in fatty acid composition and measured the changes in blood lipids (mainly total plasma cholesterol) that ensued (5).

From their results, these research workers derived equations that could predict the change in plasma total cholesterol when there were changes in the saturated and polyunsaturated fatty acid composition of the diet. Current public health recommendations about consumption of fats in relation to coronary heart disease (CHD) risk are still firmly based on the results of these studies in conjunction with epidemiological evidence (see Chapter 2).

Since then, many similar dietary studies have been performed and several modified prediction equations have been derived. One of the most comprehensive was published by Howell and colleagues in the *American Journal of Clinical Nutrition* (6).

A new meta-analysis

The study of Howell *et al.* (6) is described as a 'meta-analysis'. This term is used when the results of several already-published investigations are pooled to yield a new overall result. Individual human metabolic studies usually involve small numbers of subjects because of the sheer amount of work required and, more importantly, the effort required to achieve the compliance necessary by subjects to make the work, and the conclusions from it, reliable. They have what is called weak 'statistical power' and the pooling of results enables that power to be enhanced, making it more likely that effects of treatments will emerge as statistically significant.

A problem that those who embark on meta-analysis have to contend with is: which publications should be included in the analysis and which excluded?

Clearly, not all published studies are comparable in their design and objectives, even though all may be to some degree concerned with dietary fat and blood lipids. For pooled results to be valid, like must be compared with like. Even if studies are similar in design and scope, some have been impeccably conducted while others have major flaws that should preclude their inclusion. The decision to include or exclude publications from the analysis may be somewhat subjective: appropriate rules must be devised and the rules are evolving. It is clear that in some analyses abundant care has been taken whereas others are less rigorous.

There is a notable difference of opinion in regard to the type of study design that is appropriate when the final objective is to use the results as the basis for advice to individuals or populations about healthy eating. Broadly, two schools of thought have emerged. The first, typified by Hegsted (7), argues that acceptable studies are only those conducted under the most rigorous conditions in a metabolic ward. The second school (6) believes that while such studies represent the ideal in scientific terms, they do not properly represent the conditions of 'real life', and that the results are not so relevant to practical dietary recommendations. This philosophy was these authors' main motivation for doing yet another meta-analysis (they cite the results of at least five others in their paper).

Criteria for inclusion in the analysis

Howell and colleagues (6) conducted a literature search on dietary interventions in adults, published in English between 1966 and 1994. Dietary interventions had to be one or more of the following: dietary cholesterol, total fat, saturated (SFA), mono- unsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. Response variables included were: plasma total cholesterol, low-density, very-low-density and high-density lipoprotein cholesterol, and triacylglycerols (TAG). This search yielded 12 250 citations which were reduced to 224 by careful scanning of titles and abstracts. The analysis did not include the large clinical trials involving multiple risk factors (established to examine the feasibility of modifying CHD risk), studies aimed at weight reduction and experiments with fish oils, *trans* fatty acids and hydrogenated fats.

The analysis differed from most previous ones in including a larger number of studies that involved subjects following the prescribed diets in their own homes (75%) as well as those conducted under laboratory or metabolic ward conditions (25%). The authors made careful note of the conditions under which the studies were conducted, their design and the characteristics of the subjects. They were able to adjust for many potential confounding factors or test for interactions between different factors when they developed their mathematical models and predictive equations. For example, they were able to investigate the effects that might have been induced by having people of widely different ages or with widely differing initial total cholesterol concentrations in the studies included in the analysis, or for the different effects that might have resulted of the lengths of the studies, which ranged from one day to six years.

Predictive equations

From their wide-ranging analysis, the authors derived equations that predict the change in blood lipid or lipoprotein fraction that will occur over a period of time when dietary fat components are changed. Only dietary terms that had statistically significant effects on blood components were included in the equations (see **Box 1**).

Qualitatively, the predictions are similar to those of Keys (8) and Hegsted (9) in the 1960s: the main influences on plasma total cholesterol were SFA and PUFA. SFA were twice as effective at raising total cholesterol as PUFA were in lowering it. According to this analysis, current guidelines would lead to about a 5% reduction in average total cholesterol. For those with higher than average total cholesterol, the reduction would be proportionately less.

ΔTC	=	49.599ΔSFA – 23.274ΔPUFA + 0.5741 ΔDC
ΔLDL-C	=	46.755ΔSFA – 12.801 ΔPUFA
ΔHDL-C	=	7.422ΔSFA + 4.965ΔTF
ΔTAG	=	0.1626ΔDC – 12.035ΔPUFA – 10.376ΔTF

Box 1.1. Equations predicting the change in blood lipid or lipoprotein fraction that will occur over a period of time when dietary fat components are changed. Derived by Howell *et al.* (6). In these equations, changes in blood components are expressed in units of micromoles per litre; changes in dietary components are as percentage of total energy except for DC, which is expressed as mg per day. DC = dietary cholesterol; TF = total fat; SFA = saturated fatty acids; PUFA = polyunsaturated fatty acids; TC = plasma total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; and TAG = triacylglycerols.

Dietary cholesterol

Although there was an effect of dietary cholesterol on total cholesterol, it was weak (Keys' original 1957 equation (5) did not contain a term for dietary cholesterol but his 1965 equation did). This analysis predicted a decrease of only 0.057 mM total cholesterol (2.2 mg/dl) when dietary cholesterol was reduced by 100 mg per day. In the authors' list of comparable studies, the Hegsted equation of 1965 (9) stands out as being the only one to predict a substantial effect of dietary cholesterol on plasma cholesterol.

The senior author's own work (10) has shown that in a majority of people, homeostatic mechanisms operate to maintain blood cholesterol within a certain range. This occurs by feedback inhibition of cholesterol biosynthesis in the liver by dietary cholesterol. When dietary cholesterol is abundant, liver biosynthesis is shut down; conversely, when there is little cholesterol in the diet, the enzymes of cholesterol biosynthesis increase in activity to maintain a supply of cholesterol for membrane structures, synthesis of bile acids and steroid hormones. These people are the so-called non-responders; in others for whom homeostatic mechanisms are less precise, blood cholesterol responds much more to increases in dietary intake. Interestingly, the effect of dietary cholesterol on LDL was not significant. I suspect this was mainly because the number of studies in which LDL was measured was small in comparison with those that recorded total cholesterol, with consequent loss of statistical power.

Saturated fatty acids

The influence on SFA on both total cholesterol and LDL-cholesterol was consistent with previous findings, although weaker than previous equations have predicted. This study did not seek to distinguish the effects of SFA with different lengths of carbon chain. Although this is a drawback because we know that there are large differences (see later section), it is understandable since few studies investigated chain length effects and the statistical power would have been weak. Few predictive equations have previously been derived for HDL-cholesterol and it is noteworthy that the concentration of this lipoprotein fraction was mainly influenced by total fat and SFA.

Whereas these facts were known, their significance for dietary advice has certainly not been sufficiently appreciated or stressed. The main scientific justification for dietary advice to reduce intakes of SFA has been the lowering of LDL-cholesterol predicted by the metabolic studies discussed above. A raised LDL-cholesterol is regarded as a major risk factor for CHD whereas a raised HDL-cholesterol is thought to protect against the development of the disease. Most metabolic studies that contributed to concepts of risk reduction through dietary fatty acid modification recorded only total cholesterol rather than LDL-cholesterol and it has been assumed that total cholesterol could be used legitimately as a surrogate for LDL-cholesterol. However, the analysis of Howell and colleagues underlines the fact that reduction of SFA should also reduce HDL-cholesterol. In other words, reducing SFA intakes will reduce risk through its lowering of LDL-cholesterol but not nearly so much as has been assumed because of the concomitant counteracting effect on HDL-cholesterol.

Monounsaturated fatty acids

It is noteworthy that none of the predictive equations contained a term for MUFA, indicating that these fatty acids had no significant effects on blood concentrations of lipids and lipoproteins. This is important in view of the intense interest in the past few years on the dietary influence of MUFA (see later section) and the preoccupation with the health-giving properties of olive oil and the 'Mediterranean diet'. The authors did not comment on this controversy in their discussion.

Clearly, when a change is made in one type of fatty acid at constant energy intake, the contribution of another type of fatty acid will also change. MUFA usually make the largest dietary contribution of any fatty acid type and the lack of a significant contribution to blood lipids may simply reflect the relatively minor changes that occurred in this fraction in the studies included in the analysis.

Polyunsaturated fatty acids

As expected, PUFA featured as a highly significant cholesterol-reducing term in the equations for total cholesterol and LDL- C. Whereas some studies have suggested that the lowering effect of PUFA disappears at about 6% of dietary energy, this analysis did not find any such limiting concentration. Surprisingly, PUFA also appeared as a significant but weak term in regard to lowering blood TAG concentration. This may reflect the well established triacylglycerol-lowering effect of *n*–3 PUFA (see Chapter 4). However, this is not entirely clear, since the authors excluded studies on fish oils so that the PUFA term in their equations must mainly reflect the effects of *n*–6 PUFA, which have not normally been regarded as triacylglycerol-lowering. This illustrates the distinct disadvantage of not distinguishing the types of PUFA. Once again, the authors had to deal with the studies that were available and few of these would have distinguished different PUFA families. However, it seems likely that in future, more attention will need to be given to the differential effects on blood lipid fractions of different types of PUFA.

Total fat

Total fat occurred as a dietary term in the equations predicting changes in HDL-cholesterol and TAG. This is consistent with the well known tendency for low fat/high carbohydrate diets to lower HDL-cholesterol and raise TAG. It has been convincingly demonstrated that total fat does not affect total cholesterol or LDL-cholesterol when the ratio of SFA to PUFA is maintained constant (see later section). It is debatable whether a predictive equation for fasting TAG is useful. The modern concept is that a prolonged elevation of TAG immediately after a meal is an important indicator of CHD risk, not fasting TAG (see later section and Chapter 2).

Conclusions

A problem with predictive equations is that they represent average responses of large numbers of people, whereas individuals respond in markedly different ways to changes in dietary fatty acids and cholesterol (see later section). Such responses depend very much on the ability of the body's homeostatic mechanisms to damp down the more extreme effects of dietary change by re-directing body fat metabolism. Howell and colleagues (6), after all their meticulous analytical work, concluded that "The data supporting a relation between dietary fat and cholesterol intake and elevated plasma lipid concentrations should be evaluated not as mean values but on the basis of individual patient responses". Such a philosophy will be anathema to those

public health professionals who argue that advice to populations is more effective than to individuals.

A problem with giving specific advice to individuals who need treatment, rather than to whole populations many of whom do not, is in identifying who needs (dietary) treatment. Widespread screening is expensive. Plasma cholesterol is one of many risk factors for CHD and the most appropriate action may be for general practitioners to identify diet-responsive individuals only when their patients also have sufficient other risk factors: high blood pressure, excessive smoking, lack of exercise, central obesity and poor glucose tolerance.

Having described the derivation of a general set of equations, based on decades of research, that can be used to predict average responses of blood lipids to dietary lipids, I will now describe, in a little more detail, the responses of blood lipids to each of the different classes of dietary fatty acids and to cholesterol and total fat intake.

Cholesterol in the diet and in the blood: a more detailed appraisal

What is cholesterol?

Cholesterol is a natural substance that belongs to a class of biologically-important chemical compounds, the sterols, characterized by a basic structure of four rings of carbon atoms to which are attached a hydroxyl group and a hydrocarbon side-chain (1). The ring structure gives the molecule rigidity and, together with the side chain, confers fat-like or hydrophobic qualities rendering the molecule insoluble in water. The hydroxyl group confers a degree of chemical reactivity on the molecule. For example, fatty acids can combine with the hydroxyl group to form cholesteryl esters.

Where do we find cholesterol?

Different sterols are characteristic of different organisms. Cholesterol is an important component of the body tissues of most animals, whereas it is rare in plants and absent from microorganisms. It is present in all animal cell membranes as the free sterol and in the blood in the form of cholesteryl esters. It is also dissolved in fat deposits, such as the adipose tissue and in lipids that accumulate in liver, muscle, some other organs and the walls of blood vessels as part of a disease process. The human body contains rather more than 100 g of cholesterol and most foods of animal origins contain some cholesterol.

Where does the human body obtain its cholesterol?

About three-quarters of the body's need for "new" cholesterol every day is made in the body (approximately 750 mg) and about a quarter (250 mg) is obtained from the diet. Many organs in the body synthesize cholesterol (1) but most is made by the liver. The 27 carbon atoms in cholesterol are derived from the simple substance acetic acid which arises from the breakdown of the dietary carbohydrates. The synthesis takes place in nearly 40 steps, each catalysed by a different enzyme. One particular enzyme is controlled so that the body produces not too little and not too much. All diets except the most strictly vegan contain cholesterol and in the UK the average daily intake is about 350–450 mg. Eggs are the most highly concentrated source, each containing about 300 mg. Other major sources are fats of animal origin such as lard, butterfat, fish and meats of all kinds.

Cholesterol is relatively poorly absorbed and only about half of what is present in the normal diet is absorbed into the blood. There are, however, wide differences between the capacities of individuals to absorb and metabolize cholesterol. That which is absorbed into the blood from the food is "sensed" by the tissues that make cholesterol and by this means, the total amount synthesized by the body can be reduced so as to keep overall body levels fairly constant. Some people's capacity for regulation of cholesterol metabolism is so defective that severe over-production occurs leading to tissue damage and disease.

What is the importance of cholesterol?

The main function of cholesterol in its native form is as a component of biological membranes (1). The backbone of a membrane is a double layer of lipids into which the vital proteins are inserted. The membrane can be stretched and compressed and this flexibility, or 'fluidity' as biologists call it, is determined by the physical properties of the lipids. Cholesterol plays a key role in regulating fluidity: too much and the membrane is too rigid, too little and the membrane is too flexible to function properly. Of all the sterols occurring in nature, only cholesterol will allow animal membranes to function as required. Therefore, cholesterol is what we call an 'essential metabolite' for without it our bodies would not function properly and we would die. It is not, however, an essential nutrient, since it can be made by the body and need not be present in the diet.

Cholesterol is also important for the formation of bile salts which behave as emulsifiers, solubilizing the dietary fats so that they can be digested and

absorbed in the small intestine. It is the starting point for the formation of a multitude of steroid hormones such as the male and female sex hormones and can also be converted, in the presence of the sun's ultraviolet light, into vitamin D in the skin.

How is cholesterol delivered to where it is needed?

Whether cholesterol is absorbed from the diet or produced by the liver, it has to be transported in the body to where it is needed. Its insolubility in water poses a severe problem, which has been overcome in the body by combining it with specific proteins that render it soluble. The range of lipoproteins so formed was discussed in an earlier section.

LDL carry cholesterol to tissues where their cholesterol is off-loaded for building membranes or for conversion into the various types of hormones. The protein component (called apo-B) interacts with another protein called a 'receptor' located in the membrane of the tissue where the cholesterol is to be off-loaded, rather like a key fits into a lock. The piece of membrane around the receptor is broken off and engulfed by the cell. The cholesterol is released and is incorporated into the cell's internal membranes. It also prevents the cell producing more of its own cholesterol. If a cell has too much cholesterol, this can be removed by an HDL particle colliding with the cell and picking up the cholesterol that is excess to requirements. When the HDL particles pass the liver they interact with receptors that are specific for HDL and the cholesterol is taken into the liver and processed in a variety of ways.

The effects of overabundance of cholesterol in blood

It is difficult to define an optimum concentration of cholesterol in blood. It is not like glucose, whose concentration is controlled between well-defined limits, above or below which the effects of hyperglycaemia or hypoglycaemia are readily apparent as all diabetics know. The range of blood cholesterol concentrations is much broader and the effects of straying much above or below 'the normal range' are not readily apparent in the short term. Different communities may have widely different distributions of blood cholesterol and this may depend on a combination of interacting factors including genetics, diet, personality, exercise patterns, and other aspects of lifestyle. In the UK the average plasma cholesterol is about 6 mM (6 millimolar, equivalent to about 230 mg/100ml).

The best evidence for the adverse effects of cholesterol in the blood is provided by patients with the disease familial hypercholesterolaemia who may have concentrations of blood cholesterol several times higher than 'normal',

mainly in the form of LDL. This is due to a faulty gene that results in defective receptors for LDL, so that LDL are not removed properly from the blood and the body's own production of cholesterol is not properly switched off. When the concentration of blood cholesterol is so high, the substance infiltrates into many tissues including the walls of the arteries and contributes to a build up of lipids that is characteristic of arterial deposits. People with familial hypercholesterolaemia generally die of coronary disease very early in life. The pathology of arterial lesions in these patients, however, is quite distinct from that in people with atherosclerosis, a disease of ageing that progressively affects all people throughout the world to different degrees.

There is little published information on the effects of having a too low plasma cholesterol concentration. A very low blood cholesterol has been associated with an increased risk of cancer, although not all reports confirm this and more data are needed before a convincing case can be made. Low blood cholesterol is also associated with anaemia and this may be connected with a greater fragility of red blood cells due to a reduction in their cholesterol content.

Can we have too much cholesterol in the diet?

Aside from those patients who have genetically-determined hypercholesterolaemia, the body's production of cholesterol normally responds to the amount absorbed from the diet. Moreover, at very high cholesterol intakes, the fraction absorbed decreases, so tending to limit absorption. Even within a 'normal' population there are widely different individual responses to dietary cholesterol, either because of differences in absorption efficiency or in ability to regulate production. Thus, it is well recognized that there are 'hypo-responders' or 'hyper-responders' (i.e. those whose plasma cholesterol is either little affected or shows a marked increase in response to dietary cholesterol). One will always find individuals who appear to be hyper-responsive in one experiment and hypo-responsive in another because everyone's blood cholesterol level fluctuates, independently of diet, over a period of time. Moreover, in experiments to test this response, cholesterol has generally been supplied in the form of eggs so that other components of the diet have been changed in addition to cholesterol, making interpretation difficult. Of the various dietary factors that affect plasma cholesterol, cholesterol in the food plays a minor role compared with certain types of saturated fatty acids, proteins, complex polysaccharides, ascorbic acid and alcohol. Moreover, diet has to be put in perspective alongside other lifestyle factors and heredity. Appreciating this perspective is important in view of the over-emphasis, not to say hysteria, on dietary cholesterol in the USA and increasingly in the UK.

Saturated fatty acids and hypercholesterolaemia: are all saturated acids equal?

I will confidently proclaim at the beginning: all saturated fatty acids (SFA) are not equal. The same applies to unsaturated fatty acids as I shall discuss in the next sections.

The importance of chain length

For those who care to read the original literature (an occupation for which, sadly, few have time these days), it is clear that suggestions that saturated fatty acids differed in their cholesterol-raising capacities were already apparent in the 1950s and certainly in the 1960s. Nevertheless, the use of a single all-embracing term for saturates in the Keys (8) equation has tended to obscure this fact and it is only recently that serious attention has been given to these differential effects. The Keys equation predicted changes in plasma total cholesterol from changes in the intake of saturates and polyunsaturates (**Figure 1.2**). According to this formula, saturates are twice as effective at raising total cholesterol as polyunsaturates are in lowering it.

$$\Delta\text{plasma cholesterol} = 2.7\,\Delta S - 1.35\,\Delta P + 1.5\,\Delta Z$$

(where S and P are percentages of total energy provided by saturated fatty acids and polyunsaturated fatty acids respectively, and Z is milligrams dietary cholesterol per 1000 kilocalories).

Figure 1.2. The Keys Equation.

Early work indicated that fatty acids with chain lengths up to and including ten carbon atoms (short-chain and medium-chain fatty acids) do not influence plasma cholesterol (11). This is because they are absorbed directly into the blood supplying the liver and rapidly metabolized in that organ, unlike the longer-chain acids which are absorbed as 'chylomicrons'. Likewise, in early experiments, cocoa butter had a far smaller effect on plasma cholesterol than had been anticipated, and it was concluded that stearic acid had low cholesterol-raising activity. This has since been confirmed by the experiments of Bonanome and Grundy (12). The stearic acid was given as a synthetic fat in a liquid formula diet and compared with two other formulas that contained high proportions of palmitic and oleic acids. The authors concluded that stearic acid was as effective as oleic acid in lowering plasma cholesterol when either replaced palmitic acid in the diet. Stearic was absorbed as efficiently as oleic

acid, so that lack of total cholesterol-raising activity was unlikely to have been due to poor absorption. A possible explanation was the rapid desaturation of stearic to oleic acid, once stearic acid has been assimilated into body tissues.

Lauric (12:0), myristic (14:0) and palmitic (16:0) acids have, thus, generally been regarded as the three 'cholesterol-raising' fatty acids and the major plasma lipoprotein fraction affected is LDL. Palmitic is quantitatively the most significant since it is the principal saturated fatty acid in most diets, occurring widely in animal and plant fats. Keys and colleagues (8) found roughly equal effects of lauric, myristic and palmitic acids, while Hegsted and co-workers (9) found effects in the order myristic > palmitic > lauric.

Recently, the differential cholesterolaemic effects of SFA of chain lengths C_{12}, C_{14} and C_{16} have been re-evaluated. In carefully controlled experiments with different species of monkeys chosen to simulate human 'high', 'medium' and 'low' responders, Hayes and colleagues (13) found that 12:0 and 14:0 were considerably more potent than 16:0 in raising plasma total cholesterol and LDL-cholesterol. The relative potencies of 12:0 and 14:0 remain in question; published animal results suggest that 14:0 is more effective than 12:0.

Influence of triacylglycerol structure

Natural fats are characterized by a stereospecific distribution of fatty acids on the three positions of the glycerol backbone rather than a random distribution. The way in which fatty acids are distributed may influence plasma cholesterol irrespective of the overall composition of the fatty acids (14). Thus linoleic acid is more hypocholesterolaemic (15) and some saturates more hypercholesterolaemic (16) when present at position 2 than in positions 1 or 3. The fact that stearic acid is normally esterified at position 1, rarely at position 2, may in part explain the neutral effect of this fatty acid on blood cholesterol. Butter is much less hypercholesterolaemic when the positions of its fatty acids are randomized by interesterification (17).

Mechanisms

Several mechanisms by which specific saturated fatty acids raise LDL cholesterol while specific unsaturated fatty acids either lower it or restrict the rise, have been discussed (11). Dietary fatty acid composition may influence: (i) the excretion of bile acids that occurs at each passage of the entero-hepatic circulation; (ii) the production of cholesterol and of apoB-containing lipoproteins; (iii) the catabolism of LDL; (iv) the cholesteryl ester content of each LDL particle in the plasma.

The apoB receptor plays a major role in regulating the rate of removal of LDL as well as its rate of synthesis from VLDL. Hepatic receptors for apoB account for most of the capacity to remove LDL. The binding capacity of the apoB receptor is genetically-determined but the number of receptors expressed is influenced by dietary and hormonal factors. Grundy and Denke (11) discuss a model in which an increase in absorbed cholesterol reduces the activity of LDL-receptors which, in turn, retards the uptake of LDL and VLDL remnants. An increased conversion of VLDL remnants into LDL as well as a reduced uptake of LDL results in increased plasma concentrations of LDL. The influence of the non-specific endocytosis and scavenger pathways remains uncertain.

The 'hypercholesterolaemic' SFA appear to suppress the receptor-mediated clearance of LDL from plasma (11). The reduced activity of the LDL receptors reduces the rate of catabolism of LDL as well as enhancing the rate of conversion of VLDL remnants to LDL. Caution has to be exercised in extrapolating results from experimental animals to man. LDL receptor activity may be low in man compared with other animals. Consequently, LDL-cholesterol concentrations could be primarily determined by rates of LDL synthesis rather than by rates of removal. Using radioactively-labelled apolipoproteins to follow the kinetics of LDL synthesis in human subjects, several laboratories have demonstrated a marked reduction in LDL synthetic rates when linoleic acid replaced saturated fatty acids in the diet and a slight rise in fractional catabolic rate (18).

Hayes and Khosla (19) have developed an hypothesis to explain the differential effects of SFA and the apparent discrepancies between previous publications. The effects of particular combinations of fatty acids are discussed at: (i) the level of individual fatty acid molecules interacting with apoB/E receptors; and (ii) the resulting consequences for the production or removal of lipoprotein particles by the liver. They propose that the different hypercholesterolaemic SFA have different thresholds at which they exert an effect on plasma cholesterol, which are dependent on: (a) the level of 18:2 in the diet; (b) the level of dietary cholesterol; and (c) on the initial concentration of LDL-cholesterol in the plasma of the experimental subjects. The interaction of these various factors operates at the level of the LDL apoB/E receptors on cell surfaces which are critically important in the removal of LDL particles from plasma. At a level of 18:2 in excess of about 6.5% of dietary energy, the LDL apoB/E receptors are 'up-regulated'. That is, they actively interact with apoB/E and remove LDL particles from plasma, maintaining a relatively low plasma concentration of LDL. As the dietary level of 18:2 is gradually reduced

below 6.5%, 14:0 and 12:0, begin to override the effects of 18:2 and start to 'down-regulate' the receptors. ApoB/E receptor activity is reduced, LDL particles are less efficiently removed and plasma concentrations of LDL begin to rise. Because of its lower potency, more 16:0 is required to observe an effect, and then only when 18:2 levels in the diet are at the lower end of the intake distribution.

This hypothesis no doubt will be refined as more experimental data become available in man and dose-response effects are established. It begins to explain the discrepant results in the literature, coming from experiments employing widely differing levels of 18:2 and SFA, different ratios of the individual SFA, different cholesterol intakes and widely different initial plasma total cholesterol and LDL-cholesterol concentrations. Thus, discrepancies between the original studies of Keys and Hegsted (8,9), which observed no cholesterolaemic effect of 18:1 and more recent studies (20,21), which observed an hypocholesterolaemic effect (see later section for more detail), can be explained. Keys and Hegsted (8,9) exchanged 18:1 for 18:2 at levels of the latter between 1 and 6% of energy and in the presence of relatively large amounts of 14:0. More recent studies (20,21) worked at levels of 18:2 above the 6% threshold in the presence of relatively small amounts of 14:0. At low levels of 14:0, minimal 18:2 is required to ensure maximum apoB/E receptor activity so that 18:1 appears to be as efficient as 18:2 in maintaining a low plasma LDL when exchanged for what is effectively an 'excess' of 18:2.

The hypothesis does not explain why the various SFA differ in their potencies but a plausible explanation, which is amenable to scientific test, might postulate an effect of SFA that depends on their interaction with a hydrophobic amino acid sequence in the receptor protein that needs a C_{14} chain length for optimal interaction. Either side of this, interactions are reduced: on the low chain-length side by reduced Van der Waals forces and on the higher chain-length side by the physical restriction upon the size of chain that can be accommodated.

Thus, it seems that dietary SFA-plasma lipid interrelationships have been grossly oversimplified. Old simplistic statements, such as: "saturated fats raise plasma cholesterol", need to be reappraised and the usefulness of the polyunsaturates/saturates (P/S) ratio can be seriously questioned. New concepts emphasize the need to focus on individual dietary fatty acid interactions rather than `fats' in general or complex mixtures of SFA and unsaturated fatty acids. The impact of any given dietary fatty acid on cholesterol metabolism can be ameliorated or exacerbated by the composition of the accompanying nutrients and level of energy intake.

Trans fatty acids: their influence on blood lipids and their wider health implications

Trans acids in the public eye

Until the mid 1980s, the phrase '*trans* fatty acids' would have been familiar only to biochemists and lipid technologists. The publication in 1984 of the report on Diet and Cardiovascular Disease (22) from the UK Department of Health's Committee on Medical Aspects of Food and Nutrition Policy (COMA) changed all that by introducing the term to health professionals and those involved in health education. The subsequent discussions about nutrition labelling, which included a debate about whether to include *trans* fatty acids in the scheme, brought these compounds to the attention of an even wider audience. Since 1984, there has been a proliferation of original papers and reviews of the health implications of the consumption of *trans* fatty acids. Each review has presented a somewhat different interpretation of the evidence (23–27), so a fairly detailed survey will be presented here. What are *trans* fatty acids, why have they risen to prominence and what are their implications for public health?

Trans fatty acids occur in nature

Fatty acids with ethylenic double bonds in the *trans* geometrical configuration are found naturally, although in much less abundance than the *cis* form (25). They may be monounsaturated or polyunsaturated. The latter may have double bonds that are methylene-interrupted or conjugated. *Trans* isomers occur as short-lived intermediates in the biosynthesis of saturated fatty acids or as stable end-products. An example of the latter, *trans*-3-hexadecenoic acid is a ubiquitous, though minor, component of the lipids of photosynthetic tissues. Some seed oils (e.g. tung) may contain up to 80% of *trans* acids such as α-eleostearic acid (cis-9, *trans*-11, *trans*-13-octadecatrienoic acid) although they are not important food fats.

Trans fatty acids are also produced by the process of biohydrogenation of dietary polyunsaturated fatty acids by rumen microorganisms of ruminant animals. The tissues of these animals are, therefore, richer in *trans* fatty acids than those of simple-stomached animals fed a normal diet and so are the food products derived from them. During biohydrogenation, the *cis*-double bonds of the original all-*cis* polyunsaturated fatty acids are isomerized. This may involve a shift in position along the hydrocarbon chain (positional isomerization) or a change in geometrical configuration or both. The more highly unsaturated

the starting fatty acid, the greater the variety of isomers formed. In milk the *trans*-monounsaturated fatty acids are quantitatively the most important and may constitute up to 21% of the total monoene fraction. The *cis* double bond is mainly found in position 9, whereas *trans* unsaturation is present in positions 6–16 of octadecenoic acid with a predominance of the 11-*trans*-isomer.

Isomerization produced by industrial processing

Highly unsaturated oils, such as those found in many seeds and fish, are generally unsuitable for food fats because of their low melting points and ready susceptibility to oxidative deterioration. The objective of industrial hydrogenation is to reduce the degree of unsaturation, thereby raising the melting point of the oil. As in biohydrogenation, a proportion of the *cis* double bonds in the natural oils is isomerized during the hydrogenation to *trans* and there is a migration of double bonds along the chain. When the highly unsaturated fish oils are used as starting materials, the mixture of isomers is extremely complex.

Monoenoic acids represent the major fraction with *trans* unsaturation at positions 6–12 with a predominance at position 10. With vegetable oils, such as soybean oil, as the starting materials, *trans* unsaturation in the diene fraction is found mainly in *cis*-9,*trans*-12-18:2, *trans*-9,*cis*-12-18:2 and *cis*-9,*trans*-13-18:2. Improvements in catalytic hydrogenation have reduced the amounts of *trans*,*trans*-dienes in modern margarines to virtually zero.

How much *trans* fat do we consume?

In the late 1980s, estimates of intake varied between 5 and 7 g/day in the UK (23). About half of this amount was derived from ruminant products and the other from industrially processed fats. Since then intakes from both sources appear to have fallen.

In the mid-1990s it was estimated (24,28) that average intakes in the UK were 4–6 g per person per day representing 5.5% of dietary fat intake or 2% of dietary energy. These figures are lower than those available before 1991 and may have arisen partly as a result of different (and probably better) methods and partly because of a true decrease in consumption. Intakes of ruminant fats have tended to decrease and the *trans* fatty acids content of hydrogenated fats has also fallen as a result of changes in raw materials and processing methods. In the USA, at the same time, average intakes were based on estimates from limited data but appeared to be higher (8–13 g per person per day). In 1990 it was estimated that intakes in The Netherlands were 17 g/day (29).

Consumers who prefer margarines and cooking fats made from hydrogenated fish oils (perhaps because they are inexpensive) and who choose a diet that is generally high in fat, might consume much greater quantities of *trans* fatty acids than the average consumer. In the survey of British adults (28), consumption in the top 2.5% of the distribution was 12 g per day and a few individuals consumed considerably more.

Metabolism of *trans* fatty acids

When *trans* fatty acids are included in the diet, they can be found in most tissues of the body. There is no evidence that they are digested, absorbed, transported or oxidized differently from their *cis*-counterparts (25). There is some selectivity with regard to the complex lipids into which *trans* fatty acids are incorporated. They are generally found more extensively in triacylglycerols because of their widespread deposition in body fats. They occur mainly in positions 1 and 3. In other tissues, such as heart, liver or brain, as much or more may be incorporated into phospholipids. The *trans*-octadecenoic acids behave like saturated fatty acids and are preferentially incorporated in position 1 of phosphoglycerides in contrast to oleic acid which is randomly distributed. However, patterns of incorporation are extremely complex, since there is selectivity with regard to the geometry and the position of the double bond (25). These conclusions have been reinforced by the work of Emken and colleagues (cited in references 24,25). These researchers gave various deuterated *cis* and *trans* fatty acids to human subjects and examined, by mass spectrometry, their incorporation into plasma lipids and lipoproteins. Only minor differences were observed.

How might *trans* fatty acids be harmful?

Interactions with essential fatty acids.

When young animals are given a diet that lacks essential fatty acids (EFA), overt signs of EFA deficiency are seen that can be reversed by feeding as little as 1% of the dietary energy as linoleic acid. When the diet has only marginally sufficient linoleic acid, the addition of non-essential fatty acids (whether saturated, *cis*- or *trans*-monounsaturated) can result in the appearance of deficiency signs. In some experiments, the animals showed progressive signs of EFA deficiency as the amounts of dietary *trans* fatty acids were increased. The *trans,trans* isomer appeared to be the most potent. There are at least two mechanisms that might account for this effect. *Trans* fatty acids have been shown to inhibit desaturase enzymes involved in the conversion of linoleic acid to longer-chain more highly polyunsaturated fatty acids, like

arachidonic acid, which are themselves the precursors of important hormone-like compounds, the eicosanoids. Again the *trans,trans* isomer was the most potent inhibitor (23,25).

Alternatively, oleic acid (*cis*-9-octadecenoic acid) and some *trans*-acids compete for the desaturases that convert linoleic acid into its higher metabolites, thus diverting linoleic acid away from its normal pathway. In one experiment with rats, diets containing rather high proportions of *trans,trans* and *cis,trans* isomers of linoleic acid resulted in lower concentrations of essential fatty acids in liver and blood platelets and a lower rate of production of certain eicosanoids by platelets (30). This could have important implications for blood clotting and thrombosis since these processes are influenced by the balance between the eicosanoids produced from different EFA. The practical implications of this particular experiment are difficult to assess however, since the diets used did not closely resemble any practical human diet and the major effects were again with the *trans,trans* isomer which is only a very minor component of human diets. A more recent experiment, again using rats as models, explored the effect of different levels of dietary *trans*-monounsaturated fatty acids and different levels of dietary essential fatty acids on eicosanoid metabolism and, therefore, was more relevant to human nutrition (31). *Trans* fatty acids did not directly influence the enzymes of eicosanoid biosynthesis and the eicosanoid products formed from EFA when at least 2% of dietary energy was provided by linoleic acid.

Effects on biological membranes

Trans fatty acids have physical properties that tend to resemble those of the saturated fatty acids of similar chain length because the shape of the double bond allows closer packing of the hydrocarbon chains than the kinked configuration that is characteristic of *cis*-double bonds (27). Thus, if *trans* fatty acids replaced *cis*-unsaturated fatty acids in membranes, the membrane may become less 'fluid', a phenomenon that has indeed been demonstrated with microorganisms and cells grown in culture in a medium enriched in *trans* acids. However, in whole living animals, *trans* acids tend to replace saturated fatty acids located at position 1 of phosphoglycerides and there is little evidence for a change in membrane properties, certainly at normal dietary intakes of *trans* acids (23).

Influence on plasma cholesterol

The justification for the recommendation by the COMA Panel on Diet and Cardiovascular Disease to equate *trans* fatty acids with saturated fatty acids for

the purposes of their recommendations may have been the assumption that they would, like certain saturated fatty acids, raise plasma cholesterol (22). At that time (1984) there was poor evidence that *trans* acids did have an adverse effect on plasma cholesterol. Two studies suggested a raising effect and one found no effect compared with a control diet in which the predominant fatty acid was oleic acid. These studies were not particularly well designed and could not be used as good evidence either way (29). The study by two Dutch authors, Ronald Mensink & Martijn Katan (29) caused a revision of those views since it was well controlled and very carefully done. It is also a step forward in that it investigated the effects of diets on lipoprotein fractions and their corresponding apolipoproteins as well as on total cholesterol. Thirty-four women and twenty-five men were given three natural mixed diets of identical nutrient composition except that 10% of daily energy intake was provided either as oleic acid, *trans*-isomers of oleic acid or saturated fatty acids. The three diets were consumed for three weeks each in random order. The authors found that the effect of *trans* fatty acids on the plasma lipoprotein profile was at least as unfavourable as that of the cholesterol-raising saturated fatty acids because they not only raised LDL-cholesterol but also lowered HDL-cholesterol concentrations. Current thinking is that higher LDL concentrations but lower HDL confer a greater risk for coronary heart disease.

The main weakness of the study, like many others, is that it gave little information about the diets being consumed prior to the start of the experiment or the plasma lipoprotein profiles of the subjects while they were consuming their pre-study diets. The lipid profiles on the experimental diets are not compared against the pre-study values: the concentrations resulting from the consumption of the 'saturated' and the 'trans' diets are compared with the values on the 'cis' diet. These authors have previously demonstrated that oleic acid has a cholesterol-lowering effect (see later section) so that it is to be expected that the effects of fatty acids with a cholesterol-raising tendency may be exaggerated if oleic acid is used as a reference. The limited data on pre-study plasma lipid concentrations presented by the authors indicate that total cholesterol changed little as a result of the *trans* diet while the saturated diet raised plasma lipids only slightly.

In trying to assess the relevance of these results to practical nutrition and health we need to place the intake of *trans* acids in this experiment (14 g/day) in context with those of the normal Dutch population (17 g/day) and those in the UK (5–7 g/day). These results suggest that it may be prudent for patients at increased risk of atherosclerosis to avoid a high intake of *trans* acids but do not alter the conclusions of the British Nutrition Foundation (BNF) Task Force

on *Trans* Fatty Acids (23) that current levels of intake in the UK do not pose a problem for most of the population.

The Dutch study of Mensink and Katan (29) on the effects of *trans* fatty acids on human plasma lipoproteins was criticized in the USA because it employed a high intake of *trans* fatty acids (11% of total dietary energy). In a US study, Judd and colleagues (32) investigated the effects of levels of *trans* of 3.8 and 6.6% of dietary energy on plasma lipoproteins. Taking these results together with other data, there is a dose related effect of C_{18} *trans* monoenes on plasma LDL, at least in the range 3–11% of dietary energy. More information is required on the physiological effects of *trans* fatty acids from hydrogenated fish oils.

In summary, dietary C_{18} fatty acids with a single *trans* double bond (which contribute the majority of *trans* fatty acids in products derived from ruminants and the industrial hydrogenation of vegetable oils) increase the concentration of LDL-cholesterol in the blood when compared with a similar amount of dietary C_{18} *cis*-monounsaturated fatty acid (oleic acid, normally the most abundant dietary fatty acid). The effect is directly related to the proportion of *trans* fatty acids in the dietary fat and is a little lower than that of the C_{14} and C_{16} saturated fatty acids. Unlike the saturated fatty acids, however, which tend to raise HDL-cholesterol slightly, the *trans* fatty acids significantly lower HDL-cholesterol.

Since the current consensus of scientific opinion is that raised LDL-cholesterol and lowered HDL-cholesterol independently increase the risk of CHD, the current view is that consumption of high levels of *trans* fatty acids should increase CHD risk, at least as judged by their influence on plasma lipoprotein concentrations.

However, at the level of 2% of dietary energy common in many European countries, it is unlikely that *trans* fatty acids contribute more than a small part of CHD risk.

There is also evidence from at least two studies that *trans* acids in the diet lead to increases in the concentration of plasma Lp(a) especially in those individuals who already have high baseline plasma concentrations of this lipoprotein (24). There is no firm evidence about the influence of *trans* fatty acids on thrombosis — the formation of a clot which, if it occludes a major artery supplying the heart, leads to a heart attack. A brief summary of current knowledge of the influence of *trans* fatty acids consumption on cardiovascular disease risk can be found in Chapter 2.

Conclusion

Where guidelines on *trans* intakes have been issued, the advice has usually been to avoid increasing the consumption of *trans* fatty acids. The food industry is already responding by reducing the *trans* content of many products. Average intakes are likely to fall and the possibility that individuals might consume amounts that are harmful, although real, is diminishing.

Cis-Monounsaturated fatty acids: are they really 'neutral'?

Background

The 'Keys equation', discussed in an earlier section does not contain a term for monounsaturated fatty acids (MUFA). Neither Keys' (5,8) nor Hegsted's (7) groups could detect a significant effect of MUFA in either raising or lowering plasma cholesterol. For the next two decades, MUFA were ignored as being uninteresting and 'neutral' in respect of cholesterolaemia but recently, this assumption has been re-evaluated.

The rekindling of interest in monounsaturates

Until the publication of a paper by Mattson and Grundy in the mid-1980s (20), those interested in the dietary control of blood lipids had concentrated on the cholesterol lowering effects of linoleic acid. By the early 1990s, the overall view was that, if the aim was to control the blood lipoprotein pattern, MUFA had several advantages over linoleic acid. High intakes of oleic acid were equally compatible with maintenance of low total and LDL-cholesterol (see below); they do not lower HDL-cholesterol; unlike linoleic acid, they are not susceptible to oxidation (see Chapter 3) and, less scientifically, they seemed to typify people's idea of 'The Mediterranean diet'. The latter was becoming a fashionable concept and was seen to be associated with good health and a low risk of coronary heart disease.

Technical developments in monounsaturated oils

The nutritional interest in MUFA rapidly led to two main types of technical developments. Not so long ago, the only widely used high-oleic oil was olive oil. Several plant species that have naturally produced oils rich in linoleic acid have now been bred to produce oils rich in oleic acid. Examples are high-oleic safflower and sunflower. Many years earlier, low-erucic rape had provided us with what is essentially a high-oleic oil, but the purpose of this

introduction was specifically to breed out the erucic acid: its replacement with oleic was secondary and not the original purpose of the exercise. A second development has been the incorporation of these new oils into a range of monounsaturated spreads and cooking oils that are promoted as having a healthy image.

The introduction of these oils and spreads has in turn re-stimulated the interest in exploring their nutritional properties. Now that they are readily available from supermarkets, they can be incorporated into everyday diets for free-living subjects in research trials, whereas many of the earlier experiments used special commercial preparations of high-oleic oils in metabolic experiments.

What do we mean by monounsaturates?

In practical terms, when we speak of dietary *cis*-MUFA, we really mean only oleic acid, *cis*-9-octadecenoic acid. Small amounts of MUFA are contributed by palmitoleic acid and the C_{20} and C_{22} monoenes in fish oils but these are quantitatively trivial compared with oleic acid. This is not to say that they may not have important biological activities, which may be different in some respects from those of oleic acid, but in this chapter the reader can assume that 'MUFA' means 'oleic acid'.

It is important to recognize that oleic acid is not essential in the diet. As far as we know, human tissues have active 9-desaturase activity and the body can synthesize abundant oleic acid. Indeed, when we analyse tissues, especially the adipose tissue stores, we find that oleic acid predominates.

Influence on blood lipids

Mattson and Grundy (20) compared the effects of a 'liquid formula diet', comprising either predominantly SFA, MUFA or PUFA on plasma lipids and lipoproteins. The diet containing predominantly oleic acid was as effective in lowering LDL-cholesterol as linoleic acid in subjects with normal triacylglycerol concentrations but with a suggestion that oleic acid did not reduce the concentration of HDL-cholesterol as did linoleic acid. Neither type of unsaturated fatty acid had a strong effect on lipoproteins in patients with elevated triacylglycerol concentrations.

There were several shortcomings of this study. There were only 20 subjects, of whom only 12 had normal triacylglycerol concentrations. The power of the study to detect significant differences was, therefore, limited. Although it was a clever idea to compare two different variants of safflower oil,

one rich in oleic acid, the other in linoleic acid, the results are not clear-cut since the 'monounsaturated oil' contained 17% linoleic acid, which might have been all that was required to exert a cholesterol-lowering effect. Finally, the relevance of liquid formula diets to normal life is difficult to assess.

Mensink and Katan (21) overcame some of the above criticisms by comparing the effects of natural diets in which the fat component contained either predominantly SFA, MUFA or PUFA (**Table 1.1**), given to healthy young people under well controlled conditions. Thirty-one women and 27 men received a diet rich in SFA for 17 days; for the next 36 days, 29 subjects were given a diet in which MUFA predominated (olive oil/safflower oil mixture) while the remaining 29 received a diet in which *n*–6 PUFA predominated (sunflower oil).

Table 1.1. Fatty acid composition of the diets in Mensink and Katan's study (21).

Component	Type of diet		
	SFA	MUFA	PUFA
Protein (%E)	13.1	13.4	13.1
Fat (%E)	36.7	37.4	37.6
SFA (%E)	19.3	12.9	12.6
MUFA (%E)	11.5	15.1	10.8
PUFA (%E)]	4.6	7.9	12.7
Carbohydrate (%E)	49.1	47.8	48.5
Cholesterol (mg/MJ)	33.4	35.8	35.3

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = polyunsaturated fatty acids; %E = as percentage of energy.

Plasma LDL-cholesterol decreased on average 18% in subjects on the MUFA diet and 13% in those on the PUFA diet. There were no significant changes in HDL-cholesterol in either men or women.

Designing dietary experiments with natural ingredients with the aim of comparing the effects of different fatty acids is fraught with difficulty. Often, research workers have compared animal and vegetable fats, forgetting that the former contain cholesterol while the latter do not. Cholesterol may be a confounding factor. These authors have avoided this particular pitfall but nevertheless, different oils, as well as having different fatty acid compositions,

contain different types and amounts of minor non-saponifiable components, which may have powerful effects on blood lipids despite their small concentration. For example, tocotrienols present in some vegetable oils, are inhibitors of the enzyme HMG-CoA reductase, the rate-limiting step in cholesterol biosynthesis. There is hardly a publication in the literature that has managed to avoid this or a similar problem.

An example of the difficulty of interpreting results because several variables, rather than just one, have been changed is provided by reference (33). The authors used a mixture of natural fats to compare the effects of SFA against MUFA and high fat content against low fat content on plasma lipoproteins in ten elderly men. Diets 1 and 2 contained 40% of their energy as fat distributed as follows — diet 1: SFA, 19; MUFA, 15; PUFA; 6, and diet 2: SFA, 7; MUFA, 27; PUFA, 6. Diet 3 had only 20% of its calories as fat and a ratio of SFA:MUFA:PUFA of 7:7:6. Compared with the 'high saturates' diet both the 'high MUFA' and 'low fat' diets reduced total cholesterol by 13.5% and LDL-cholesterol by 19%. The low-fat diet resulted in a significant reduction in HDL compared with either of the high-fat diets. A good dietary study should endeavour to eliminate as many confounding variables as possible. The main flaws of this study were the huge differences between diet 1 and diets 2 and 3 in both cholesterol content (900 compared with 200 mg/day) and in P/S ratio (0.3 compared with 0.86). As the authors say in their discussion, the cholesterol-lowering effects of the high MUFA and low-fat diets could simply be due to differences in cholesterol intake. They failed to discuss, however, the differences in P/S ratio which were also huge. It is impossible, therefore, to draw firm conclusions about the specific effects of MUFA from this and several other papers.

The general confusion as to whether modification of blood lipids is best served by reducing the total fat content of the diet or by simply changing the composition of the fatty acids has clearly encouraged researchers interested in MUFA to compare high-fat diets rich in MUFA with low-fat diets as well as with high-fat diets rich in PUFA or SFA. The work of Grundy, cited above, is an example. Two rather better controlled experiments (34,35) came to a similar conclusion, namely that a high-fat diet that contained predominantly MUFA was as effective as a low-fat diet in reducing total cholesterol and resulted in lower triacylglycerol concentration than the low-fat diet. They disagreed, however, on the effects on HDL.

Mensink and Katan (35) found that HDL concentration fell significantly in subjects given the low-fat diet and rose slightly in subjects given MUFA compared with concentrations in the same subjects when they followed their

habitual diet. Baggio *et al.* (34), however, found no such differences in HDL. Both studies, though otherwise well controlled, were unsatisfactory in not giving sufficient data on the subjects' habitual diet and the lipid profiles resulting from that dietary pattern. This is a fault common to most studies, however.

To summarize, dietary lipids containing a high proportion of MUFA maintain relatively low plasma cholesterol and LDL-cholesterol concentrations even when present in the diet as a relatively high proportion of energy. They do not reduce plasma HDL-concentrations nor do they elevate plasma VLDL or total triacylglycerol concentrations (11). Since LDL-cholesterol is now well recognized as an important risk factor for CHD, the significance of maintaining low plasma LDL is clear. There is also a school of thought that holds that a low plasma HDL is a more important risk factor than LDL. Therefore, any strategy that lowered HDL-cholesterol as well as total cholesterol and LDL-cholesterol would have no advantage and might actually do harm. Some studies have shown that high intakes of PUFA may lower HDL-cholesterol as well as total cholesterol and LDL-cholesterol and have advised against high intakes of PUFA (14). However, HDL-lowering by PUFA is encountered only when the ratio of PUFA to saturated fatty acids is well in excess of 1.0, which is well above the intake recommended by the COMA guidelines (reference 36 and Chapters 2 and 4).

A possible advantage of MUFA is that they are much less susceptible to oxidation than PUFA. High intakes of the latter may well raise requirements for vitamin E and other antioxidant nutrients (see Chapter 3).

Many people are resistant to 'being told what to eat' and despite nagging worries about heart disease, do not want to be restricted to a high-PUFA or low-fat cholesterol-lowering diet. Perhaps the most promising aspect of the recent research on MUFA is that it should allow much greater flexibility and range of choice of diets and, therefore, help to make dieting less of a chore and eating more pleasurable.

Until recently, when effects of dietary fatty acids on blood lipids have been investigated, almost all emphasis has been on fasting levels. There are two main approaches. Either the experimental subjects are studied in a laboratory setting under strictly controlled conditions. The diets are formulated to be simple in composition and are often extreme in composition to increase the chance that a highly significant change will result from consumption of the 'test' fatty acid. The alternative is to study subjects eating normal diets in their own homes. Such experiments are more difficult to control than when

conducted under laboratory conditions but have the advantage that they are a better reflection of 'real life' and the results, though perhaps less scientifically rigorous, may be more appropriate for formulating public health policies.

Professor Christine Williams and her colleagues at the Hugh Sinclair Unit of Human Nutrition at Reading University are developing and perfecting techniques for the study of dietary fats and health in the home setting. As part of their strategy, they have developed collaborations with food manufacturers to produce a range of foods enriched in specific fatty acids or fatty acid types. In this way, subjects are provided with 'test' fatty acids in a palatable form and this is likely to enhance their compliance with the dietary protocols over lengthy time periods. In a recent study (37), this team evaluated the cholesterol-lowering efficacy of a diet rich in MUFA compared with a 'control' diet whose fatty acid composition approximated that of the national UK diet.

The 30 middle-aged and 23 young men in the study were apparently healthy but all had a history of heart disease in the family. Diets were given for 8 weeks and contained 38% of energy as fat (close to the current national average). After a so-called 'washout period' of 4–6 weeks, in which the subjects had free choice of diet, they were transferred to the opposite diet for another 8 weeks. The control diet contributed 13% energy as MUFA and 17% as saturates (SFA), whereas for the 'test' diet the contributions were 18% and 10% respectively. The polyunsaturates (PUFA) content was 7% in each. Fat was provided by specially manufactured spreads, ready meals, biscuits, puddings and breads, which apart from their fatty acid compositions were identical for both diets. Mean fasting total and LDL-cholesterol concentrations were significantly lower after the MUFA diet than the control by 0.29 and 0.38 mM respectively. In middle aged men the difference was due to a decrease in LDL-cholesterol after the MUFA diet and no change after the control diet, whereas in young men there was not only a decrease after the MUFA diet but also an increase after the control diet. This probably reflects differences in the composition of their habitual diets.

Another recent interest has been in comparing the metabolic effects of different MUFA-rich oils. Ruiz-Gutierrez and her colleagues (38) compared the influence of diets rich in olive oil or high-oleic sunflower oil on the composition of human VLDL. The content of MUFA in the two oils was similar but their triacylglycerol composition differed. The olive oil contained slightly more SFA and slightly less PUFA than the high-oleic sunflower oil (**Table 1.2**). In both oils, the *sn*–2 position was mainly occupied by oleic acid but there were notable differences between other fatty acids in their occupancy

Table 1.2. Comparisons of lipids in olive and high-oleic sunflower (HOSO) oils.

Lipid	Olive	HOSO
Major fatty acids (% total fatty acids)		
Total saturates	12.4	9.6
18:1*n*–9	77.6	78.5
18:2*n*–6	8.0	11.4
18:3*n*–3	0.8	0.1
Major triacylglycerol molecular species (%total)		
POO	30.5	12.1
OOO	49.9	65.1
OLL	0.3	3.1
Major fatty acids in position *sn*–2 (% total)		
18:1*n*–9	94.1	92.9
18:2*n*–6	4.1	6.8
18:3*n*–3	0.6	ND

Compiled from reference 38. Only the major fatty acids have been listed; P = palmitic; O = oleic; L = linoleic; ND = not detected.

of this position. Thus high-oleic sunflower oil had more linoleic acid in position 2 whereas olive oil had more α-linolenic acid in this position (Table 1.2). The OOO triacylglycerol molecular species predominated in both oils but much more so in high-oleic sunflower oil than in olive oil (Table 1.2). By contrast, there was a much greater proportion of POO in olive oil than in high-oleic sunflower oil (O = oleic; P = palmitic).

Diets containing these oils were given to 22 young people in a crossover design similar to that of Williams and colleagues described above (37). Each oil contributed 40% of total energy. Changes in lipids were compared with those resulting from a control low-fat diet. As might be expected, there were major changes in the molecular species of triacylglycerols present in VLDL after giving the high oleic oils compared with the low-fat control diet. More unexpected were significant differences in triacylglycerol molecular species when comparing the two high-MUFA oils. Interestingly there was a much higher proportion of OOO in the VLDL triacylglycerols after giving olive oil even though this molecular species was present in higher proportion in the high-oleic sunflower oil. PPP was significantly decreased after giving olive oil

but not after high-oleic sunflower oil. Perhaps more importantly, the molecular species containing α-linolenic acid esterified at position 2 was highly enriched after giving olive oil but not after high-oleic sunflower oil.

The authors suggested that the differences in composition of VLDL might be of major importance in helping to explain the beneficial effects of olive oil in reducing atherogenic risk but did not elaborate a mechanism by which this might occur. A major difficulty in interpreting the results of comparisons between two oils is that the oils differ not just in fatty acid or triacylglycerol composition but also in a number of minor components: tocols, tocotrienols, phytosterols, ubiquinones etc. Whereas these are present in small quantities compared with fatty acids, they may nevertheless exert biologically significant effects.

Practical implications

What are likely to be the implications of this latest research into dietary MUFA on public health and nutrition policy? There has been vigorous debate (39) over the past year or so on whether the 'healthiest' diets are those low in fat (and therefore, by definition, rich in carbohydrates) or those relatively high in fat but in which oleic acid predominates. Arguments for the low-fat diet are that it is compatible with low total and LDL-cholesterol concentrations in the blood and it is less conducive to obesity. Proponents of the higher fat MUFA diet express concern about the tendency of low-fat diets to reduce HDL-cholesterol. High MUFA diets would maintain a high HDL-cholesterol concentration and, it is argued, most people would find them more palatable. Other research suggests that a possible disadvantage of high-fat high-MUFA diets may be an increased tendency for factor VII levels to be raised (see Chapter 2). This discussion illustrates the need to look broadly at the effects of dietary fats on many different risk factors, not simply their influence on blood lipoproteins.

Dietary fat, blood lipids and health. Which is more important: total amount of fat eaten or its composition?

Guidelines for a healthy diet universally recommend a reduction in total fat intake from the current 40% or more of dietary energy to 35% or less. For those who are overweight or who have difficulty in maintaining weight, this may be sensible advice for reasons that I have outlined in Chapter 5. Since the advice is frequently given in the context of recommendations aimed at reducing blood cholesterol and the risk of coronary heart disease, the implication

is that reducing fat consumption will automatically result in a reduction in blood lipids. What is the evidence for this assumption?

Given the large volume of research published on the subject of diet and blood lipids since the 1950s (reviewed above), there have been surprisingly few studies on the changes in blood lipids in response to changes in the level of fat in the diet. A proper dose-response relationship seems never to have been established. In the few studies that investigated blood lipid and lipoprotein concentrations at different levels of fat intake, the composition of the fat in the different dietary groups was almost invariably different, thereby precluding a clear answer to the question of whether level or composition of the fat was the more important factor. This criticism applies to studies conducted by established experts several decades ago (9), as well as some very recent studies (40).

Content or composition?

Recognizing this problem, Nelson and colleagues designed an experiment in which only the level of fat differed, not the fatty acid composition (41). These authors gave 11 healthy young men (age 20–35) diets containing either 22% or 39% of their energy as fat for 50 days and compared the blood lipid and lipoprotein profiles. The study was well designed. All subjects were initially given the same high-fat diet for 20 days. This ensured that as far as possible they all started off with similar dietary status. Six were then given the low-fat diet for 50 days and the other 5 remained on the high-fat diet for the same period. The groups were then 'crossed over' for the remaining 50 days of the study, so that each subject received both diets during the experiment and essentially acted as his own control.

Details of fat intakes are given in **Table 1.3**. At each level of fat, the fatty acid composition was exactly the same. Only natural foods were used: there were no supplements. For the duration of the study all subjects were confined to a metabolic suite so that every aspect of the study could be monitored and controlled. There were no opportunities to consume foods other than those provided and all subjects were required to consume all food that was given.

It is of course difficult to ensure that fatty acid intakes on two dietary regimens are precisely the same. The authors managed this remarkably well. There appeared to have been a 2% higher proportion of linoleic acid in the high-fat than in the low-fat diet, which was reflected in the higher ratio of polyunsaturated to saturated fatty acids (Table 1.3) but this was not statistically significant. The intake of arachidonic acid in the high-fat group was statistically higher but the amounts involved were very small indeed (0.2% against 0.4%

Table 1.3. Fat and fatty acid intakes in the study of Nelson *et al.* (41).

Nutrients	Low-fat	High-fat
Macronutrients (% energy)		
Protein	16	16
Fat	22	39
Carbohydrate	62	45
Cholesterol (mg/day)	360	360
Fatty acids (% energy)		
Saturated	6.4	10.6
Monounsaturated	9.2	15.5
Polyunsaturated	6.6	12.6
Polyunsaturates/saturates	1.0	1.2

of total fatty acids). The effectiveness of the dietary control was reflected in the similarity of fatty acid compositions of the plasma, red blood cell and platelet lipids in the two groups. Of the major fatty acids, only linoleic acid was slightly higher in the high-fat group in line with its slightly higher proportion in the diet.

Blood lipids

The major blood lipid and lipoprotein classes in the two dietary groups are illustrated in **Table 1.4**. None of the dietary group comparisons was significant in respect of the cholesterol fractions. The blood triacylglycerol concentration was, however, significantly higher after consumption of the low-fat diet than after the high-fat diet by about 25 mg/dl ($P<0.002$).

If the Keys (5) or Hegsted (9) equations had been applied to this study, it would have been predicted that blood total cholesterol would have risen from 173 mg/dl on the low-fat diet to about 196 mg/dl on the high-fat diet. The authors discussed several reasons for the discrepancy. The Hegsted and Keys studies used many more subjects than Nelson's. However, Nelson and colleagues dismissed the idea that 'statistical power' was a problem on the grounds that they traded large numbers for much more careful control of experimental conditions, which is certainly true. They also rejected the notion that the discrepancy arose because the mean blood cholesterol concentration in this study was much lower than in the original Keys and Hegsted studies.

Table 1.4. Blood lipids and lipoproteins in the study of Nelson *et al.* (41) (mean mg/dl, n = 11).

Time period	Diet	TC	TAG	HDL-C	LDL-C
At entry	normal	176.3	85.8	46.3	112.8
After 20 days	HF	172.5	75.3	44.8	112.6
After 50 days	LF	173.2	91.5[1]	40.5	114.5
After 50 days	HF	176.9	66.4	43.2	119.5

TC = total cholesterol; TAG = triacylglycerol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; HF = high-fat; LF= low-fat; [1]P <0.002 comparing day 50 with day 20.

Whereas this is so, the subjects in Nelson's study with the highest cholesterol values did not show any tendency to change in response to fat level more than those with average or low cholesterol levels.

It is well established that people respond differently to dietary fatty acids. Thus, some people experience a large rise in cholesterol when they consume butter, whereas others show no response or even a fall; likewise the same individuals demonstrate a large fall in response to consumption of corn oil, or no response or even a rise (42). It could also be argued that the Nelson study, by chance, included only people who were non-responders to dietary fat. Despite the small number studied, this seems unlikely because they were randomly selected from the general population.

Therefore, the most reasonable explanation is that the component of blood cholesterol concentration that is sensitive to dietary fat (other dietary and non-dietary factors also influence blood total cholesterol) depends almost entirely on the composition of the dietary fat, not on the proportion of energy supplied as fat. It has to be said that the authors chose to study a dietary fat composition with a very high ratio of polyunsaturates to saturates (1.0). This is much higher than normally experienced in Western countries and somewhat higher than anticipated by current guidelines for healthy eating (36). It would be interesting to see the work repeated with a more realistic polyunsaturates/saturates ratio to establish that the same results would be obtained.

Misleading messages

One clear lesson to be learned from this study is that health education messages are misleading when they suggest significant improvements in blood lipid profiles can be obtained simply by fat reduction without reference to the type of fat. This does not mean of course that many people could not benefit

from total fat reduction if they have a weight problem, but that is a different issue.

There could be an interesting spin-off from these results for the design of human nutrition studies. An example is in regard to the controversy about the effect of dietary *trans* fatty acids on blood lipid concentrations. A major problem is that, if the total fat in the diet is to be maintained constant (as has always been assumed to be necessary) then to obtain different levels of dietary *trans* fatty acids, they have to be exchanged for some other fatty acid — usually saturated or *cis*- monounsaturates. Under these conditions, it is not clear whether any observed effect is due to a change in *trans* fatty acids or to changes in the fatty acids with which they have been exchanged. If total fat can now be regarded as being irrelevant, then an appropriate experimental design would be to maintain all dietary constituents constant and merely add increasing amounts of *trans* fatty acids to the diet.

Changes in blood lipid after a meal: significance for health

The use of fasting blood samples

At the beginning of this chapter I described the results of a 'meta-analysis': a quantitative assessment of studies of the influence of dietary fats on blood lipids and lipoproteins. A consistent feature of such investigations has been that the subjects' blood samples were taken after an overnight fast. This was done routinely on the grounds that the fats present in a recent meal would have influenced circulating lipids in complex and diverse ways so that the results of different investigations would not have been comparable. As a result of this virtually unchallenged approach, concepts have developed about the prediction of ischaemic heart disease risk solely from knowledge of fasting patterns of plasma lipoproteins.

In recent years, thinking on this subject has been undergoing considerable change, with the result that many research workers in this field now emphasize the importance of postprandial changes in lipoprotein profiles (43,44). There are several reasons for this change in approach. Firstly, most of us are in a postprandial state for the greater part of each 24 hour period (**Figure 1.3A**). In developed countries, the fasting state is irrelevant in relation to long-term health. Secondly, the way in which lipoprotein particles that carry fat in the bloodstream are processed immediately after a meal is now thought to influence the two main aspects of the natural history of heart disease: atherosclerosis and thrombosis (references 43–45 and Chapter 2). It is important therefore to understand postprandial metabolism of lipids in more detail.

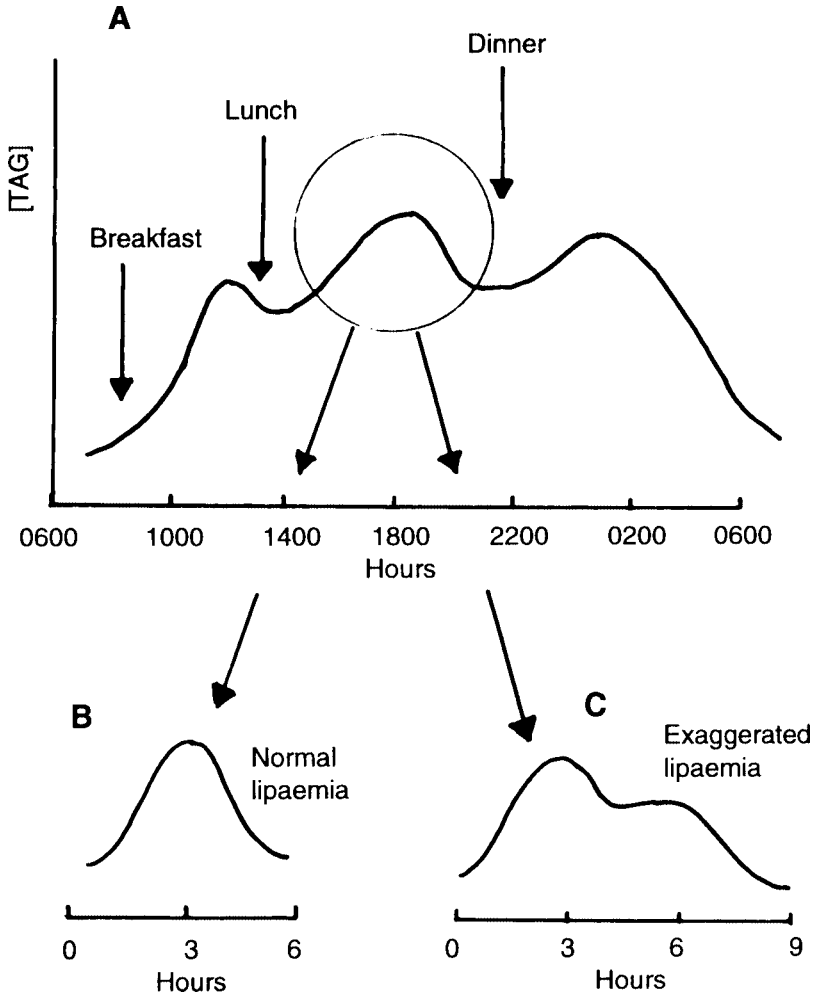

Figure 1.3. Postprandial changes in plasma triacylglycerol (TAG) levels.

Normal metabolic processing of fats after a meal

Several lipid-rich particles involved in transporting fats in the blood (see also first section of this chapter) are key players in postprandial lipid metabolism (**Figure 1.4**).

- Chylomicrons carry TAG that have been resynthesized in the intestinal cells immediately after fat absorption. They have a broad range of particle sizes and are large enough to cause an opalescent appearance of plasma soon after a fatty meal. The particles are mainly composed of TAG stabilized by a surface coat of phospholipids, cholesterol and apo-B_{48}. Normally, their concentration in plasma rises quickly after a meal, reaching a peak (lipaemia) two to three hours after the meal, which then subsides (Figure 1.3).
- Chylomicron remnants are generated from chylomicrons as a result of the lipolysis of triacylglycerols by the enzyme lipoprotein lipase. This activity occurs in many tissues but in well nourished people most postprandial processing of chylomicrons occurs at the surface of the adipose tissue. Lipolysis is incomplete. As the particles become smaller due to loss of TAG, the proportion of phospholipids, cholesterol, and protein increases and the particles are less susceptible to enzymic attack. Remnants are removed from plasma in two ways. In healthy people, the preferred route is by interaction with receptors on the surface of liver cells followed by further degradation in the liver. Alternatively, they can be processed by interaction with high density lipoproteins (see below and Figure 1.4).
- Non-esterified fatty acids are generated during the lipolysis of chylomicron TAG by lipoprotein lipase. A proportion enters the adipose tissue to be re-synthesized into TAG for storage, whereas the remainder is transported in the plasma attached to the protein albumin.
- High-density lipoproteins (HDL) show much less dramatic, yet important, fluctuations in concentration than chylomicrons. They exist in several forms and are in the business of acting as vehicles for lipid exchange. Primary or 'nascent' HDL are produced by the liver and pick up cholesterol from peripheral tissues to become cholesterol-rich HDL. In the plasma, fatty acids are transferred from phosphatidylcholine to HDL-cholesterol to form HDL that is rich in cholesteryl ester. In turn, the cholesteryl esters can be exchanged for TAG to form larger TAG-rich HDL particles, a process termed 'neutral lipid exchange'.
- Very-low-density lipoproteins (VLDL). Whereas the chylomicrons appear in the circulation immediately after absorption of fat from a meal, VLDL come later into the picture. They carry lipid that has been synthesized in the liver, mainly from circulating non-esterified fatty acids.

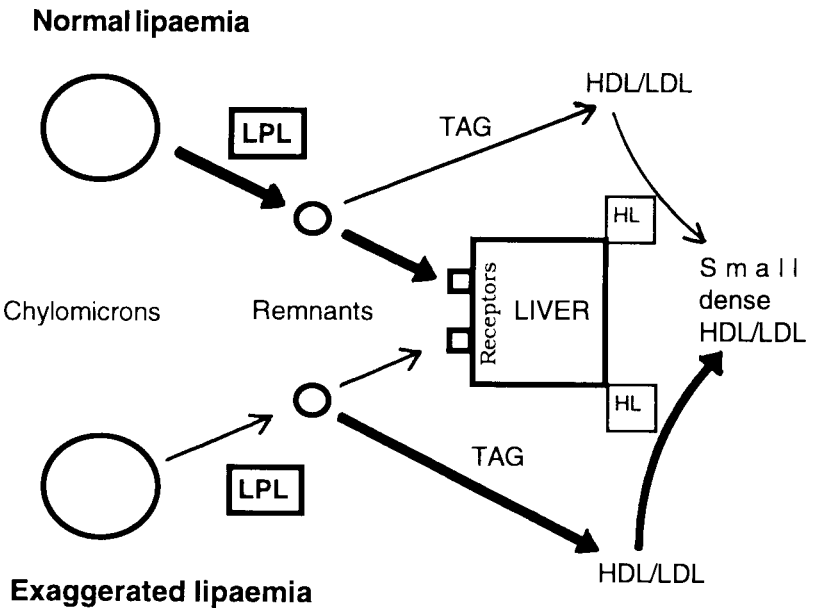

Figure 1.4. Postprandial metabolism of lipoproteins. Thick lines indicate active pathways; thin lines indicate less active pathways. LPL: lipoprotein lipase; TAG: triacylglycerol; HL: hepatic lipase; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Exaggerated lipaemia

I have described the usual comparatively rapid processing of fats after a meal (normal lipaemia, **Figure 1.3B**). It is not uncommon to find an exaggerated lipaemic response (43) in which the postprandially raised TAG concentration persists for much longer (**Figure 1.3C**). An important reason for TAG persistence may be a reduced activity of lipoprotein lipase resulting in slower clearance of TAG-rich particles. Such 'TAG intolerance' is frequently observed in people with maturity onset diabetes (so-called non-insulin dependent, or type 2 diabetes). In this type of diabetes, insulin production is not a problem but lipoprotein lipase (whose activity is controlled by insulin) is less able to respond to insulin under these conditions and so the removal of chylomicrons is sluggish. Lipaemia is further exaggerated because the partially hydrolysed remnant particles are poorly taken up by the receptors on the surface of the liver cells Their consequent persistence in the plasma provides greater opportunity for 'neutral lipid exchange' referred to above.

Exaggerated lipaemia, therefore, involves the appearance in the plasma of new TAG-enriched HDL particles and cholesterol-enriched remnant particles. The TAG in the HDL particles are rapidly hydrolysed by a lipase present in the liver (hepatic lipase) to form small dense HDL particles, which in turn are rapidly degraded, thereby reducing the total HDL concentration in the plasma. Similar hydrolysis of remnant TAG leads to the formation of small dense LDL.

Postprandial lipoprotein particles and ischaemic heart disease risk

In 1979, Zilversmit (cited in reference 43) proposed that chylomicron remnants have the potential to cause cholesterol deposition in arterial walls. This hypothesis has been extended into the present concept of the 'atherogenic lipoprotein phenotype', which integrates all that we known about postprandial lipid changes described above (46). The main features of the atherogenic lipoprotein phenotype are shown in **Table 1.5**.

Table 1.5. Some characteristics of the atherogenic lipoprotein phenotype (LDL = low-density lipoprotein; HDL = high-density lipoprotein).

Exaggerated postprandial lipaemia
Raised fasting plasma triacylglycerols (>1.5mM)
Presence of small dense LDL
Reduced fasting HDL-cholesterol (<1mM)
Reduced proportion of large HDL
Presence of cholesteryl ester-rich remnant particles
Insulin resistance

Studies *in vitro* have demonstrated that macrophages (white blood cells, among whose functions is to consume 'foreign bodies') have receptors for remnant particles and are subsequently transformed into the cholesterol-rich foam cells characteristic of atherosclerotic plaques. In prospective epidemiological studies, men at the top of the distribution of plasma TAG concentration and at the bottom of the distribution of HDL concentration were 2–3 times more likely to develop ischaemic heart disease (44). Several studies have shown that the extent of atherosclerosis is associated with prolonged exposure to TAG-rich lipoproteins (44). The present concept is that a major

predisposing factor to arterial disease is an impaired TAG tolerance as reflected in a metabolism that fails to remove TAG rapidly from the circulation after the consumption of a fatty meal (43).

Genetic basis of the atherogenic lipoprotein phenotype

Research suggests that the atherogenic lipoprotein phenotype has a strong genetic basis (46). Of particular importance are genes that code for various lipase enzymes: lipoprotein lipase and hepatic lipase, described above, and the hormone sensitive lipase in adipose tissue that is involved in the mobilization of fatty acids from the fat stores. Equally important are those genes that code for various apoproteins, especially apoprotein E (apoE), which is important in the recognition of several lipoproteins by their receptors on cell surfaces. There are several variants of the apoE gene in the population and one in particular seems to confer increased susceptibility to ischaemic heart disease. Variations in the genes for different enzymes and receptors influence the efficiency with which various lipid-rich particles are removed from the circulation. However, it is not enough to ascribe a certain metabolic profile as being 'genetically determined'. A driving force in current nutrition research is the concept that an individual's metabolism is the result of interactions between genes and environmental (nutritional) factors: each individual's metabolic response to food is determined by his or her genetic background. One person's response to a fat-rich meal may therefore be rapid clearance and reversion to a background postprandial state (Figure 1.3B); another's will be a massive lipaemia that only slowly reverts to a 'normal' postprandial state (Figure 1.3C). Most importantly, interactions between nutrients and genes are not simply passive in the sense that a given gene results in a constant metabolic capacity. Interactions can be active in the sense that nutrients themselves can affect the way in which genes are expressed and therefore the level of metabolic activity that results.

Influence of dietary fatty acids on postprandial lipid changes

There are surprisingly few published studies on the effect of meal composition on immediate postprandial metabolism. Even these are difficult to compare because different fats and oils and different levels of fat were given in the experiments. It can be concluded that similar postprandial lipaemic responses are observed when meals containing predominantly saturated, monounsaturated or *n*–6 PUFA are compared but that a meal containing a significant quantity of *n*–3 PUFA has the effect of attenuating postprandial lipaemia (43).

In addition to acute effects of fatty acids in a single meal, there is also evidence that habitual dietary fat intake can influence postprandial changes in any one meal. Again, it was found that when the background diet had included relatively high intakes of *n*–3 PUFA for extended periods, triacylglycerol tolerance to high-fat intakes in a single meal was much improved (43). It would appear that diets that regularly contain about 2 g of long-chain *n*–3 PUFA from fish oils daily result in reduced postprandial lipaemia of about 30%.

In practical terms, this level of *n*–3 PUFA intake is condiderably more than that in most human diets. In the UK, for example, average intakes are only 0.2 g daily of long-chain *n*–3 PUFA. Intakes of α-linolenic acid are higher (about 1.3 g/day) but the effect of α-linolenic acid on postprandial lipaemia is unknown. The appropriate levels of intake, the proportion of precursor to long-chain PUFA and of *n*–6 to *n*–3 PUFA are important matters for future research in regard to the question of triacylglycerol tolerance, which is increasingly being regarded as a key aspect of lipid nutrition.

Endpiece

Perceptive readers of *Lipid Technology* will be aware that I have frequently expressed scepticism about a major role for dietary lipids in the development of ischaemic heart disease. That remains my position, even though the work on postprandial metabolism undoubtedly provides a more rational framework for understanding nutritional influences on existing cardiovascular disease than more conventional approaches. One factor that has not been addressed here is the interaction between physical activity and nutrition and this has been both insufficiently researched and acknowledged. The tendency towards triacylglycerol intolerance would become less significant if activity levels in the general population were to rise even modestly.

References

1. Gurr, M.I. and Harwood, J.L. (1991) Lipid Biochemistry: An Introduction, 4th ed, Chapman and Hall, London.
2. Frazer, A.C. *et al*. (1949) *British Journal of Nutrition*, 3, 358–363.
3. Lehner, R. and Kuksis, A. (1996) *Progress in Lipid Research*, 35, 169–201.
4. Field, F.J. and Mathur, S.N. (1995) *Progress in Lipid Research*, 34, 185–198.
5. Keys, A. *et al*. (1957) *Lancet*, ii, 959–966.
6. Howell, W.H. *et al*. (1997) *American Journal of Clinical Nutrition*, 65, 1747–1764.
7. Hegsted, D.M. *et al*. (1993) *American Journal of Clinical Nutrition*, 57, 875–883.
8. Keys, A. *et al*. (1965) *Metabolism*, 14, 776–787.
9. Hegsted, D.M. *et al*. (1965) *American Journal of Clinical Nutrition*, 17, 281–295.

10. McNamara, D.J. *et al*. (1987) *Journal of Clinical Investigation*, 79, 1729–1739.
11. Grundy, S.M. and Denke, M.A. (1990) *Journal of Lipid Research*, 31, 1149–1172.
12. Bonanome, A. and Grundy, S.M. (1988) *New England Journal of Medicine*, 318, 1244–1248.
13. Hayes, K.C. *et al*. (1991) *American Journal of Clinical Nutrition*, 53, 491–498.
14. Gurr, M.I. *et al.* (1989) *Nutrition Research Reviews*, 2, 63–86.
15. Tamamoto, I. *et al.* (1971) *Atherosclerosis*, 13, 171–184.
16. McGandy, R.B. *et al.* (1970) *American Journal of Clinical Nutrition*, 23, 1288–1298.
17. Cristophe, A. *et al.* (1978) *Archives Internationales de Physiologie et de Biochimie*, 86, 413–415.
18. Illingworth, D.R. *et al*. (1981) *Arteriosclerosis*, 1, 380A.
19. Hayes, K.C. and Khosla, P. (1992) *FASEB J.*, 6, 2600–2607.
20. Mattson, F.H. and Grundy, S.M. (1985) *Journal of Lipid Research*, 26, 194–202.
21. Mensink, R.P. and Katan, M.B. (1989) *New England Journal of Medicine*, 321, 436–441.
22. UK Department of Health and Social Security (1984). Diet and Cardiovascular Disease. Report on Health and Social Subjects 28. Committee on Medical Aspects of Food Policy. London: Her Majesty's Stationery Office.
23. British Nutrition Foundation (1987). Report of the Task Force on *Trans* Fatty Acids. London: British Nutrition Foundation.
24. British Nutrition Foundation (1995) Report of the Task Force on *Trans* Fatty Acids. London: British Nutrition Foundation.
25. Gurr, M.I. (1996) *Nutrition Research Reviews*, 9, 259–279.
26. Danish Nutrition Council (1995) *Clinical Science*, 88, 375–392.
27. Kris–Etherton, P.M. *et al*. (1995) *American Journal of Clinical Nutrition*, 62, 655S–708S.
28. UK Ministry of Agriculture Fisheries and Foods (1994). The Dietary and Nutritional Survey of British Adults — Further Analysis. London: Her Majesty's Stationery Office.
29. Mensink, R. and Katan, M.B. (1990) *New England Journal of Medicine*, 323, 439–445.
30. Hwang, D.H. and Kinsella, J.E. (1979) *Prostaglandins*, 17, 543–558.
31. Zevenbergen, J.L. and Haddeman, E. (1989) *Lipids*, 24, 555–563.
32. Judd, J.T. *et al.* (1994) *American Journal of Clinical Nutrition*, 59, 861–868.
33. Grundy, S.M. *et al.* (1988) *American Journal of Clinical Nutrition*, 47, 965–969.
34. Baggio, G. *et al.* (1988) *American Journal of Clinical Nutrition*, 47, 960–964.
35. Mensink, R.P. and Katan, M.B. (1987) *Lancet*, 2, 122–125.
36. Department of Health (1994). Nutritional Aspects of Cardiovascular Disease. Report on Health and Social Subjects, 46. Her Majesty's Stationery Office, London, UK.
37. Willams, C.M. *et al.* (1999) *British Journal of Nutrition*, 81, 439–446.
38. Ruiz–Gutierrez, V. *et al.* (1998) *Journal of Nutrition*, 128, 570–576.
39. Connor, W.E. *et al.* (1997) *New England Journal of Medicine*, 337, 562–566.
40. Nestel, P.J. *et al.* (1995) *American Journal of Clinical Nutrition*. 62, 950–955.

41. Nelson, G.J. *et al*. (1995) *Lipids*, 30, 969–976.
42. Kris–Etherton, P.M. *et al.* (1993) *Metabolism*, 42, 121–129.
43. Williams, C.M. (1997) *Proceedings of the Nutrition Society*, 56, 679–692.
44. Sethi, S. *et al.* (1993) *Nutrition Research Reviews*, 6, 161–183.
45. Vorster, H.H. *et al.* (1997) *Nutrition Research Reviews*, 10, 115–135.
46. Griffin, B.A. and Zampelas, A. (1995)Nutrition Research Reviews, 8, 1–26.

Chapter 2

Dietary Fats and Cardiovascular Disease

This chapter examines the scientific basis for the view that restriction of consumption of saturated fatty acids is a primary requirement in a strategy to reduce or prevent heart disease and for the opposing view that such a strategy is largely irrelevant.

The 'lipid hypothesis' is outlined and the evidence supporting it is briefly discussed. This is the concept that over-consumption of saturated fatty acids results in an elevated concentration of cholesterol in the blood, which in turn increases the risk of coronary heart disease mainly by causing severe narrowing of arteries supplying blood to the heart. The scientific evidence comes mainly from experimental studies with animals, epidemiological studies in different human populations and human intervention trials in which the fat component of the diet was modified considerably.

Much of the chapter is devoted to a systematic and critical examination of the evidence. Firstly, fat consumption is examined in relation to the prevalence of coronary heart disease as assessed from research published worldwide. This section considers information from prospective and case-control studies and also examines communities in the developing world that have traditionally eaten diets rich in saturated fatty acids. It is concluded that there is little association between dietary saturated fatty acids and coronary heart disease.

Next, changes in coronary heart disease (CHD) mortality in different countries worldwide during the 20th Century are outlined and compared with changes in consumption of total fats and different fatty acids. There have been rises, peaks and declines in CHD in most developed countries during this century and, more recently, rises in developing countries. Although there have also been changes in fat consumption, the timing of these changes cannot explain the disease changes. Although in small scale well supervised experiments, significant reductions in blood cholesterol can be obtained by

modest changes in the dietary fat composition, the results of large scale community studies reveal that quite extreme diets are needed to achieve worthwhile cholesterol lowering. Even then, the demonstrable rewards in terms of reductions in non-fatal coronary events or CHD deaths are modest or non-existent and there is little evidence for any overall saving of lives.

A recent epidemiological study has revealed that in the USA, deaths from coronary heart disease steadily declined in the period 1987–94, whereas the number of new cases identified per year did not change. Since what have been assumed to be the major heart disease risk factors (high blood cholesterol, high blood pressure and smoking) all declined significantly before and during the same period, the importance of these 'risk factors' in causing the disease would seem to have been overstated.

Coronary heart disease and stroke are two types of blood vessel disease, the first affecting the heart, the other the brain. Several 'risk factors' including high blood pressure, smoking and diabetes are common to both but a high blood cholesterol concentration, although a recognized risk factor for heart disease is less obviously linked to stroke. Despite this, it has been almost universally assumed that high intakes of total fat and of saturated fatty acids are associated with stroke risk and that reduction of these dietary constituents will self-evidently be helpful in preventing stroke.

Several publications have presented evidence that this is not so. The most recent study in the USA found that men with the highest intakes of saturated fatty acids had the lowest incidence of stroke when followed for 20 years. This inverse association remained significant after adjusting for all other known risk factors for stroke. Despite attempts by the authors and editorial writers in the same issue of journal to rationalize these findings in terms of public health policy, the results underline the futility of expecting to be able to prevent cardiovascular disease by issuing general guidelines on dietary fat intakes.

In 1994, the UK Department of Health's Committee on Medical Aspects of Food and Nutrition Policy (COMA) reviewed scientific research on diet and cardiovascular disease and made public health recommendations. As in earlier reports by this committee, the focus was still on dietary fat modification as a means of preventing cardiovascular disease, with the emphasis on the reduction of total fat and saturated fatty acids. However, distinctions have now been made between the effects of n–3 and n–6 polyunsaturates and between saturates and acids with trans unsaturation; a ceiling of 10% of dietary energy has been put on intakes of polyunsaturates.

This chapter argues that the scientific basis for the focus on fat modification is weak and that the committee has been selective and inaccurate in its citation of the scientific evidence in support of its case. Scientific objectivity has been warped by the demands of public health policies and there are clear parallels with the controversy over the role of salt in hypertension.

Background to the debate

Current public health policy

A major shift in emphasis in UK public health policy has been evident during the last years of the 20th Century, away from a health service that concentrates on curing the sick towards one that puts more stress on preventing illness occurring. Because, rightly or wrongly, it is perceived that infectious diseases have ceased to be the predominant health problem, emphasis is being placed on the prevention of chronic diseases, the most important of which are said to be coronary heart disease (CHD), cancer, diabetes and obesity.

In 1992, a UK Government document entitled 'Health of the Nation' (1) made recommendations for changes in lifestyle that were designed to meet certain health targets. One such target was: "To reduce death rates for both CHD and stroke in people under 65 by at least 40% by the year 2000", and to achieve this the aims were to reduce smoking, increase physical activity and change eating and drinking habits. Of all the many aspects of nutrition that could have been addressed, the focus was overwhelmingly on modification of dietary fat, the targets being:

- To reduce the average percentage of food energy derived by the population from saturated fatty acids by at least 35% by 2005 (from 17% in 1990 to no more than 11%).
- To reduce the average percentage of food energy derived by the population from total fat by at least 12% by 2005 (from about 40% in 1990 to no more than 35%).

Other targets that involved nutrition to some degree were concerned with reducing the prevalence of obesity, reducing the proportion of the population consuming more than a certain level of alcohol and reducing blood pressure. There was no indication of the precise nutritional strategy recommended for reduction of obesity and blood pressure but the text implied an assumption that these conditions would be improved by a combination of reductions in fat, alcohol and sodium consumption and increases in physical activity.

To help implement these strategies, the Department of Health appointed a Nutrition Task Force, which aimed to:

- inform and educate consumers of all ages, including children;
- train specialists to ensure that the advice they give is up-to-date, consistent and balanced;
- develop guidance for caterers on healthy catering practice;
- work with the food industry to reduce the fat content of products where feasible and promote the consumption of foods such as bread and other cereal products, potatoes and other vegetables, and fruit.

The focus on fat and especially saturated fatty acids was consistent with recommendations published in the UK Department of Health's 'Dietary Reference Values for Food Energy and Nutrients for the United Kingdom' (2) (see also Chapter 4). The latest review of diet and cardiovascular disease (CVD) from the Department of Health's Committee on Medical Aspects of Food and Nutrition Policy (3) also focused strongly on saturated fatty acids. Similar recommendations are common throughout the world (4).

The scientific debate

It is common for 'expert' committees who publish these recommendations to state that the views represent an overwhelming consensus of international scientific opinion and are based on the best scientific evidence available at the time. A recent report on the diets of Scots (5) (who have one of the highest rates of CHD mortality in the world) states that: "Evidence linking specific saturated fatty acids to increases in blood cholesterol is overwhelming, as is the causal role of the elevated levels of the cholesterol-containing low-density lipoproteins in promoting heart disease". This report however, acknowledged, somewhat defensively, that opinion is not unanimous on this issue but patronizingly castigated sceptical scientists for confusing the public. It stated: "Academic scepticism, backed by careful reading of the literature is a bonus in research workers involved in clinical research, but can become irresponsible in those whose task it is to improve the health of the Scottish people". In other words, truth and the scientific method are all very well for scientists in the seclusion of their own laboratories but can be thrown out of the window when it comes to public health.

There can be no doubt that controversy on this issue does exist amongst scientists and physicians. This has been expressed in terms of conflicting views on:

- whether the recommended fat modifications will in fact reduce plasma cholesterol enough to have an impact on the disease;
- whether, even if the recommendations were applicable to men, they would be at all relevant to women;
- whether dietary recommendations aimed at reducing CHD should be directed at the total population or individuals most at risk;
- whether dietary recommendations for CHD are equally applicable to other forms of CVD such as stroke;
- whether it is worthwhile or positively harmful to have general population screening for blood cholesterol; and
- whether lowering cholesterol might not actually do more harm than good for those who are not at particularly high risk.

Furthermore, there are those who question the whole scientific basis for 'the lipid hypothesis'. These points of controversy have been discussed by myself and others (6–11).

The aim of this chapter is to examine in considerable detail the scientific basis for the proposition that changes in the amount and type of fat (particularly saturated fatty acids) in the diet will significantly reduce an individual's risk of developing CHD. Furthermore, if adopted as a public health measure, it will reduce the national mortality and morbidity from this disease. I will discuss evidence for and against this proposition.

Cardiovascular disease

Cardiovascular disease is a broad term that embraces diseases of the blood vessels of the heart (CHD), brain (cerebrovsacular disease, stroke) and the limbs (peripheral vascular disease). Put in over-simplified terms, CVD is usually a culmination of two processes: atherosclerosis and thrombosis. In atherosclerosis there is an accumulation of material ('plaque')in the walls of arteries of cells, comprising connective tissue, lipids, calcium and debris resulting from cellular breakdown. The overall effect is to cause thickening of the arterial wall and narrowing of the arterial lumen through which blood flows. At a certain point, this 'plaque' may rupture, generating a series of biochemical events leading to the formation of a clot or 'thrombus'. When a thrombus blocks a major vessel supplying the heart, such as a coronary artery, blood (and therefore oxygen) supply to the heart is cut off (ischaemia), resulting in damage to the heart muscle. (Hence, CHD is often also called 'ischaemic heart disease', IHD.) Such damage in a restricted area may lead to a non-fatal myocardial infarction (heart attack). More extensive damage may

cause death. Various symptoms resulting from arterial disease may occur without the patient experiencing a heart attack. These may involve the muscular pains of angina or the effects of irregular heart beat (arrythmias).

In 'stroke', the affected blood vessels are in the brain. There are two main types of stroke, and it seems likely that the natural history of each, and at least some of the risk factors involved in their development, are different. In haemorrhagic stroke, bleeding occurs from a damaged vessel into the surrounding brain tissue; in ischaemic stroke, a thrombus (blood clot) forms and blocks a vessel, thus cutting off blood supply to an area of the brain. The latter is the more common form in western developed countries (about 80–85% of all stoke) and the sequence of events is similar to what happens in a heart attack. The course of the ensuing illness depends on the part of the brain that is affected and the degree of ischaemia but paralysis, speech defects and death are common.

Evidence in support of the lipid hypothesis

The lipid hypothesis

The lipid hypothesis had its origins in the early part of this century in attempts to reproduce some of the pathology of atherosclerosis by giving animals diets rich in cholesterol. It was noticed that the animals developed high concentrations of lipids in the blood but it was not until the 1950s and 1960s that the influence of dietary fats on blood cholesterol in animals and man began to be studied in a systematic way (12). Keys and his colleagues also embarked on their classical epidemiological investigation — The Seven Countries Study — that produced cross-cultural evidence for associations between dietary saturated fatty acids (SFA), blood cholesterol and CHD mortality (13). Together, this early work gave rise to the lipid hypothesis, which for convenience will be considered in four parts.

- Diets containing a high content of fat/SFA/cholesterol lead to high blood concentrations of cholesterol [especially low-density lipoprotein (LDL) cholesterol].
- This results in high morbidity and mortality from CHD.
- Reducing the amount of fat/SFA/cholesterol in the diet will reduce blood cholesterol (especially LDL-cholesterol) concentrations.
- This in turn will result in a lower risk of CHD and eventually a lower morbidity and mortality from the disease.

It is important to bear in mind that CHD is a complex disease with many contributory components. The hypothesis as stated here over-simplifies the issues. As knowledge has developed, attention has switched from the role of cholesterol itself to the lipoprotein fractions of which it is a component and, more recently, from the influence of diet on the atherosclerotic to the thrombotic component of the disease. Furthermore, there has been, and continues to be, confusion over whether it is the amount or type of dietary fat that is most important in determining risk. In this brief review, the emphasis will be on composition of the dietary fat but readers will find further detail of the effects of type versus quantity of dietary fats in Chapter 1.

In the literature, reports deal with either plasma, serum or blood cholesterol. Although these are not synonymous, I will consistently use the term 'blood cholesterol' for simplicity. I have referenced what I consider to be the most important points. However, where statements have not been referenced in the text, readers can consult my more detailed reviews (6,14). In the following sections I will present evidence for each of the four parts of the lipid hypothesis outlined above, providing evidence mainly from animal experiments, human intervention studies and epidemiology.

Fat consumption and blood lipids

In some animals there is a marked rise in blood cholesterol in response to cholesterol in the diet, whereas in others the rise is minimal.

Inclusion of crystalline cholesterol in human diets does not elicit a rise in blood cholesterol and most experiments have relied on supplementing the diet with eggs. Meta-analysis of 68 clinical trials representing 1490 subjects demonstrated that blood cholesterol rises an average 2.3 (SD, 0.2) mg/dl (0.059 mM) for every 100 mg/day increase in dietary cholesterol (15). Some epidemiological studies also suggested a linear association between dietary and blood cholesterol. It is clear that, like other animals, some individual human beings respond strongly, others weakly or not at all, to dietary cholesterol.

Cross-cultural epidemiological studies have generally demonstrated strong correlations between average consumption of SFA and mean blood cholesterol concentration (13). The weak or absent correlations within countries have been explained, according to some authors, by the relatively narrow range of intakes compared to those between countries. The cross-cultural epidemiological results are confirmed by much experimental evidence that diets rich in SFA raise cholesterol to a significant extent

(16). Keys and Hegsted developed formulas to predict the extent of change in blood cholesterol that would result from changes in SFA and polyunsaturated fatty acid (PUFA) intakes expressed as percentage of energy and dietary cholesterol expressed as mg/1000 kcal. These have been recently refined and updated (17) (see also Chapter 1).

Three SFA are considered to be 'cholesterol raising': lauric (12:0), myristic (14:0) and palmitic (16:0). Their activities depend on a broad range of dietary interactions (see Chapter 1). Early research regarded the monounsaturated fatty acid, oleic acid (18:1), as neither raising nor lowering cholesterol. Several recent studies have indicated that monounsaturates, when substituted for SFA, were as effective as *n*–6PUFA in maintaining low blood cholesterol. PUFA of the *n*–3 family lower blood triacylglycerols (which some consider to be another risk marker for CHD, see Chapters 1 and 4) but not cholesterol. A recent reappraisal of the effects of monounsaturates with *trans* unsaturation concluded that they were almost as effective as the C_{12}–C_{16} carbon SFA in raising blood cholesterol, while also lowering high-density lipoprotein (HDL) cholesterol (18). It is widely considered that an important index of CHD risk is the ratio of LDL-cholesterol to HDL-cholesterol.

Raised blood lipids and CHD risk

Animal experiments as early as the 19th Century provided some of the first indications that fatty substances in blood could lead to atherosclerosis. Many authors have considered that these changes seen in rabbits, rats, pigs and non-human primates, among others, sufficiently resemble atherosclerosis in man to provide sound evidence for a primary role for circulating lipids, especially LDL, in the initiation of atherosclerosis. Nearly all animal research has been concerned with atherosclerosis since it is unusual, though not entirely unknown, to find CHD or coronary thrombosis in animals.

Patients with familial hypercholesterolaemia, an inherited disorder characterized by abnormally elevated concentrations of LDL, have a high risk of CHD, one report suggesting that there is a 52% chance of fatal or non-fatal CHD in men by age 50. Many authors have described extensive atherosclerosis in persons with familial hypercholesterolaemia, whose blood cholesterol concentration may reach 10–15 mM in heterozygotes and up to 30 mM in homozygotes compared with 5–6 mM in the general population. These findings are said to provide the most persuasive evidence for a direct link between blood LDL and CHD (19).

Turning to epidemiology, the Seven Countries Study (13) found a strong correlation between blood cholesterol concentration and mortality from CHD.

Several 'cohort' or 'prospective' studies have shown that individuals with a high blood cholesterol concentration at baseline are more likely to develop CHD over the next 10–20 years than those with lower values. These have demonstrated a strong graded relationship between blood cholesterol and CHD, occurring in both sexes and independent of all other risk factors (20). Other studies of populations that migrated from areas of low to high CHD incidence also supported the idea that there is a strong influence of dietary fat and blood cholesterol on CHD mortality.

Reducing blood lipids by modifying dietary fat

Numerous experiments with animals indicate that replacing SFA by unsaturated fatty acids or carbohydrates generally results in lower concentrations of total blood cholesterol. There are wide differences in responsiveness, rabbits tending to exhibit exaggerated responses, dogs and rats a limited response and non-human primates a wide range of responses.

A comprehensive review of small scale experimental dietary studies in human beings suggests that for every 1% energy from SFA replaced by linoleic acid (18:2), there is an average reduction in blood cholesterol of 0.13 mM (5 mg/dl) (16). Intakes of up to 12% of energy as 18:2 do not affect blood HDL but amounts of 18:2 (but not 18:1) above this concentration cause a reduction in HDL.

Many intervention trials, conducted to test the lipid hypothesis (mainly in subjects at high risk), have also provided information on the extent of blood lipid changes consequent upon modifying the dietary fat. Reductions in blood cholesterol of up to 16% have been achieved over periods of 1–5 years. These have been influential in supporting arguments for the benefits of extensive changes in dietary fat in developed countries (21).

Reducing CHD risk by reducing blood cholesterol

The best evidence relating modification of blood lipids to changes in CHD morbidity and mortality has come from intervention trials set up specifically to test the lipid hypothesis. In these, lipid lowering was achieved either by means of drugs or by dietary change. In general, it has been easier to demonstrate clear lipid lowering effects with drugs. They lend themselves more readily to double-blind placebo controlled design, their action is more specific and adherence by subjects to the treatment is likely to be stricter. One study has even used surgical intervention (removal of part of the gut) to reduce lipid levels with positive results.

There have been many primary (involving people without obvious CHD) and secondary (involving people who have already had a heart attack) intervention trials using diet to lower blood cholesterol (21). Some demonstrated significant reductions in CHD deaths but many more showed improvements in non-fatal coronary events. Sometimes dietary treatment was combined with modification of other risk factors, such as smoking or blood pressure, leaving the quantitative contribution of dietary change uncertain.

Nevertheless it has been claimed that, taken together, the results of dietary and drug trials show that for every 1% reduction in an individual's total blood cholesterol, a 2% reduction in CHD risk can be expected. In 1994 an influential series of publications concluded that the link between blood cholesterol reduction and CHD had been grossly underestimated. The authors calculated that a reduction in blood cholesterol of about 0.6 mM (which is about 10% of the average value in the UK) would be equivalent to a 27% reduction in CHD mortality (22).

A strong view that diet, blood lipids and CHD are intimately linked has been stated by Blackburn (23). He states: "There has probably been a small but significant drop in the population average level of total serum cholesterol in the last 20 years in the US, largely explainable by known changes in the composition of the diet during this period". Without question there has been a very dramatic fall in CHD mortality in the USA in that period.

The next sections examine contrary views and present arguments against a primary role for SFA in CHD.

Evidence against the lipid hypothesis

To assess the validity of proposed links between dietary SFA, blood lipids and CHD, I shall examine three sorts of scientific evidence:

- studies that have recorded existing dietary patterns and related them to prevailing disease;
- studies that try to match changes in diet over periods of time with changes in disease incidence during the same periods; and
- intervention studies in which specific diets have been imposed and subsequent responses in blood lipids and/or the occurrence of coronary heart disease events have been recorded.

What can be learned from observation of diet and disease patterns?

Several different epidemiological approaches have been used to assess diet–disease links. In a simple cross-sectional study one may observe food and nutrient intakes in a representative sample of a population and relate those to prevailing patterns of the disease of interest. Such surveys may involve comparing communities in many different countries or within a single country. The former has the advantage that diets vary widely, offering more chance of detecting a diet–disease association, but the disadvantages that dietary information may not be comparable and that many factors other than diet will differ. In the latter, there is a better chance of controlling different variables, but differences in dietary habits might not be large enough to observe associations. Clearly, if current disease had been influenced by previous diet, this method has limited value.

A case-control study may be more informative. In these investigations, people with diagnosed CHD (cases) are matched against controls chosen to be as similar as possible to cases in all respects other than suffering from CHD. Case-control studies suffer from the problem that dietary assessment is usually retrospective and therefore unreliable and that matching of cases to controls is frequently inadequate. A better method is the prospective or cohort study in which certain characteristics (for example, blood cholesterol) of a group of people (the cohort) are measured at the start of the study and the group is followed over a period of time and both fatal and non-fatal CHD events are recorded.

Reliable measurement of food and nutrient intakes is difficult. International epidemiological studies often rely on food supply information provided by government departments or international agencies and these tend to over-estimate individual intakes. Surveys of individual eating habits have used a variety of methods. A simple one is to ask the subject to record all that was eaten over the past 24 hours, which clearly may not represent long-term intake. Some methods rely on subjects weighing and recording all the food they eat over a specified period, which is tedious and may encourage people to change their eating habits to make recording simple.

Large studies now tend to employ food frequency questionnaires in which the frequency of consumption of specified foods is noted. All these methods rely on food composition tables to translate foods eaten into nutrients. These may or may not represent accurately the composition of the foods actually used by the subjects. For assessment of fatty acid intakes, it is now common to

analyse adipose tissue composition as a measure of long term intake of certain fatty acids, especially linoleic acid. This method cannot give information on the amount of fat in the diet.

International studies

The most cited study in favour of a link between saturated fatty acids and CHD is the 'Seven Countries Study' (13). Graphical representations of the results show a more or less straight line relationship between the intake of SFA as a percentage of dietary energy and either plasma cholesterol concentration or CHD (but not total) mortality. The emphasis placed on this study is hardly justified since other aspects of lifestyle in these widely differing communities were also very different. In the case of Japan, for example, the low SFA intake was accompanied by a large intake of fish oil which may have a protective effect against CHD that is not mediated through plasma cholesterol (24). Diets low in SFA may also be rich in fibre or other components that have an hypocholesterolaemic effect.

The usual interpretation of the Seven Countries Study suffers from an important logical defect. The seven countries were among 21 member states of the Organization of Economic Cooperation and Development for which statistics were available on average annual consumption of different types of fats and on CHD mortality. Keys obtained a correlation coefficient for the association between the percentage of energy as SFA and CHD mortality of +0.84 for the 7 countries he selected. Wood (25) pointed out that there are 116 280 possible ways of obtaining a sample of 7 from 21 and that fewer than 10% of samples of 7 gave a correlation coefficient equal to or greater than 0.84. Indeed, correlation coefficients obtained by Wood ranged from –0.9 to +0.9. Keys' sample, therefore suffered from a selection effect so that no valid inferences could be drawn from it concerning the relationship between consumption of SFA and CHD mortality. Wood's paper is rarely quoted and provides an example of how certain ideas, statements or publications are adopted without challenge by those who are reluctant to conceive that a favourite concept may be wrong or is certainly in need of more rigorous testing.

The present day equivalent of the Seven Countries Study is the World Health Organization (WHO) MONICA project. This monitors 50 populations in 26 countries worldwide including both developed and developing countries. It has taken account of many different 'risk factors' and has not focused simply on blood cholesterol and dietary fat. Within Europe, the MONICA study has shown, for example, that there is an eight-fold difference in CHD mortality between the West of Scotland and Catalonia, but no significant difference in

blood cholesterol concentration. A recent progress report (26) found that three factors that have hitherto been considered to be the most important in terms of predicting heart disease mortality, namely smoking, high blood pressure and high blood cholesterol do not reflect well the variation in CHD or total mortality between populations.

The French Paradox

The WHO MONICA Study includes two centres in France: Toulouse and Strasbourg. Renaud and de Lorgeril (27) regarded the situation in France as paradoxical, in that there is generally a very low mortality in that country from CHD but a relatively high intake of SFA. They, therefore, examined the literature for factors that might account for this apparent anomaly. They concluded that the high wine consumption in France (about 20–30 g alcohol from wine per day) may be partly responsible. This level of intake is judged to decrease the risk of CHD by at least 40%. Many authors have assumed that the main protective effect of alcohol is through its effect in elevating plasma high-density lipoproteins (HDL) (28) although it was appreciated that an influence on HDL could explain only part of the effect of alcohol on CHD (29). HDL are particles that scavenge cholesterol from membranes and tissues where it is in excess and transport it to the liver for disposal. A low concentration in plasma is associated with increased risk of CHD. Renaud and de Lorgeril (27) dismissed this argument on the grounds that HDL concentrations in France are no higher than in other countries. They concluded that the effect is mediated through platelet activation: alcohol has been shown significantly to reduce platelet aggregation at levels of intake associated with reduced risk of CHD. Platelets are the cellular elements in the blood that clump together to seal a wound and prevent bleeding. However, an excessive tendency for platelets to aggregate in some people may lead to conditions that give rise to a coronary thrombosis.

In their paper, the authors highlighted the 'French paradox' (Switzerland is also in a similar position) by showing a correlation (Figure 1 in reference 27) between CHD and calories from dairy fat. The correlation coefficient is +0.73, significant at $p<0.001$. The authors claimed that dairy fat is the only food that is significantly correlated with CHD mortality. France and Switzerland were anomalous in falling well below the straight-line plot of CHD mortality against dairy fat consumption, while the UK was anomalous in the opposite direction, i.e. having a high CHD mortality but a somewhat smaller intake of dairy fat than France and Switzerland. When the authors re-plotted the graph (Figure 2 in reference 27) adding in a factor for wine consumption, the correlation was even higher ($r = +0.87$) and more highly significant ($p<0.001$). The points on

the graph representing the UK, France and Switzerland were now much closer to the line. This, the authors argued, demonstrates that, while the SFA in milk fat are normally responsible for an increased risk of CHD, wine drinking in France is able to counteract this effect through its influence on the thrombotic phase of CHD.

Renaud and de Lorgeril's paper addresses an important point and, perceptively, recognizes that the large differences in coronary heart disease (CHD) between different geographical locations are more likely to result from influences on the thrombotic than the atherosclerotic phase of the disease. However, the authors do not convincingly make a case either for a causal role for 'dairy' fat or for a protective role for wine.

Simple correlations between 'dairy' fat and CHD mortality can never provide convincing scientific evidence because of the problems of selection bias and of potential confounding factors. The same problem (25) occurred in relation to the famous Seven Countries Study of Keys (13) discussed above. In previous publications, but not in this one (why?), Renaud and de Lorgeril distinguished between fat from cheese and that from other milk products (30). When cheese fat was included, the correlation was not significant. They postulated that the high concentration of calcium in cheese, but not in other milk products inhibited absorption of saturated fatty acids on the basis of experiments with rabbits (31). There is no experimental evidence for this distinction in man, nor should there be a distinction theoretically. While it is true that the calcium in cheese, because of the low pH, is more soluble than that in milk, where it is bound strongly to phosphate centres, the distinction is lost once the digesta from these different foods enters the duodenum where the higher pH allows strong interaction again between the calcium and phosphate centres.

Regarding the 'wine effect', the authors discussed the lack of evidence for a significant distinction between the effects of alcohol in wine and beer. However, they provided no evidence whatever that the total alcohol consumption in areas they cite as having high CHD mortality (e.g. Belfast, Strasbourg) is actually significantly lower than in Toulouse. In another study that compared risk factors for CHD in Toulouse and Strasbourg, it was speculated that the higher alcohol consumption in Strasbourg might account for the higher CHD mortality in that city! (32).

There are dangers in trying to formulate an all-embracing hypothesis to account for the unexplained patterns of a disease as complex as CHD. The authors get themselves into a logical tangle trying to explain the anomaly of

low CHD mortality and high dairy fat consumption in France both in terms of (i) a distinction between fatty acids in cheese versus other dairy products and (ii) an effect of alcohol in wine but not in other alcoholic beverages. If it were true that cheese fatty acids are not well absorbed, then the same amount of dairy fat in France, coming mainly from cheese, should result in a lower plasma cholesterol in France than in the UK if, indeed, dietary fat is a major determinant of plasma cholesterol. The European Monica Study, however, clearly illustrates that the average plasma cholesterols in the two countries are very similar (33). The distinction between fatty acids taken as cheese and those taken as liquid milk is spurious. The other argument, that the difference is due to the effect of alcohol on platelet aggregation, is equally suspect, as discussed above. The authors need to explain why alcohol from wine could somehow affect platelets differently from alcohol from other sources. The author's suggestion that wine is drunk with meals and, therefore, counteracts the adverse effect of the meal fatty acids on platelet function remains unconvincing until corroborated by experiment. There are, of course, components of wine other than alcohol that might explain an influence of wine on cardiovascular disease risk but these were not adequately discussed by the authors.

There remain many anomalies, and CHD will continue to appear paradoxical while investigators retain their fixation that diet is all important. Many other lifestyle factors more important than diet have to be plugged into the equation.

Migration studies

Too much confidence has also been placed in migration studies that often show that those who migrate from one country to another adopt the disease pattern of their adopted country. Confidence in the frequently made assumption that this is due to changes in diet, especially regarding intakes of SFA, must be limited because other lifestyle characteristics also change (6). The changes in plasma lipoproteins and disease patterns cannot be attributed to diet with any confidence.

Prospective studies

Among the longest running of the prospective studies has been The Framingham Study, which has followed the fortunes of a large number of people in a small New England, USA, town since the late 1940s. Although it has provided much information, now often taken for granted, about important risk factors for CHD, it has never been able to demonstrate any relationship between plasma cholesterol concentration and intakes of fat or cholesterol (6).

Another large American prospective study has reported on associations between consumption of *trans* fatty acids and CHD mortality. The investigators of the Nurses Health Study (34) reported that of 85 000 American nurses, those consuming the highest levels of *trans* fatty acids had a 50% greater risk of CHD than those consuming the lowest amounts. This association remained after correction for known risk markers (age, smoking, blood pressure etc) and for intakes of saturated fatty acids, linoleic acid, cholesterol, vitamin E, carotene and dietary fibre. The association was significant only for *trans* fatty acids from hydrogenated vegetable oils, not from ruminant fats. The increase in risk was remarkably high considering that there was a difference of only 3.3 g between the highest and lowest quintiles of consumption. It is doubtful whether the food frequency questionnaire used to assess food and nutrient intakes would have been sensitive enough accurately to discriminate between such close levels of intake.

In view of the apparent anomaly of high risk from industrially-produced *trans* fatty acids and no risk from ruminant *trans* fatty acids (ruminant fats contain *trans* fatty acid isomers that are qualitatively similar to those in hydrogenated vegetable oils but in different proportions) it is prudent to consider whether the *trans* fatty acids content of hydrogenated fats might have been acting as a marker for socio-economic status (cheaper brands of margarine usually have a higher *trans* fatty acid content) or for other aspects of an unhealthy lifestyle. Such a problem of 'confounding factors' is an important reason why the results of epidemiological studies alone cannot be considered as conclusive evidence of the influence of specific dietary components on health. Two European epidemiological studies published in 1995 (35,36) which used the *trans* content of adipose tissue biopsy samples as an index of long-term intake rather than the food frequency questionnaire methods, found no association between *trans* intake and CHD.

The Caerphilly Study in South Wales, an area of high CHD mortality, found that diet accounted for only 2% of the variance in plasma total cholesterol. Moreover, this study has found no significant relationship between intakes of any nutrient and the risk of a new CHD event over a 5-year period (37).

Several studies, designed prospectively, have also provided cross-sectional data that allow us to compare fat intakes of people with CHD (cases) and healthy controls. As **Table 2.1** shows, there have generally been no differences between fat intakes expressed either in g/day or as % of energy intake when controls were compared with people who had suffered a CHD event. In several of these studies, people free of CHD had higher energy intakes.

Table 2.1. Dietary fat intakes in major prospective studies.

Study[1]	Men who experienced:					
	no CHD event			an incident CHD event		
	Number	Fat consumption		Number	Fat consumption	
		g/day	% energy		g/day	% energy
1	780	114	39	51	106	40
2	7982	95	35	163	92	36
3	6632	86	33	309	87	35
4	287	129	41	50	118	40
5	827	142	42	30	130	42
6	891	144	39	110	140	40
7	2197	103	40	137	99	41

[1] For details and references, see reference (6)

I reported above that the apparent lack of association between dietary fat and CHD *within* countries (as compared with international studies) has frequently been "explained" as being due to the small dietary differences that occur within countries (6). However, it is undeniable that within many countries (for example the UK) there are very large differences in CHD prevalence between regions. If dietary SFA were the main cause of CHD, as many claim, then large differences in SFA intake between regions ought to be evident. In the UK there is no evidence for large differences between regions but there is evidence for large differences between individuals. Thus, an important UK dietary survey (38) revealed that the average intake of SFA among men was 16.5% of dietary energy but some people were eating as little as 10.6% and others as much as 22.4%. If SFA were a major factor in causation of CHD, it is likely that these differences would be sufficient to reveal an association if there were one.

Communities with naturally high SFA intakes

In many Asian communities — particularly the Philippines, Polynesia, and parts of India — coconut, a highly saturated vegetable oil, has traditionally made a substantial contribution to food and energy intakes. Prior and colleagues studied the inhabitants of the Polynesian islands of Pukapuka and Tokelau (39). The percentages of energy derived from fat in these communities were about 35% and 50% respectively and in each community the fat was provided mainly by coconut. SFA provided about 28 and 48% of total energy respectively

and PUFA about 2% in each community. These data, obtained by 7-day weighing of household food items, were reflected in the adipose tissue fatty acid composition. The proportions of 12:0, 14:0 and 18:2 in the adipose tissue were about 11%, 17% and 3% respectively in both communities compared with 0.3, 4 and 3% in New Zealanders of European origin. There can be no doubt that coconut oil had dominated the diets of these communities over a very long period and that, as a result, intakes of the 'cholesterol-raising' fatty acids were far higher than in the West.

Plasma cholesterol concentrations of Pukapukan men and women were 4.5 mM and 4.6 mM, while those of Tokelauans were 5.5 mM and 5.7 mM. The higher levels in Tokelauans were judged by the authors to result from their higher intakes of SFA. Nevertheless, these values are considerably lower than those found in New Zealand (on average about 5.9 mM), where SFA intakes are lower, and *much lower* than would be predicted from application of the Keys/Hegsted formulae (see Chapter 1).

When Tokelauans migrated to New Zealand, their plasma cholesterol concentration increased (40), despite a *decrease* in their consumption of saturated fatty acids (45% to 21% of energy). However, their consumption of cholesterol increased (85 mg to 340 mg/day). It is clear that, most other lifestyle attributes being equal, differences in SFA intake may affect plasma cholesterol to some extent but that overall the influence of SFA is not dominant.

These reports also record that the people in these communities have extremely low rates of CHD despite their high total fat and SFA intakes. The same is true in Melanesia. In Africa too, CHD is rare among the Masai despite their high consumption of SFA and Nigerians, with a high consumption of SFA from palm oil, albeit with relatively low total fat intakes, also suffer little CHD (41).

Therefore, despite constant propaganda that SFA are a cause of CHD, it seems that there is little evidence from studies of what people are actually eating to confirm this view. The studies cited here have been concerned mainly with cross-sectional data, which are snapshots of diets and disease prevalence at any one time. In the next section I will discuss what can be learned by comparing changes in CHD over a period of time with corresponding changes in dietary habits.

Trends in heart disease mortality since 1950 in the UK

Coronary heart disease (CHD) is the most common cause of death for men in the UK. In 1991, 81 611 men died from CHD in England and Wales (42),

accounting for approximately 29% of all male deaths. (In the UK, population data and statistics are often collected for the combined region of England and Wales separate from those for Scotland and Northern Ireland.) Since the late 1970s, however, the death rate from CHD for men in England and Wales has been falling steadily. CHD is mainly a cause of death for older men; in 1991, 78% of male deaths from CHD were in those aged 65 and over (42).

Figure 2.1 shows how the death rate from CHD has changed for men aged 45–54 in England and Wales since 1950. For 20 years the mortality from CHD rose steadily up to a peak in the mid-1970s. This rise was particularly noticeable among younger men. In the youngest age group (35–44 years) the rate doubled between 1950 and 1972, while in the same period it went up by only 30% for men aged 55–64. Since reaching this peak, the male mortality rate from heart disease has dropped appreciably, and for younger groups it is now back to the levels of the 1950s and for the older groups considerably below those levels.

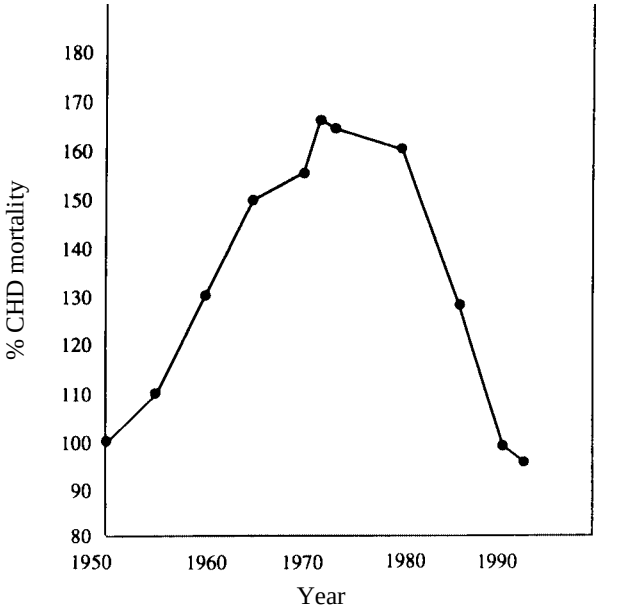

Figure 2.1. Coronary heart disease mortality in men aged 45–54 in England and Wales as % comparison with the 1950 level. (In the UK, population data and statistics are often collected for the combined region of England and Wales separate from those for Scotland and Northern Ireland.).

Of course, since the population has grown (especially in the older age groups due to increased life expectancy) the number of deaths from CHD has increased. This explains why there were 74% more deaths from heart disease in 1991 than in 1950.

Trends in heart disease mortality since 1950 in several other countries

Figure 2.2 shows that trends similar to those that have occurred in the UK have also taken place in the USA, Australia and New Zealand, among others (43). Of particular interest is that in a large country like the USA the peak in heart disease deaths and the subsequent sharp fall occurred at different times in different parts of the country (44). Thus, while the national decline did not start until about 1968, the peak was reached first in California around 1950, followed by the North Eastern states but did not start in the South Eastern states until well into the 1970s. Another important observation is that, in each area, the trends and their timing have been similar among white and black people and

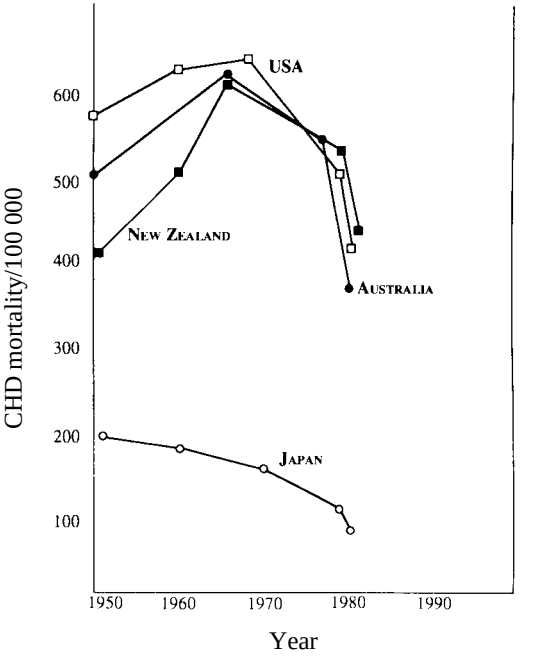

Figure 2.2. Comparison of coronary heart disease mortality rates in men aged 45–65 in four countries (expressed as deaths per 100 000).

in the different social classes, although the absolute rates of mortality between different sections of the population may have been very different. On average, a massive 39% decrease in CHD mortality occurred in the USA between 1970 and 1985 and the figures for Australia and New Zealand were 54% and 34% respectively (45).

In several Eastern European countries, however, CHD mortality has been rising and has not yet peaked.

Heart disease statistics for Japan are particularly interesting. This country has always had a remarkably low rate of CHD compared with Western industrialized countries but even this low rate has been decreasing since 1950 and continues to do so (43,45).

Two observations about these figures are particularly important. The first is that they apply only to men. Heart disease death rates have always been lower in women in all countries and although the direction of changes among women have been broadly similar, the rates of change have not. The second point is that only mortality rates have been discussed since the data are relatively easy to obtain and are reasonably accurate in the countries mentioned.

Information on the incidence of non-fatal CHD events such as myocardial infarction or angina pectoris is less readily available and less reliable but what little evidence there is suggests that incidence has not fallen nearly so much as mortality.

Trends in fat consumption since 1950 in the UK

Figure 2.3 shows the percentage of energy derived from fat, a key measure by which dietary targets are assessed. These figures are from the Ministry of Agriculture, Fisheries & Food 's estimates of UK household food supplies (46). They show that the percentage energy from fat increased by about 10% over the first 20 post-war years and has remained at about the same level since then. The steady decline in CHD mortality since the late 1970s has therefore occurred during a period when the proportion of fat in the diet did not change.

The percentage of food energy derived from *saturated* fatty acids (first calculated in 1972) changed little during the 1970s, then started to fall around 1980. This fall did not precede the decline in overall male death rates from CHD and occurred well after the decline in CHD mortality for middle-aged men.

Because, in simple terms, dietary saturated and polyunsaturated fatty acids have opposing effects on blood cholesterol (one of the markers of heart

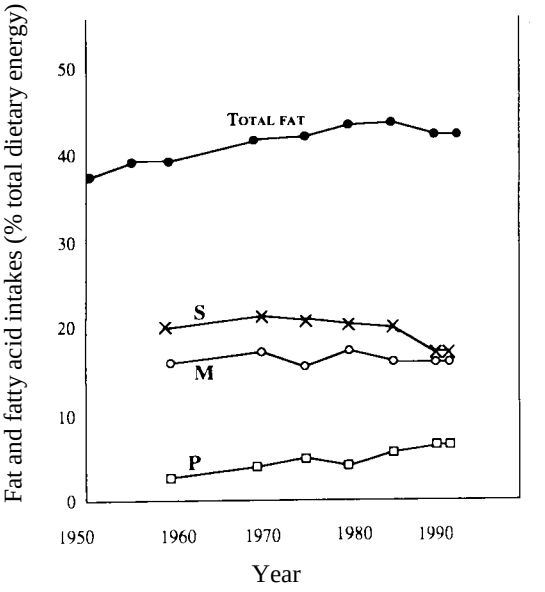

Figure 2.3. Changes in fat and fatty acid intakes as % of total dietary energy in the UK. These figures are from the UK Ministry of Agriculture, Fisheries & Food's estimates of household food supplies. S = saturated fatty acids; M = monounsaturated fatty acids; P = polyunsaturated fatty acids.

disease risk) many people have argued that trends in the ratio of polyunsaturated to saturated fatty acids (P/S) are of more significance than trends in saturated fatty acids alone. Figure 2.3 illustrates that there has been a steady upward trend in this ratio since 1950, which is still continuing, during which time the mortality from CHD has risen, peaked and then fallen. Dietary intakes of monounsaturated fatty acids, whose health effects have received a considerable upsurge of interest since the late 1980s, have been relatively stable.

Trends in fat consumption since the 1950s in some other countries

Figure 2.4 illustrates that, expressed as a proportion of energy intake (47), total fat consumption in the USA, Australia and New Zealand has been remarkably stable while the Japanese have experienced a considerable rise in fat intake. In Japan, fat represented only 11% of calories in 1960 but had risen to 25% by the early 1980s (48). The increase was represented in all the main

classes of fatty acids, saturates (3% to 14%), monounsaturates (4% to 15%) and polyunsaturates (4% to 8%). During that time, heart disease mortality has peaked and then fallen precipitately in the 'Western' countries and fallen steadily from an already low baseline in Japan.

The changes can be summarized as follows.

- Since the mid-1970s, the male death rate from coronary heart disease has fallen in England and Wales and is now lower than in 1950.
- Total fat increased as a proportion of dietary energy up to 1970 and has remained constant while the male death rate from CHD has fallen in England and Wales.
- The death rate in middle-aged men started to decline before saturated fatty acid intakes began to fall as a percentage of energy and the ratio of polyunsaturates to saturates has risen steadily during a period when heart disease mortality first increased, peaked and then fell.
- In the USA, Australia and New Zealand, heart disease mortality peaked on average in about 1968 and fell dramatically thereafter. The proportion of

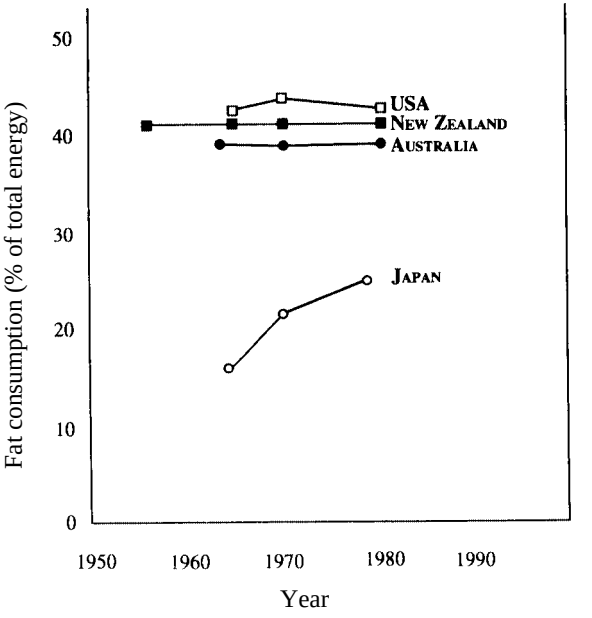

Figure 2.4. Comparison of the changes in fat and fatty acid intakes as % of total dietary energy in four countries.

energy from fat remained relatively constant during this period. In Japan, heart disease mortality, already much lower than in the West, continued to fall steadily while fat intake has risen steeply as a proportion of dietary energy.

The complete lack of correlation of changes in total and animal fat intakes in 27 different developed and developing countries has been well summarized by Yarnell and coworkers (49). The population characteristics that were best correlated with changes in total cardiovascular mortality (but not with CHD) were annual income per person and infant mortality rates. It seems likely that answers to the riddle will need to be sought in terms of socio-economic conditions that prevailed sometime in the past.

Time trends in coronary heart disease statistics: new epidemiological evidence

The review by Yarnell *et al.* (49) on cardiovascular risk factors pointed up important differences between correlations of risk factors and cardiovascular mortality and correlations of risk factors and incident coronary disease. More recently this important distinction — between mortality and disease incidence — has been brought into sharp focus by a publication from the Atherosclerosis Risk in Communities (ARIC) Study in the USA (50). This study, which started in 1987, examined the incidence of and mortality from coronary heart disease in four areas ('communities') throughout the USA. It found that overall CHD mortality in hospital declined by 5.1% per year, whereas mortality out of hospital declined by about 3.6% per year. There were differences between men and women and between black and white people but the trends were similar.

In contrast to these mortality statistics, there was no evidence of a decline in the incidence of hospital admissions for people with a first heart attack among men or women, black or white. Indeed hospital admissions increased somewhat for black people. Rates of recurrent heart attacks decreased and survival after a heart attack improved. These data suggest that reduction in mortality is a result of improvements in the treatment and secondary prevention of heart attacks rather than improvements in the rates of occurrence of first heart attacks and a reduction in underlying arterial disease.

The importance of distinguishing incidence and mortality

Before the publication of the ARIC study, the overwhelming emphasis of publications had been on mortality rather than on non-fatal heart attacks or other manifestations of CHD morbidity. Mortality statistics, with differing degrees of reliability, are collected in most countries, whereas few data on incidence (defined as new cases of heart disease appearing in the population

per year) have been available and their reliability was questionable. The ARIC study has broken new ground by collecting reliable data on CHD incidence. Because such work is in its infancy, the collection period has been only 8 years and there is some question as to whether these incidence data are entirely representative of the whole USA. Nevertheless, within its limitations, the work was carefully done and provided good evidence that incidence has not fallen, and may have actually risen somewhat in black people. Set against a background in which strenuous efforts have been made for at least the last 30 years to educate people about the risk factors for CHD and how they might be reduced, these findings prompt closer examination of those risk factors and how they have changed.

Risk factors for CHD

Rosamond and colleagues (50) did not attempt to measure changes in risk factors and did not make more than passing reference to them in their discussion. As early as the 1960s, the principal risk factors for CHD were identified (to most people's satisfaction) as hypertension (high blood pressure), hypercholesterolaemia (high blood cholesterol) and cigarette smoking. Although many other potential risk factors have been identified subsequently, and ideas about the roles of different components of blood cholesterol have become more sophisticated, these 3 major risk factors remain, in the 1990s, the principal targets of public health programmes.

Data from the US National Health and Nutrition Examination Surveys, for the period 1976 to 1994, indicate the following overall changes in these risk factors (51):

- 40% decline in the prevalence of hypertension;
- 28% decline in the prevalence of hypercholesterolaemia; and
- 25% decline in cigarette smoking.

It is clear that these very substantial reductions in risk factors, which apply to both sexes and to black and white people, have not had the expected influence on CHD incidence. (This of course makes the necessary assumption that the data of Rosamond *et al.*(50) are representative of incidence statistics for the USA as a whole.) One of several possible conclusions is that the original hypothesis (that these so-called risk factors are directly involved in causation of the disease) was wrong. In an editorial in the same issue of the journal, Levy and Thom (51) described the fact that there was no decline in the incidence of myocardial infarction in the ARIC study when the prevalence of *causal* risk factors was reduced as a "puzzling paradox". They suggested three possible explanations.

Their first suggestion was that there had been a failure of primary prevention at a national level. This was dismissed on the basis that the main risk factors (smoking, blood cholesterol, hypertension) had definitely declined. The second suggestion was that the ARIC results were valid but not representative of the USA as a whole. Third, they argued that the most probable explanation was that incidence of myocardial infarction had indeed declined across the USA but that this had not been reflected in the ARIC data, which were invalid for "a variety of complex reasons that influence all surveillance studies of trends in the incidence of myocardial infarction".

Readers will note the circular arguments employed and the failure of the editorial writers (both officials of the National Heart, Lung and Blood Institute) to recognize the simplest of all explanations: namely that when evidence continually fails to support a hypothesis, the hypothesis may need to be discarded. The paradox that these authors found so puzzling is neither "puzzling" nor a "paradox", once it is acknowledged that the so-called primary risk factors are not primarily involved in the causation of the disease. The editorial writers give themselves away when they refer to a decline in the prevalence of causal risk factors. These risk factors have been established by epidemiological studies that provide only statistical associations and cannot provide information about cause and effect relationships. No research has ever proved that high blood cholesterol causes coronary heart disease, let alone that high intakes of saturated fat do so.

Experiments with human subjects designed to reduce blood cholesterol and coronary heart disease: testing the lipid hypothesis

In this review of the evidence for and against the lipid hypothesis, I have so far described observations of people with or without heart disease in relation to characteristics such as the concentrations of lipids in their blood and the composition of lipids in their diets. Some have claimed that these observations fit a pattern that is consistent with a primary causal role for dietary saturated fatty acids (SFA) and cholesterol in CHD (e.g. see reference 3); I have argued in preceding sections that the associations are at best tenuous and at worst non-existent.

Such evidence is certainly circumstantial and, being based on statistical associations, cannot provide information about whether SFA *cause* CHD. It is common to find epidemiologists asserting this important caveat at the outset and then later breaking their own rules (3). The only true test of an hypothesis is by experiment and the following sections will discuss experiments set up to

test the hypotheses that (i) modification of dietary fat will result in lowered blood cholesterol concentration, and (ii) lowered blood cholesterol concentration will result in reduced CHD.

It would have been ideal if the hypothesis had been expressed in more specific terms i.e. "reduction of SFA intakes" or "reduced CHD mortality" so that the results could have been scientifically sharper. In practice, some experiments have reduced total fat and dietary cholesterol as well as modifying fatty acid composition. Some have chosen as their end points total CHD events, including deaths, while others have considered all-cause mortality. Some experiments have focussed on blood cholesterol as the `modifiable risk factor' (single risk factor trials) and have sought to investigate the effects of either diet or a drug or a combination of the two. Others have sought to modify several risk factors (e.g. blood cholesterol, blood pressure, body mass index, smoking habit, glucose tolerance) at the same time (multiple risk factor trials). These variations have tended towards a lack of clarity in assessing the overall results.

Will changing diet lower blood lipids ?

There is a vast amount of experimental work to show that changing the amount and type of fat in the diet of subjects under carefully controlled experimental conditions will modify plasma cholesterol. I have discussed these at some length in Chapter 1 and will not go into detail here. These studies have mainly involved relatively small numbers of subjects supervised under laboratory or metabolic ward conditions, that may have been far removed from 'normal life'. Many were conducted in institutions for patients with medical conditions (in the case of the Keys studies, with schizophrenic subjects). There is always room for doubt about the validity of extending the conclusions to the general population.

Scientifically, these studies are important in demonstrating the physiological effects of particular oils or even individual fatty acids under defined conditions. However, from the point of view of practical nutrition and the effectiveness of population strategies for the prevention of heart disease, the important question is whether advice to modify dietary fat brings about the desired change in 'real life'? A recent review examined the effects of dietary modification in 16 major intervention studies (52).

Although each study was different in many respects, they could be divided into two groups according to the stringency of the diet.

In Group 1, fat contributed less than 30% of total energy, cholesterol less than 300 mg per day and the PUFA to SFA ratio was about 1. For most people

in 'Western' countries, this would represent quite a marked change in diet but could just be considered practical. These diets resulted in reductions in cholesterol from 0.2% to 4% in nine studies, no change in one and an increase in 1% in another. Taken overall, there was little impact on plasma cholesterol.

The diets in Group 2, however, were considerably more extreme. Fat again contributed less than 30% energy, cholesterol intake was less than 200 mg/day and the ratio of PUFA to SFA was very high at 1.4. Many people would find difficulty in adhering to this diet for very long and would almost certainly need great encouragement and supervision. These diets, however, resulted in falls ranging from 6.5 to 15.5%.

These conclusions contrast sharply with the assertion in many guidelines that cholesterol will fall by 10–25% in response to diet similar to those in the Group 1 studies. Why have perceptions been so unrealistic? Too much reliance has been placed on the results of short-term experiments involving supervised captive populations. There has been a tendency to extrapolate unjustifiably from studies using more rigorous diets, from subjects with higher plasma cholesterols than the normal population and from drug trials which tend to give larger and more consistent cholesterol lowering.

It is noteworthy that the most recent official UK report on diet and cardiovascular disease (3) does not dispute the analysis presented in reference (52) but simply says that "This strongly suggests that the actual diet was not the same as that prescribed over the period of the study". This says little for the quality of the nutrition research on which the report bases its sweeping statement: "Diet is a major and modifiable cause of cardiovascular disease", or its far reaching recommendations for dietary change. It also sends the message that because the results did not coincide with the Committee's preconceived conception, the studies must have been flawed.

Does lowering plasma cholesterol reduce the risk of CHD?

The effects of modifying diet, blood cholesterol, or other 'risk factors' may be put to the test in relation to any of these aspects of vascular disease that come under the umbrella term 'coronary heart disease' (see earlier section). The direct effects on either atherosclerosis or thrombosis have not so far been investigated very thoroughly because reliable methods for studying these processes in living patients have been lacking.

Thrombosis

Experimental studies linking dietary lipids with thrombosis are still in their infancy and have been of two main types: effects on (i) platelet aggregation,

and (ii) on different components of the coagulation cascade. Platelet studies rely on tests of function *in vitro* whose relationship with events *in vivo* is uncertain. These have been inconsistent, although some studies have shown increased potential for aggregation in diets with a high intake of SFA (3). In regard to components of the coagulation cascade, the blood concentration of a protein called Factor VII coagulant is a strong and independent risk factor for CHD. It is now well established that the concentration of the activated form of this protein increases in the blood after a fatty meal, more or less in proportion to the degree of lipaemia. In some studies the reduction of fat intake as a proportion of dietary energy reduced the plasma concentration of Factor VII_c but the composition of the fatty acids had no influence (53,54). In another study, a high-fat, high-oleate meal led to greater activation of Factor VII than a low-fat meal; moreover, a meal rich in oleate had greater activating potential than one rich in palmitate (55).

Most studies have found the concentration of plasma fibrinogen (a powerful predictor of CHD) to be uninfluenced by dietary fat. The few studies so far reported on the effects of dietary lipids on the fibrinolytic system (which dissolves clots) are conflicting (56). Studies on the effects of diet on different parts of the blood coagulation system that are likely to influence thrombosis are still in their infancy. Current knowledge has been comprehensively reviewed (56).

Atherosclerosis

Studies in man, using angiographic techniques (radiological examination of arterial width) to demonstrate progression or regression of atherosclerosis in relation to various 'risk factors', have not been conclusive. Successful studies have used drugs or surgery to lower blood cholesterol (3); diet alone has not been shown to succeed. Many angiographic studies have concluded that the rate of progression or regression is unrelated to concentration of blood lipoproteins, family history, smoking habit, obesity or blood pressure: all risk factors regarded as important enough to feature in strategies for disease prevention (9). Dietary data have often been poor and simultaneous changes in other lifestyle factors often makes interpretation difficult. The fact that in some patients progression of lesions may be observed in one location and regression in another suggests strongly that local factors are more important than these 'global' risk factors.

In one study (reviewed in references 3,6) surgical intervention, but not a rigorous diet, reduced LDL concentrations significantly. Although the authors claimed that there were beneficial effects in the reduction of

atherosclerosis progression, the measurement of progression was crude and the claimed improvements in the surgical group compared with controls have to be judged against a background in which the proportion of subjects in whom the extent of atherosclerosis worsened was always vastly greater than the proportion in whom the condition improved. At best, twice as many surgical patients had a significant worsening of their disease as those who improved, even when their total cholesterol was at a 'favourable' concentration of 4.7 mM, LDL at 2.7 mM and HDL at 1.1 mM. This seems to indicate that there are more important factors resulting in atherosclerosis progression than the lipoprotein profile and runs contrary to the idea that radical decreases in plasma cholesterol result in significant regression of atherosclerosis. Most importantly, there was no significant improvement in CHD mortality in this study. Although this was not a dietary study, I have included it in this critical review because it has been cited as evidence that aggressive lipid lowering can be effective in reducing CHD risk and that by implication the results can be extrapolated to diet (3).

Coronary heart disease

I have discussed the merits and deficiencies of the intervention studies in reference (6). There have been few primary prevention trials that intervened with diet modification alone. They achieved small but significant reductions in 'coronary events' but treated small numbers of subjects and had severe design flaws. The largest published study, organized by the World Health Organization (57), involved 60 881 people. Non-fatal coronary events, coronary deaths and total deaths were respectively: 561, 450, 1341 (controls) and 505, 428, 1325 (treated) after intervention in respect of diet and four other risk factors for up to 6 years. Differences were not significant.

The most successful study (58), in Oslo, Norway, involved 1232 men with very high blood cholesterol concentrations. Risk factor interventions were by dietary fat modification (achieving 13% reduction in blood cholesterol concentration) and smoking reduction. Non-fatal coronary events, coronary deaths and total deaths were: 22, 14, 24 (controls) and 13, 6, 16 (treated). The reduction in non-fatal coronary events was significant but there are many reasons why these results of this trial should not be generalized (6).

Despite the volumes of words that have been written justifying widespread dietary change on the basis of the intervention trials, a strong conclusion must be that they fail the test: they provide little or no evidence for worthwhile benefits of dietary modification in respect to coronary deaths, none in respect of overall mortality, and only marginal benefits in regard to non-fatal coronary events.

These conclusions are brought out in analyses of the intervention trials by several authors (reviewed in 6,9) although quite a different picture is presented in other reviews (3,21). Indeed, a series of papers in the *British Medical Journal* by Law and colleagues (reference 22, and reviewed in reference 3) used sophisticated statistical techniques to demonstrate that previous estimates of benefit had been grossly underestimated.

Meta-analysis invariably involves some sort of selection of which trials will be analysed and cannot compensate for flaws in the original research. Problems have included poor design, selection by initial screening procedures of subjects most likely to respond to diet, unjustified extrapolation from drug trials to what the effects of diet may be, and from high risk individuals to the general population, failure to abide by the originally chosen criteria for statistical significance, and presentation of results in abstracts as significant when, in the text they clearly fail to reach significance (6). In reviewing the results of trials as a basis for formulating dietary guidelines, groups of 'experts' have blatantly selected those trials giving a more favourable outlook (10).

Of particular concern has been the suggestion that cholesterol-lowering, particularly by drugs, may lead to increased mortality from causes other than cardiovascular disease (3,6,8). This may explain the apparent lack of impact of cholesterol-lowering strategies on total mortality and is cited as evidence for the need for a cautious approach to the provision of population advice (59). Arguments that these concerns are unwarranted (3) do not seem entirely convincing and doubts are bound to linger. I have discussed this topic in more detail in a *Lipid Technology* article (60).

Other forms of cardiovascular disease: dietary fat and stroke

I have called this chapter 'Dietary fats and cardiovascular disease'. As outlined in an earlier section, CVD is a broad term that embraces diseases of the blood vessels of the heart (CHD), brain (cerebrovascular disease, stroke) and the limbs (peripheral vascular disease). A very large proportion of the published work on diet in relation to CVD has been concerned with CHD: very little has been published on stroke. Yet in many developed countries, stroke is the third most common cause of death, after coronary heart disease (CHD) and cancer. Despite the paucity of research, it is frequently assumed that the same risk factors are operative for CHD and stroke and that dietary associations will be similar if not identical. It is important, therefore, briefly to review work specifically on stroke.

The epidemiology of stroke

Even from the little published work on stroke, it is quite clear that the geographic distribution and time trends in mortality from stroke and CHD are quite dissimilar (61). Furthermore, the male excess in risk of CHD, especially at younger ages, is not seen with stroke and a major predictor of CHD, blood cholesterol concentration, is less consistently associated with stroke. Patterns of mortality among different ethnic groups in the UK are quite different between CHD and stroke (3). In England and Wales there was a slow but steady fall in stroke mortality between 1900 and 1950; after 1950 a much steeper decline began, which still continues (3,61). By contrast, male CHD deaths showed a slow but consistent rise between 1900 and 1920, a steep rise from 1920 to the 1950s, followed by a slow but steady fall from the early 1960s to the present day. The peak occurred in the early 1970s in middle-aged men.

These trends in stroke mortality have occurred in almost all developed countries, although the rates of change have differed somewhat. Only in the former Soviet countries has there been a rise since 1970 (61). Whereas there is always room for doubt about the reliability of mortality statistics, the magnitude and consistency of the decline in stroke mortality and the worldwide similarity in patterns speak against it being artefactual. In any case, there are few reliable data on incidence (the number of new cases per year) but where comparative statistics exist, they tend to show that incidence rates have moved in parallel with mortality rates.

Given the assumptions about the close relationship between stroke and CHD, despite these clear-cut differences in epidemiology, it is not surprising that assumptions have also been made that high intakes of fat and especially of saturated fatty acids must be implicated as risk factors for stroke.

The UK Department of Health's 1994 report 'Nutritional Aspects of Cardiovascular Disease' specifically set out to review both heart disease *and* stroke (3). Its dietary recommendations made no distinction between the two diseases and the conclusion must be that the committee were satisfied that there was persuasive scientific evidence that reduction in total and saturated fatty acids would reduce the risk of stroke. Nevertheless, they found that blood concentrations of cholesterol less than 5 mM were associated with increased risk of haemorrhagic stroke; no comment was made about the association between ischaemic stroke risk and blood cholesterol. However, an American study found only a weak association between blood cholesterol concentration and ischaemic stroke (62).

Several pieces of evidence from Japan and Hawaii (cited in reference 63) suggested that intake of both total fat and saturated fatty acids was *inversely* associated with total stroke mortality after adjustment for potential confounding factors. By contrast, a study in Sweden (64) found no associations between fat consumption and stroke.

A recent American study

Because these studies had limited statistical power and because ischaemic stroke is relatively more common in Western as compared with Asiatic countries, Gillman and colleagues (63) decided to re-investigate associations between dietary fat and stroke, using data from the now famous Framingham Heart Study. This longitudinal study of cardiovascular and other diseases and their associated risk factors began in 1948. The original cohort consisted of 5209 men and women, who have since been examined biennially. Less than 2% of subjects have been lost to follow-up.

A subset of 832 men took part in the stroke study. They were between 45 and 65 years of age in 1966–69 and were free of cardiovascular disease at that time. On average they consumed 11 MJ energy per day of which 39% came from fat, representing 114 g per day. Average intakes of the main fatty acid classes were: saturated (SFA) 44 g/day (15% energy); monounsaturated (MUFA) 46 g (16%) and polyunsaturated (PUFA), 16 g (5%). The men were followed up for 20 years during which time there were 61 cases of ischaemic stroke. Using standard statistical techniques, the authors calculated the relative risk (RR) of stroke over this period for different levels of intake of the various types of fat and for total fat. Each relative risk was given a set of '95% confidence intervals' (CI) which provide a measure of the variability of the measurements and the degree of statistical significance. Confidence intervals that span the value of 1.00 indicate that the RR is not significantly different from unity.

A summary of the findings is presented in **Table 2.2**. The risk of ischaemic stroke declined significantly from the lowest to the highest intakes of total fat, saturated and monounsaturated fatty acids, but not polyunsaturated fatty acids. No significant associations were found with haemorrhagic stroke, but the number of cases was very small. For every 3% increase in total fat consumption, the relative risk of stroke was 0.84 (i.e. a 16% reduction in risk) after adjustment for nine potential confounding factors. The corresponding values for SFA and MUFA were respectively 0.90 and 0.87. When the stroke cases were divided into those who remained free of CHD at the time of their stroke, the inverse association with fat intake remained the same. However, in stroke cases who had also developed CHD, the association disappeared (RR = 1.05, CI = 0.88–1.23).

Table 2.2. Associations between fat intakes and ischaemic stroke risk.

	Quintiles of intake[1]					*P*	RR[3]	95%CI
	1	2	3	4	5			
	Number of stroke events[2]							
Total fat	112.3	84.8	57.2	53.4	41.8	0.01	0.84	0.75–0.92
SFA	131.1	69.3	61.4	72.4	31.8	0.002	0.90	0.83–0.96
MUFA	132.7	70.0	44.2	53.0	66.2	0.01	0.87	0.81–0.94
PUFA	85.3	75.4	69.5	74.4	58.0	0.41	–	–

Data from reference 63. [1]Intakes of fat were divided into fifths (quintiles) of the distribution, quintile 1 representing the lowest intake, quintile 5 the highest. [2]Age-adjusted cumulative ischaemic stroke incidence rates per 1000 subjects. [3]Relative risk of ischaemic stroke for each increment of 3% of energy intake for total fat and for each 1% of energy from SFA and MUFA, adjusted for systolic blood pressure, cigarette smoking, glucose intolerance, body mass index, physical activity, left ventricular hypertrophy and intakes of total energy, alcohol, fruits and vegetables. CI = confidence interval. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids ; PUFA = polyunsaturated fatty acids. The P value relates to the significance of the trend from the lowest to highest values.

What do we make of this?

When published results are controversial, it is even more important than usual to ask searching questions about the strengths and weaknesses of the study. A great deal of experience and expertise has gone into the Framingham study. There has been a notably low drop-out, the follow-up was over a long period and extreme care was taken to adjust for known confounding factors. Of course there may well be some that are unknown and could not have been included. The weakest aspect of the Framingham Study has always been the measurement of dietary variables and this part of it is no exception. When using a single 24 hour recall dietary diary, there is an extremely high risk of classifying a significant proportion of participants into an inappropriate quintile of fat intake. The authors' claim that a more precise method would have revealed a stronger association may be true but, to this reviewer at least, it has a hollow ring to it.

Gillman and colleagues examined fat intakes in their subjects in quintiles of fat intake expressed as a percentage of energy. Table 2.2 in their paper reveals that absolute energy intakes differed two-fold among quintiles, 7.9 MJ being consumed by subjects in the lowest quintile of fat intake and 14.4 MJ by those in the highest. Despite these variations, physical activity and body mass index did not differ across quintiles. These data reveal inconsistencies that

should lead us to interpret measurements of fat intake with great caution. It is not generally known that the Framingham Study has never convincingly demonstrated associations between diet and cardiovascular disease. The only rigorous dietary measurements made in the study were published only in a US Government report (65), not in generally accessible journals.

Inconsistencies between different parts of the Framingham Study come to the surface in this paper. Thus, on page 2148 (column 1, second paragraph of the 'comment' section) the authors refer to a paper that found a direct association between intake of total fat and saturated fatty acids in the same data set (66). They used this 'fact' to justify the reliability of their finding that stroke and fat intake were inversely associated, since the 'expected' direct association between CHD and fat intake was found in the same men (66). However, on page 2149 (column 1, last few lines) they stated that their own data revealed a lack of association between CHD and fat intake. They used this 'fact' to dismiss the hypothesis that their results could have been explained on the basis of 'competing mortality'. The argument goes that subjects who died from CHD (which tends to occur at earlier ages than death from stroke) would be no longer susceptible to stroke. Thus, survivors of CHD, who might be candidates for stroke, would tend to have lower intakes of fat and saturated fatty acids than the whole cohort. This idea was dismissed on the grounds that there was no association between fat and CHD anyway.

An editorial in the same issue of *Journal of the American Medical Association* (67), assesses the implications of Gillman and colleagues' findings. However it misses the point by getting bogged down in a lengthy discussion of the role of blood cholesterol. This is irrelevant because the Gillman paper shows (Table 2.2) that blood cholesterol hardly differed across the whole range of fat intakes from 26–51% of energy. The editorial states that Gillman's results tend to support advocates of the Mediterranean style of diet (which is low in saturated fatty acids) and does not suggest that current dietary guidelines (reduce saturated fatty acids) are inappropriate. This is a misrepresentation of the paper's findings. Taken at face value, Gillman's results suggest that low intakes of saturated fatty acids are associated with an increased risk of stroke. The editorial is a clear example of the wishful thinking engaged in by those who are convinced that dietary fat intake is a major contributor to cardiovascular disease of all types and find difficulty in extricating themselves from this way of thinking.

The Gillman paper does not add anything to our understanding of the causes of stroke but it does underline the futility of aiming to prevent cardiovascular disease by general advice to change dietary fat consumption.

In their closing sentences, the authors acknowledge this and urge that other measures for the prevention of stroke be emphasized. Ironically, they fail to mention that the control of diabetes and hypertension by diet are equally controversial.

Is the lipid hypothesis well founded?

It will be clear from the preceding sections that my conclusion is that the lipid hypothesis is not well founded and the main reasons are summarized in **Table 2.3**.

Table 2.3. Deficiencies in the `lipid hypothesis'.

- There is insufficient correspondence in vascular pathology between (a) animals and man; (b) general atherosclerosis and lesions seen in familial hypercholesterolaemia.
- International epidemiology is flawed by confounding factors and selection biases.
- Within countries, epidemiology gives little support for a relationship between dietary fat and coronary heart disease (CHD). Fat intake does not explain total CHD, regional, sex or social class differences.
- Trends in CHD mortality are not coincident with changes in the amount and type of fat eaten.
- The hypothesis cannot explain the greater risk in women compared with men, enhanced risk in women post-menopause or consistent recent falls in mortality in women compared with more erratic changes in men in many countries. There have been no corresponding falls in CHD incidence.
- Plasma total cholesterol is a weak predictor of CHD compared with haemostatic factors. Less than 50% of CHD risk is accounted for by known 'risk factors'.
- Extrapolations from drug trials in high-risk individuals to the general population are unwarranted.
- Intervention trials have not demonstrated a major impact of dietary fat modification on CHD and none on total mortality. Only the most stringent diets achieve useful plasma cholesterol reductions.

Dietary fat and cardiovascular disease: are current guidelines justified?

Although published in 1994, the last report of the UK Department of Health's Cardiovascular Review Group of the Committee on Medical Aspects of Food Policy (COMA) remains the basis for public health policy (3). Having argued the case that causal links between dietary fats and CVD are at best tenuous and at worst spurious, it seems appropriate to end this review with a detailed examination of the COMA recommendations.

Background to the COMA report

In preceding sections I have examined the scientific evidence for and against a major role for dietary fatty acids in the development as well as the treatment of coronary heart disease (CHD). The words 'major' and 'dietary' are important. Nobody doubts that the metabolism of lipids in the body is intimately involved in the various processes contributing to the disease and it would be rash to conclude that dietary lipids have no part to play at all. My principal conclusions were that careful analysis of available evidence did not support the view that dietary saturated fatty acids caused the disease (as many have argued), or that making changes in the general population's fatty acid intakes would significantly alter the incidence of the disease or mortality from this cause.

The UK Department of Health has a standing Committee on Medical Aspects of Food Policy (COMA) that is charged with keeping abreast of research in food and nutrition that may have implications for health. It appoints panels to investigate and report on specific topics. The Panel on Diet and Cardiovascular Disease first reported in 1974, again in 1984 (68) and most recently in November 1994 (3). On this occasion its report came soon after another COMA panel's report on Dietary Reference Values on which I have commented (2). Furthermore the latest COMA report on Nutritional Aspects of Cardiovascular Disease (3) was intended to inform the work of the Nutrition Task Force that is overseeing the dietary aspects of the Department of Health's initiative known as 'The Health of the Nation' (now superseded by 'Our Healthier Nation').

The report will certainly be of national importance in the UK and is likely also to influence thinking internationally. It is, therefore, crucial that the conclusions are well based scientifically. Here I shall argue that many of the report's conclusion are not soundly based.

Comparison of the 1984 and 1994 Reports

The Cardiovascular Review Group's (CRG) brief was to review what had been recommended by the 1984 report and to advise COMA on any changes considered necessary in the light of new knowledge. When the 1984 (68) and 1994 (3) recommendations are put side by side it can be seen that there are remarkably few changes (**Table 2.4**.).

The recommended cut in SFA is more draconian (so much so that it will be virtually impossible to achieve in practice). Regarding PUFA, there is now an emphasis on the distinction between *n*–3 and *n*–6 that was not there before and a recognition that *n*–3 intakes should be increased. Probably the most important difference is that a ceiling of 10% of energy was set on PUFA intake. The reasons for this have to be teased out but mainly seem to be due to worries, albeit cautiously expressed, about potential oxidation problems. Apart from these changes in emphasis concerning PUFA, the lack of change in most recommendations implies that little new knowledge has come to light in the intervening 10 years.

Although the scope of the Report covered diet in general, the CRG clearly came to the conclusion that dietary fat was of overriding importance since no less than 7 out of the 10 nutrient recommendations are concerned with dietary

Table 2.4. COMA nutrient recommendations 10 years apart.

Nutrient	1984	1994
Total fat (%E)	31–35	35
SFA (%E)	15	10
MUFA (%E)	No recommendations	No recommendations
n–6 PUFA (%E)	3.5–6.8	No more than 10%E
n–3 PUFA (%E)	–	0.2 g/day
Trans	Incl. with SFA	No more than 2%E
Cholesterol	No recommendations	Not to rise
Complex CHO	Compensate for reduced fat	Compensate for reduced fat
Simple sugars	Not to rise	No recommendations
Salt	Reduce	< 6 g/day
Potassium	No recommendations	Increase to 3.5 g/day

%E = as percentage of energy; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids ; PUFA = polyunsaturated fatty acids; CHO carbohydrates.

fat and a third of the section devoted to 'Diet and Risk' is directly concerned with lipids. It should also be noted that although the brief was to consider cardiovascular disease (CVD), consideration was given almost entirely to CHD rather than to stroke.

Selectivity and inaccuracy in the citations

A legitimate criticism of the 1984 report was that it contained few references, so that the reader could not assess what evidence had been considered. The CRG obviously agreed because it has now cited 414 publications compared with 24 in 1984. Nevertheless a perceptive reader soon realizes that quantity does not imply quality. Too many citations are to reviews, unpublished work and flimsy abstracts.

Many publications have been cited inaccurately. An example of the sin of not reading the original paper properly comes in the section on *trans* fatty acids. The main thrust of the CRG's argument here is that there is now sufficient evidence to conclude that *trans* fatty acids consumption contributes to CVD risk. It cites a case-control study (69) as providing evidence of a direct contribution. Had the CRG's members read the paper properly, they would have seen that the relative risk did not increase in a dose-response manner across the whole range of intakes. Indeed, from the results presented, one could almost make a case for a 'protective effect' of moderate intakes of *trans* fatty acids! The statement in the Report of the results in this paper was taken word for word from the authors' abstract, suggesting the possibility that the CRG had read no further than the abstract.

The least satisfactory aspect of the Report is its selection of published material to support the case being made. Central to the CRG's case is that a high concentration of blood cholesterol, more particularly LDL-cholesterol, causes atherosclerosis and, by implication, CVD. This idea is certainly widely held. Is it true? The Report does not in fact cite any evidence that LDL-cholesterol causes atherosclerosis. The Group certainly avoids citing several publications that demonstrated that there is little or no relationship between blood cholesterol and degree of atherosclerosis (e.g. 70).

There is also a similar lack of association between plasma cholesterol concentration and CVD mortality as demonstrated by the WHO MONICA study taking values from about 40 countries around the world (71). The CRG, however, relies mainly on the highly selective Seven Countries Study(13).

The CRG is perpetuating a long tradition of citation bias. Throughout the published literature there are publications that appear to support the lipid

hypothesis and those that do not. Examination of the Science Citation Index reveals that 'supportive' trials are overwhelmingly more frequently cited than 'non-supportive' ones (10).

Relying on such selective material, the CRG is able to make the sweeping statement, not borne out by any scientific evidence cited by it, or available to me, that diet is a major cause of CVD and a principal means by which it can be prevented. The following discusses how the CRG justified its stance.

The influence of practical dietary change on blood cholesterol level

To play a central role in prevention, dietary factors must be proven to prevent CVD. No study published to date has proved that changing diet prevents CVD and no amount of 'meta-analysis' (in which the CRG puts enormous faith) can provide this proof where individual studies did not, nor can they make up for the gross deficiencies of individual studies. If we focus on the SFA–blood cholesterol–CVD links, which is the main plank of the CRG's arguments, the analysis of Ramsay *et al.*(52) clearly reveals that lowering blood cholesterol by dietary modifications proposed by the CRG is rarely as effective as claimed. It is interesting that the CRG does not contest the conclusion of Ramsay *et al.* Its actual response is: "This strongly suggests that the actual diet was not the same as that prescribed over the period of the study".

In other words it is saying that all the relevant intervention studies that employed diets that are potentially achievable by most people were fundamentally flawed in that the subjects in the experimental groups did not eat the diet that the authors reported they ate in the publication. This is either a terrible indictment of dietary studies, reflecting badly on authors, journal editors and the science of nutrition or an example of a professional committee with a preconceived view that a certain type of diet will lower blood cholesterol by a specified amount and when it finds that the scientific evidence does not bear this out, choosing to ignore the evidence. In fact, a few studies cited by Ramsay *et al.*(52) did provide convincing evidence that participants had eaten the diets they were allocated but in many others it was not at all apparent that this was the case. If the CRG were correct that the participants in these trials, given every encouragement by armies of dieticians, doctors and research workers to follow the prescribed regimen, did not actually achieve what was expected, how much less likely is it that the general population of the UK can be prevailed upon to do what it is advised?

The CRG says that the studies cited by Ramsay *et al.*(which were designed to change dietary behaviour in such a way as to reduce CVD) contrast with "the strong evidence for the effects of diet on plasma cholesterol and of reduction of plasma cholesterol on CHD risk". What is this "strong evidence"? Regarding effects of dietary fat modification on plasma lipids, the appropriate sections are 6.2.2 and 6.2.3, citing 30-year-old work (references 209–211 in the report) with patients in institutions, many of whom were schizophrenics and unlikely to have been representative of the general population, especially in terms of activity levels.

There is an enormous range of plasma cholesterol responses to dietary fats between individuals. Thus while the average response of a group of people to a diet rich in saturated fatty acids is a *rise* in blood cholesterol, this is mainly due to exaggerated responses of a few individuals: about a third of subjects can be expected to experience a fall in cholesterol (72). If the responses of individuals — which are unknown and unpredictable — to dietary components, that are recommended as being guaranteed to lower blood cholesterol, can be the opposite of what is assumed, what is the rationale or indeed the morality of recommending such changes to everyone?

Reducing CHD risk as a result of changing diet and/or blood cholesterol

The CRG quotes several pieces of evidence (from both epidemiological and experimental studies) to support its case that dietary fat modification of the type it recommends will directly improve CVD mortality and morbidity.

Much is made, for example, of migrant studies which are cited as demonstrating that genetic predisposition is not a major determinant of CVD death rate and that environmental factors, of which diet is the main focus of attention, are paramount. Study of the cited publications reveals that little detailed dietary information was available. Moreover, citations are again selective. A study of a Pacific island community showed that when the inhabitants (who have a fat consumption of 50% of energy intake and SFA consumption of 48% energy, mostly from coconut oil) migrated to New Zealand, their SFA intake decreased and their blood cholesterol increased (40). Evidence from migration studies cannot be used to make a conclusive case for SFA–CVD links when selective or inappropriate citations have been used.

Many studies, not cited by the CRG, failed to confirm that fat intakes of men who experienced a CVD event were significantly different from those free

of the disease. Secular trends in fat consumption do not give firm scientific evidence that changes in CVD mortality are in any way related and indeed, tend to argue against a relationship (see earlier section).

It is noteworthy that when one examines many of the CRG's own tables and figures, they do not appear to support the case being made. For example, in one table the % distribution of total blood cholesterol in English men and women in 1986–87 is compared with that in 1991. There were mainly upward trends in both sexes yet CHD deaths have been falling. This is not consistent with a *causal* role for blood cholesterol. In this and several other tables, the risk factor profiles simply do not match up with the disease profiles. Examining these figures and tables, an intelligent reader with little background to the diet–CVD issue, reading this report and knowing the UK's CHD mortality ranking would hardly be able to understand the rationale for advising the Report's food recommendations.

The 'evidence' discussed above comes from observational studies. The only real way to test an hypothesis is by experiment. If it were easy to reduce CVD incidence or mortality by dietary fat modification, then this should have been picked up by the many intervention trials that have been conducted, costing a great deal of taxpayers' money. Most failed dismally and it has been common to attribute this to lack of statistical power in individual studies, necessitating recourse to meta-analysis in which studies are pooled. The CRG rely heavily on this approach. Another interpretation (10) is that the overall result of no less than 26 controlled cholesterol-lowering trials demonstrates little overall benefit.

Interestingly, a study published after the COMA report came out, reported that substantial lipid-lowering by drugs had absolutely no effect on arterial disease (measured by angiography) in people with diagnosed CVD but blood cholesterol in the normal range (73). Since these represent most of the population in whom CVD occurs, it seems unlikely that the dietary recommendations in the Report could have any impact whatsoever.

Much concern has been expressed about the apparent excess of total deaths in the treated groups in many trials. The CRG dismiss this concern lightly, arguing that such deaths occurred mainly in men who did not take the treatment or who had psychiatric illness already. Little weight is given to opposing arguments (74).

Epilogue: The politicization of science

Before reading the report of the UK Department of Health's COMA Committee 'Nutritional Aspects of Cardiovascular Disease' (3), I had begun to doubt whether anyone seriously believed that saturated fatty acids *cause* heart disease but the reader is left in no doubt that this was the committee's conclusion. This seems to me to be a flagrant misinterpretation of scientific information.

From a nutritionist's viewpoint, there can be no doubt that eating too much of any dietary component — and fat is a good example — is not conducive to good health. My message is not that dietary fat modification may not be beneficial for some individuals (not least because of potential improvements in energy balance), but this is quite a different matter from claiming that a fatty diet is the reason why so many people in industrialized countries die from a heart attack. The fact that over 50% of CHD is unexplained by any of the frequently described environmental factors, let alone fat consumption, and that mortality and morbidity have not followed similar patterns in relation to fat consumption, should make scientists sceptical of such over-generalizations. It should also lead them to probe more deeply into alternative mechanisms to explain the upsurge and decline of CHD in this century.

As long ago as 1951, Page (75) wrote: "I have no doubt that we are grossly oversimplifying the problem of both the etiology and treatment of arteriosclerosis. Lest we do more harm than good, let us refrain from drawing hasty conclusions". Despite the vast increase in knowledge in the intervening 41 years, this advice still holds good.

It is time for the lipid hypothesis to be discarded and replaced by something better so that research in this field can move forward.

Unfortunately this is unlikely to happen quickly. Too many reputations are at stake and the demands of good science too often clash with the pressures of public health policy. Those who began their careers as careful scientists, all too frequently enter the public health arena with a kind of evangelistic zeal that then obscures their former scientific judgement. Readers who may feel that this writer's own judgement is under strain may like to read 'The (political) science of salt' by Taubes (76). This penetrating article reveals many uncanny parallels between the dietary fat/heart disease story and the equally controversial concept that excessive salt intake underlies the problem of hypertension and that salt reduction provides an easy solution. The article begins with a quotation from Thomas Huxley and I can do no better than to conclude with it: "Science . . warns me to be careful how I adopt a view which jumps with

my preconceptions and to require stronger evidence for such a belief than for one to which I was previously hostile. My business is to teach my aspirations to conform themselves to fact, not to try and make facts harmonize with my aspirations" (Thomas Huxley, 1860).

A few years ago I visited a friend whom I had not seen for some time. He had recently moved and I had never been to his new house, which was deep in the country in a street called 'Dark Lane'. When I arrived it was dark and I was tired. I turned the car into Dark Lane and before long realized that I was in deep trouble: the lane had become little wider than the car and I needed to extricate myself, which was only done with difficulty and a certain amount of humiliation. The car and my temper were seriously damaged.

Reading the COMA report on diet and cardiovascular disease, and many like it, causes me to conclude that many scientists dealing with this subject have gone deep into their own 'Dark Lane' from which it is difficult, if not impossible, to retreat. Reputations have been built on the diet–heart concept; enormous amounts of our money have been spent on shoring up a dubious hypothesis. That is why each report that is produced looks pretty much like the last and why protagonists can say complacently that numerous learned committees around the world have come to the same conclusion. Of course they have: they all start from where the last left off and they cannot afford to do other than support the party line. This latest offering from the UK Department of Health's COMA Committee is little different from the rest. Its obsession with dietary fat and certain foods rich in saturated fatty acids may result in garbled messages to the public which may have the effect of discouraging rather than encouraging sensible eating. If a central policy involves getting people to change their diets so that they may suffer less CHD, then such advice had better be based on good scientific evidence and it should be known that such advice will work.

References

1. Department of Health (1992). The Health of the Nation: a strategy for health in England. Her Majesty's Stationery Office, London.
2. Department of Health (1991) Dietary reference values for food energy and nutrients for the United Kingdom. Report on Health and Social Subjects, 41, Her Majesty's Stationery Office, London.
3. Department of Health (1994) Report on Health and Social Subjects 46. Her Majesty's Stationery Office, London.
4. World Health Organization Study Group. (1990) Technical Report Series *797* WHO, Geneva.

5. The Scottish Office Home and Health Department (1993). The Scottish Diet. Report of a Working Party to the Chief Medical Officer for Scotland.
6. Gurr, M.I. (1992) *Progress in Lipid Research*, 31, 195–243.
7. Hulley, S.B. *et al*. (1992) *Circulation*, 86, 1026–1029.
8. Oliver, M.F. (1993) *British Journal of Clinical Pharmacology*, 47, 26–29.
9. Rosenman, R.H. (1993) *Homeostasis*, 34, 1–44.
10. Ravnskov, U. (1992). *British Medical Journal*, 305, 15–19.
11. Stehbens, W.E. (1990) *Medical Hypotheses*, 31, 105–113.
12. McGandy, R.B. and Hegsted, D.M. (1975). In: The Role of Fats in Human Nutrition, pp.211–230 (Vergroesen, A.J. ed.). Academic Press, London.
13. Keys, A. (1980) Seven Countries, Harvard University Press, Cambridge, Massachusetts, USA.
14. Gurr, M.I. *et al*. (1989) *Nutrition Research Reviews*, 2, 63–86.
15. McNamara, D.J. (1990) In: Meat and Health, Advances in Meat Research, 6, pp.63–87 (Pearson, A.M. and Dutson, T.R. eds), Elsevier Applied Science, London.
16. Grundy, S.M. and Denke, M.A. (1990) *Journal Lipid Research*, 31, 1149–1172.
17. Hegsted, D.M. *et al.* (1993) *American Journal of Clinical Nutrition*, 57, 875–883.
18. Mensink, R.P. and Katan, M.B. (1990) *New England Journal of Medicine*, 323, 439–445.
19. Goldstein, J.L. and Brown, M.S. (1983) In: The Metabolic Basis for Inherited Disease, 5th edn, pp.672–712 (Stanbury, J.B., Wyngaarden, J.B., Fredrickson, D.S., Goldstein, J.L. and Brown, M.S. eds), McGraw–Hill, New York.
20. Marmot, M.G. and Mann, J.I. (1987) In: Ischaemic Heart Disease, pp.1–31 (Fox, K.M. ed), MTP Press, Lancaster, UK.
21. Holme, I. (1990) *Circulation*, 82, 1916–1924.
22. Law, M.R. *et al.* (1994) *British Medical Journal*, 308, 363–366.
23. Blackburn, H. (1987) In: Hypercholesterolemia and Atherosclerosis: Pathogenesis and Prevention, pp.53–98, (Steinberg, D. and Olefsky, J.M. eds), Churchill Livingstone, New York.
24. Hirai, A. *et al.* (1982) *Thrombosis Research*, 28, 285–298.
25. Wood, P.D.P. (1981) *Statistician*, 30, 131–136.
26. Stewart, A.W. for the World Health Organization MONICA Project (1994) *International Journal of Epidemiology*, 23, 505–516.
27. Renaud S. and de Lorgeril M. (1992) *Lancet*, 339, 1523–1526.
28. Moore, R.D. and Pearson, T.A. (1986) *Medicine*, 65, 242–267.
29. Langer, R.D. *et al*. (1992) *Circulation*, 85, 910–915
30. Renaud, S. and de Lorgeril, M. (1989) *Journal of Internal Medicine*, 225 (S1), 39–46.
31. Renaud, S. *et al*. (1983) *Atherosclerosis*, 47, 187–198.
32. Douste-Blazy, P. *et al*. (1988) *Acta Medica Scandinavica*, Suppl 728, 137–143.
33. Gey, K.F. *et al*. (1991) *American Journal of Clinical Nutrition*, 53, 326S–334S.
34. Willett, W.C. *et al.* (1993) *Lancet*, 341, 581–585.
35. Aro, A.V. *et al*. (1995) *Lancet*, 345, 273–278.

36. Roberts, T.L. *et al.* (1995) *Lancet*, 345, 278–282.
37. Fehily, A.M. *et al*. (1993) *British Journal of Nutrition*, 69, 313–314.
38. Gregory, J. *et al*. (1990) The Dietary and Nutritional Survey of British Adults, Her Majesty's Stationery Office, London.
39. Prior, I.A. *et al.* (1981) *American Journal of Clinical Nutrition*, 34, 1552–1561.
40. Stanhope, J.M. *et al*. (1981) *Journal of Chronic Diseases*, 34, 45–55.
41. Kesteloot, H. *et al*. (1989) *Atherosclerosis*, 78, 33–38.
42. OPCS Monitor, Series DH2, Her Majesty's Stationery Office, London.
43. World Health Statistics Annual, WHO, Geneva.
44. Stallones, R.A. (1980) *Scientific American*, 243, 43–49.
45. Uemura, K. and Pisa, Z. (1988) *World Health Statistics Quarterly*, 41, 155–177.
46. Annual Reports of the National Food Survey Committee, Ministry of Agriculture, Fisheries and Food, Her Majesty's Stationery Office, London.
47. United Nations Food and Agriculture Organisation Food Production Yearbook, 1982, FAO, Rome.
48. Lands, W.E.M. *et al.* (1990) *American Journal of Clinical Nutrition*, 51, 991–993.
49. Yarnell, J.W.G. *et al.* (1993) *Cardiovascular Risk Factors*, 3, 344–353.
50. Rosamond, W.D. *et al*. (1998) *New England Journal of Medicine*, 339, 861–867.
51. Levy, D. and Thom, T.J. (1998) *New England Journal of Medicine*, 339, 915–917.
52. Ramsay, L.E. *et al*. (1991) *British Medical Journal*, 303, 953–957.
53. Marckmann, P. *et al.* (1990) *Atherosclerosis*, 80, 227–233.
54. Sanders, T.A.B. *et al.* (1981) *Clinical Science*, 61, 317–324.
55. Oakley, F.R. *et al.* (1998) *American Journal of Clinical Nutrition*, 68, 1202–1207.
56. Vorster, H.H. *et al*. (1997) *Nutrition Research Reviews*, 10, 115–135.
57. World Health Organization European Collaborative Group (1986) *Lancet*, i, 869–872.
58. Hjermann, I. *et al*. (1981) *Lancet*, ii, 1303–1310.
59. Oliver, M.F. (1992) *European Journal of Clinical Investigation*, 22, 441–442.
60. Gurr, M.I. (1989) *Lipid Technology*, 1, 21–22.
61. Gale, C.R. and Martyn, C.N. (1997) *Journal of the Royal Society of Medicine*, 90, 138–143.
62. Benfante, R. *et al*. (1994) *Stroke*, 25, 814–820.
63. Gillman, M.W. *et al*. (1997) *Journal of the American Medical Association*, 278, 2145–2150.
64. Lapidus, L. *et al*. (1980) *American Journal of Clinical Nutrition*, 44, 444–448.
65. Gordon, T. (1970) The Framingham Study: an epidemiological investigation of cardiovascular disease (eds Kannel, M.B. and Gordon, T.). The Government Printing Office, Washington DC.
66. Posner, B. *et al.* (1991) *Archives of Internal Medicine*, 151, 1181–1187.
67. Sherwin, R. and Price, T.R. (1997) *Journal of the American Medical Association*, 278, 2185–2186.

68. Department of Health and Social Security (1984). Report on Health and Social Subjects, 28, Her Majesty's Stationery Office, London.
69. Ascherio A. *et al.* (1994) *Circulation*, 89, 94–101.
70. Lande KE, Sperry WM. (1936) *Archives of Pathology*, 22, 301–312.
71. Keil U. and Kuulasmaa K. (1989) *International Journal of Epidemiology*, 18, Suppl.1, S46–S55.
72. Kris–Etherton P.M. *et al*.(1993) *Metabolism*, 1993, 42, 121–129.
73. Sacks, F.M *et al*. (1994) *Lancet*, 344, 1182–1186.
74. Oliver, M.F. (1992) *European Journal of Clinical Investigation*, 22, 441–442.
75. Page, I.H. (1951) *Journal of the American Medical Association*, 147, 1311–1318.
76. Taubes, G. (1998) *Science*, 281, 898–907.

Chapter 3

Nutritional Significance of Lipid Peroxidation

Lipid peroxidation is initiated when a hydrogen atom is removed from a lipid molecule to generate a lipid radical. Interaction with oxygen generates peroxy and hydroperoxy radicals that are capable of maintaining a chain reaction until termination occurs by interaction with other radicals, for example antioxidants. When such processes occur in food lipids, the result is rancidity and deterioration in product quality. Nutritive value is reduced (by removal of essential fatty acids and antioxidant nutrients) and some oxidation products may be toxic. Lipid peroxidation occurs in a controlled manner as a natural part of several metabolic pathways, the most important being in the conversion of polyunsaturated fatty acids into prostaglandins and leukotrienes as described in Chapter 4. However, when the structural integrity of cells breaks down, for example as the result of an injury, lipids can suffer peroxidative damage from attack by reactive oxygen species that are formed during oxidative metabolism in the body.

Lipid peroxides can cause damage to macromolecules such as proteins and nucleic acids unless restrained by the body's normal antioxidant defence system. This consists of small antioxidant molecules, many of which are present in foods and some of which are essential nutrients; enzymes that destroy reactive oxygen species; and enzymes that repair damage after it has occurred. Long-established methods for estimating peroxide and conjugated diene intermediates and carbonyl and other end products of lipid peroxidation are unreliable and non-specific. Current trends are to couple separation methods such as high-performance liquid chromatography with specific identification techniques such as mass spectrometry. Methods for estimating secondary damage to proteins and DNA are also being developed.

There is evidence that reactive oxygen species may cause tissue damage in the living body when antioxidant defence is not functioning properly and this

chapter highlights current research into the links between radical attack and several chronic diseases. These include atherosclerosis, thrombosis, cancer, respiratory disease, arthritis, cataract and diabetes. The ageing process may be in part a result of continual damage inflicted upon cellular proteins and nucleic acids.

Background

The effects of lipid oxidation are well known to consumers who have smelled or tasted rancid food and to manufacturers whose products have become unsaleable. The same phenomenon is put to good use in the manufacture of paints and varnishes. The most susceptible lipids are the polyunsaturated fatty acids and because of their importance in nutrition, nutritionists have long been concerned with the potential adverse effects of lipid oxidation in foods. Traditionally such adverse effects have been examined in terms of the reduction in nutritive value and potential toxicity of the oxidized food components. More recently, interest has turned to the possibility that those same lipids that can be oxidized in foods, can also undergo oxidative changes in the body itself, with subsequent damage to surrounding tissues.

This topic is of particular significance because dietary guidelines as part of preventative measures against cardiovascular disease or other health problems are now common in many developed countries. Dietary fat is almost invariably brought into sharp focus in these guidelines and the benefits of exchanging unsaturated for saturated fatty acids are usually emphasized. However, because of the susceptibility of unsaturated fatty acids to oxidation under certain conditions, the potential dangers of oxidized lipids to health have frequently been debated by scientists and naturally, such debates have been highlighted in the press. Thus in 1989 a headline proclaimed (1): “Scientists do an about-turn on polyunsaturates”.

This chapter will briefly describe nutritional and toxicological effects of oxidation of food lipids and outline current concepts of the association between lipid oxidation in the body and long-term health.

Lipid peroxidation

The process of lipid peroxidation is initiated when a hydrogen atom is removed from a methylene group in the hydrocarbon chain of a lipid molecule

1. **Generation of an initiator radical**

a) $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{\bullet} + OH^{-}$

b) $H_2O \xrightarrow{irradiation} H^{\bullet} + OH^{\bullet}$

2. **Initiation**

$$\begin{matrix} R- & COOH \\ | & \\ H & \end{matrix} + OH^{\bullet} \longrightarrow \begin{matrix} R^{\bullet}- & COOH \\ & \end{matrix} + H_2O$$

3. **Propagation**

$$R^{\bullet}- COOH + O_2 \longrightarrow \begin{matrix} R- & COOH \\ | & \\ OO^{\bullet} & \end{matrix}$$

$$\begin{matrix} R- & COOH \\ | & \\ OO^{\bullet} & \end{matrix} + \begin{matrix} R\text{-}COOH \\ | \\ H \end{matrix} \longrightarrow \begin{matrix} R- & COOH \\ | & \\ OOH & \end{matrix} + R^{\bullet}- COOH$$

4. **Termination**

$$\begin{matrix} R- & COOH \\ | & \\ OO^{\bullet} & \end{matrix} \longrightarrow \text{Interaction of lipid radicals to form polymers}$$

$$\downarrow$$

Interaction with antioxidant radicals

Figure 3.1. Mechanisms of lipid peroxidation.

(**Figure 3.1**). The chemical species formed in this reaction is very reactive and is known as a (free) radical. Important initiators are 'singlet oxygen' (formed from ground-state oxygen by sensitizers such as chlorophylls, bilirubin, porphyrins and haem compounds in the presence of light) or hydroxyl radicals (generated from superoxide anions by catalysis with ferric ions). These substances are common in foods and in biological tissues.

Next the process of propagation gives rise to rearrangements of the lipid molecules involving double bond migration and further reaction with oxygen to form peroxy and hydroperoxy radicals. Once the process has been initiated, it can continue by a chain reaction of propagation steps.

The chains can be terminated in several ways. Two lipid radicals can combine to form a dimer, and eventually polymeric products, or peroxy radicals can undergo cyclization followed by decomposition of the cyclic peroxides into malondialdehyde and other low molecular weight carbonyl compounds, oxyacids and hydrocarbons. Termination can also be achieved by the presence of 'chain-breaking antioxidants' which are themselves capable of forming radicals that unite with lipid radicals and so terminate the oxidative reaction. The most important natural antioxidant is α-tocopherol (vitamin E) but several synthetic antioxidants (e.g. butylated hydroxytoluene, BHT) are used in the food industry.

Although emphasis is usually placed on the peroxidation of polyunsaturated fatty acids because of the ease with which they undergo free-radical formation, it has been realized for some time that cholesterol, generally regarded as chemically rather inert, can autoxidize in the presence of oxygen. Even 'analytical grade' cholesterol contains small quantities of oxygenated products but the cholesterol in dehydrated foods such as powdered eggs and dried whole milk is highly susceptible to autoxidation when stored in air. Thirty or more products have been detected. Much of the food we eat is processed in some way and any food containing unsaturated lipids is likely to undergo some oxidation. The nature of the resulting products depends on the degree of exposure to air, the temperature achieved, the time of heating, the composition of the fat and the presence of components (e.g. antioxidants) with which radicals can interact to terminate chain reactions.

Lipid peroxidation also occurs in living tissues, the intermediate steps being catalysed by specific enzymes, thereby generating a variety of flavour and aroma compounds in foods of both animal and plant origin.

Significance of oxidation of lipids in foods

Nutritional effects

Because the most susceptible oils are those rich in polyunsaturated fatty acids, one nutritional effect of oxidation is to reduce the essential fatty acid content of edible fats. The overall nutritional significance, however, is likely to be minimal since losses are usually small in relation to the total content of polyunsaturated fatty acids supplied by these susceptible oils. More serious is the loss of the so-called 'antioxidant nutrients', vitamin E, various carotenes and vitamin C, in cooked foods. As well as reducing the protective effect of these substances in the food itself, the overall dietary antioxidant intake will be reduced (2).

Nutritional effects may also occur through interaction of radicals or other food lipid oxidation products with other important nutrients, mainly proteins and vitamins. Lipid radicals can interact with several amino acids in protein molecules to induce the formation of carbon-centred protein radicals. The end products of such reactions may be polymers formed by protein cross-links. Damage to amino acid residues can also occur by protein scission, the most sensitive components being histidine, cysteine/cystine, methionine, lysine, tyrosine and tryptophan. Activated oxygen species (e.g. superoxide and singlet oxygen) which are generated during the catalytic decomposition of lipid hydroperoxides, are also capable of reacting with these sensitive amino acids, particularly at sulphydryl and amide groupings, thereby reducing the nutritive value of the proteins. Secondary products of lipid peroxidation (e.g. the aldehydes, epoxides and ketones) can also react with amino acids. Lysine is particularly susceptible to degradation by reaction with aldehydes. As well as reducing the nutritive value of the proteins, these reactions can lead to the production of brown colouration and unpleasant odours and tastes.

Other nutrients particularly vulnerable to attack by peroxidation products are the vitamins that act as antioxidants (E, C, and the carotenes). These are destroyed by the process of radical scavenging and this effectively increases the requirement for these nutrients in the diet. Those that ha ve sensitive sulphydryl or amide groups will undergo reactions similar to those described for amino acids thus reducing their potency as vitamins. For example, the folic acid content of foods is reduced in the presence of lipid hydroperoxides and the folic acid status of animals fed such diets is significantly reduced. Folic acid is particularly important in protection against anaemia in pregnant women.

Possible toxic effects

In addition to these nutritional effects, possible toxic effects also have to be considered. Reactive species like radicals and peroxides are potentially damaging to cells but as dietary components they are unlikely to be toxic unless they are absorbed and incorporated into body tissues. Studies in animals with radiolabelled peroxides suggest that little if any lipid peroxide is absorbed intact and, therefore, cannot enter tissues and cause oxidative damage. Yet other research has demonstrated effects of feeding lipid peroxides to animals that might be interpreted as toxicity. These included increases in relative weight of the liver, increases in malonaldehyde, peroxide and carbonyl concentrations in tissues and tissue chemiluminescence and decreases in the tissue alpha-tocopherol and linoleic acid concentrations. Supplementing the diet with α-tocopherol can protect against these changes.

If lipid hydroperoxides are not absorbed, how are these changes mediated? The answer seems to be that lower molecular weight products of hydroperoxide decomposition, such as hydroperoxy alkenals, are absorbed and are more damaging than the long-chain hydroperoxides. There is, however, some evidence of gut damage caused by feeding rats linoleate hydroperoxides, leading to impairment of nutrient absorption and greater tumour incidence.

Research is needed to ascertain whether significant amounts of intact lipid peroxides, hydroperoxides, or hydroxyalkenals are absorbed by human beings. Preliminary evidence suggests that this may be so. There is little evidence that the dimeric and polymeric materials produced by prolonged cooking of oils are absorbed or are toxic.

The toxicity of oxidized cholesterols has been demonstrated by including them in the diets of rabbits. The frequency of finding dead aortic smooth muscle cells was much increased and the authors of one paper (cited in reference 2) suggested that studies of the induction of atherosclerosis by cholesterol that had been stored in air at room temperature should be re-evaluated since the cholesterol used in experimental diets almost certainly contained significant quantities of oxidized sterols that could contain the atherogenic factor(s) rather than pure cholesterol itself.

In summary, products of lipid peroxidation in foods can cause damage to tissues if those tissues are not protected by sufficient antioxidant. In practice, the most vulnerable sites of damage are in the gut, since only the smaller molecular weight products of lipid peroxidation are absorbed into the blood and have access to body tissues. Even then, damage is minimal if sufficient antioxidant is also absorbed and probably one of the prime dangers from lipid

oxidation in foods is not so much the oxidation products themselves as the concomitant destruction of protective substances such as tocopherols, carotenoids, ascorbic acid and folates. While every precaution should always be taken to avoid lipid peroxidation, it should be borne in mind that food fats are likely to become inedible, because of the development of off-flavours and aromas as well as deterioration in appearance, long before concentrations of lipid oxidation products have reached toxic levels (2).

Lipid peroxidation and the body's defence against it

Oxygen is essential to life, but its use in biology poses certain problems. During oxygen metabolism, forms of the element and its compounds are produced (**Figure 3.2**) that are much more reactive than O_2 (triplet or ground-state oxygen). With the exception of singlet oxygen and hydrogen peroxide, these are normally radicals and have one or more unpaired electrons (**Table 3.1**). Collectively they are termed 'reactive oxygen species' (ROS). Recent research also indicates that several species that contain nitrogen ('reactive nitrogen species') also contribute to processes (see below) in which ROS are involved.

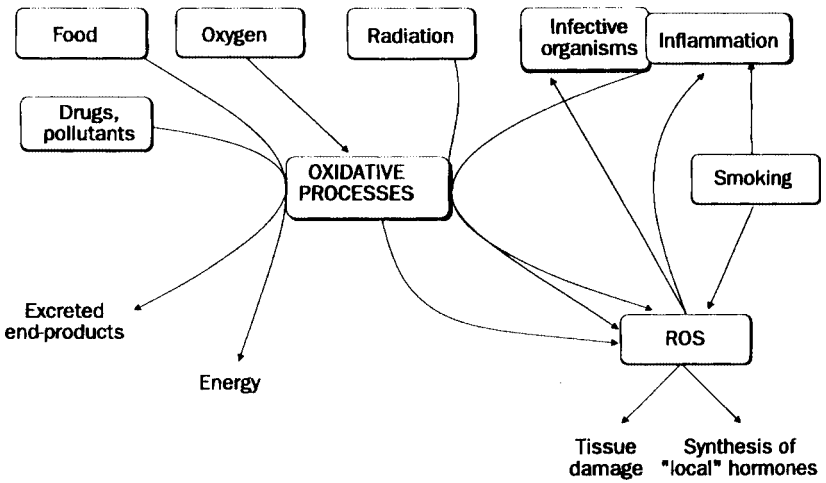

Figure 3.2. Production of reactive oxygen species (ROS) in the body.

Table 3.1. Principal reactive oxygen species (ROS) produced in the body.

Non-radicals:	hydrogen peroxide	H_2O_2
	singlet oxygen	$^1O_2$
Radicals:	superoxide	$O_2^{-\bullet}$
	hydroxyl	$OH^{\bullet}$
	lipid peroxyl	$ROO^{\bullet}$
	lipid alkoxyl	$RO^{\bullet}$

ROS are produced in the body in diverse metabolic pathways (Figure 3.1). These include the normal oxidative metabolism of absorbed food components in the mitochondria and peroxisomes, and the metabolism of drugs and contaminants in the endoplasmic reticular membranes of cells by oxidase enzymes involving cytochrome P_{450}. Bacterial killing by macrophages is also reliant on the production of ROS. Cigarette smoke, abnormal oxygenation and radiation also contribute to the ROS flux.

The enzymic conversion of polyunsaturated fatty acids into eicosanoids and leukotrienes (3) involves radical mechanisms that generate lipid peroxide intermediates. Lipid peroxidation is initiated when a radical abstracts a hydrogen atom (which itself is a free radical) from the hydrocarbon chain of a fatty acid, or the ring system of steroids to produce a lipid radical (Figure 3.1). Many ROS can initiate lipid peroxidation but it is thought that the most important initiating species in the human body are the highly reactive $OH^{\bullet}$ (hydroxyl) radical and singlet oxygen (4). Hydroxyl radicals are formed by irradiation of water or by the scission of hydrogen peroxide (which is a product of several metabolic pathways) catalysed by iron or copper ions (Figure 3.1). Singlet oxygen is formed when certain photosensitizing compounds absorb light in the presence of oxygen. Such compounds include the naturally occurring porphyrins, bilirubin and riboflavin, as well as some dyes and drugs. Singlet oxygen may also be formed when two lipid peroxyl radicals interact with each other.

Any fatty acid or steroid can be the target for peroxidation but the methylene-interrupted double bond sequences of polyunsaturated fatty acids (PUFA) are particularly sensitive because of the reactivity of the methylene groups. Once a lipid radical has been formed it undergoes molecular rearrangement to form a *cis–trans* conjugated diene system followed by attack

of the carbon-centred radical by oxygen to produce a lipid peroxyl radical. The latter may then abstract a hydrogen atom from an adjacent lipid chain to produce another lipid radical, itself being converted into a hydroperoxide. Additionally, singlet oxygen, generated by the interaction of two lipid peroxyl radicals can also initiate the production of further lipid radicals. Thus the propagation of a potentially endless supply of lipid radicals can continue until the chain reaction is terminated by destruction of the radical by one of several forms of antioxidant defence.

Antioxidant defence against damage by lipid peroxides

Metabolic reactions that involve lipid radical intermediates, such as those of the cyclooxygenase and lipoxygenase reactions (3) occur within the confines of an organized biological membrane. Membrane-bound enzymes ensure that reactive and potentially damaging intermediates are rapidly and locally metabolized into harmless end-products. Antioxidants, like α-tocopherol, built into the structure of the membrane help to maintain the integrity of the membrane and protect the PUFA against peroxidation.

Once a tissue has been damaged, by whatever cause, and membranes have been disrupted, several factors contribute to uncontrolled lipid peroxidation. These are:

- separation of the unsaturated chains from the vicinity of the antioxidant;
- release of catalysts of radical formation, especially iron and copper ions; and
- release of hydrolytic and oxidative enzymes that may otherwise not have come into contact with lipid substrates.

Lipid peroxides, unless quickly destroyed, have the potential to cause damage to the genetic material of the cell, DNA, and to many important proteins, giving rise to disease states as will be described later. Three types of biological defence systems have evolved to limit inappropriate exposure to ROS. These are summarized in **Table 3.2** and briefly described here.

- Primary (sometimes called ‘preventive’) antioxidants inactivate radicals before they have a chance to initiate peroxidation chains. They may be small molecules like vitamin A, but are often complex enzyme systems, like catalase, that decomposes hydrogen peroxide, or superoxide dismutase, which destroys superoxide radicals, thought to be the most abundant ROS in the body. The requirement of the antioxidant defence enzyme glutathione peroxidase for selenium explains the role of this element as an antioxidant nutrient.

Table 3.2. Types of antioxidant defence.

Primary	'Preventive'	Catalase (Fe) Superoxide dismutase (Cu/Mn) Glutathione peroxidase (Se) Transition metal binding proteins Vitamin A Riboflavin
Secondary	'Chain -breaking'	Vitamin E Vitamin C Carotenoids Riboflavin
Tertiary	'Repair systems'	Enzymes for repair of protein and DNA damage with Mg, Zn, folate, vitamin B_{12} as cofactors

- Secondary (sometimes called 'chain-breaking') antioxidants trap radicals that are formed in an oxidation chain once it has started. These may be small molecules that are themselves able to form radicals, combining with chain propagating radicals to terminate the chain. Many are essential nutrients: the so-called antioxidant nutrients, ascorbic acid (vitamin C), tocopherols (vitamin E) and the carotenoids.

- Tertiary defence systems are those devoted to the repair of damage already caused to proteins and DNA. Many enzymes involved in repair processes are dependent for their activity on essential nutrients including zinc, folates and cobalamin (vitamin B_{12}).

Antioxidants often have multiple roles in the defence system. Thus, glutathione peroxidase has a primary role in the conversion of H_2O_2 into water but can also act in a chain-breaking manner in concert with vitamin E in detoxifying organic peroxyl radicals. Riboflavin plays a role in secondary defence as a cofactor for the enzyme glutathione reductase, required for the regeneration of glutathione and a primary role in maintaining tissue integrity. Vitamin C can remove newly formed ROS in the aqueous phase as well as its more widely recognized role in acting synergistically with lipid-soluble vitamin E in regenerating the active form.

A principal aspect of primary defence is in the sequestration of transition metal ions by various binding proteins, in which form they are incapable of catalysing the conversion of H_2O_2 and superoxide into $OH^{\bullet}$.

Whereas almost all attention has been directed towards the well-known antioxidant nutrients, vitamins E and C and β-carotene, it is becoming increasingly apparent that foods contain a multiplicity of compounds (not necessarily nutrients) with antioxidant activities. These include other carotenoids such as lycopene, other tocols such as the tocotrienols, flavonoids and many phenolic compounds (e.g. gallic acid and catechins).

The concept of oxidant stress

As detailed above, ROS are produced in the body continually as part of normal metabolism and a complex and generally efficient defence system exists to protect against their potentially damaging effects.

However, the body may become vulnerable to damage from ROS under certain conditions, as when:

- the defence system is inadequate, either because intakes of antioxidant nutrients are low, or malnutrition or disease has resulted in an inability to synthesize the enzymes needed to destroy ROS or repair existing damage;
- exposure to oxygen is particularly large —this may occur in the reperfusion of cardiac muscle after an infarct or in premature babies who need ventilation with extra oxygen yet whose lungs are too immature to cope with the stress;
- exposure to other pro-oxidants is particularly large, for example, in the condition of iron overload; or
- tissues are injured, physically or during infectious diseases or inflammatory reactions. This leads to disruption of cellular organization, and release of enzymes, pro-oxidants and the generation of abnormal concentrations of ROS.

The concept of oxidant stress and its role in the progression of many diseases is assuming great importance in medical and nutritional research. If further progress is to be made, the appropriate methods for measuring ROS in the living body and for identifying antioxidant activities in foods and their relative effectiveness in the body need to be improved. The next section will review the current state of the art in antioxidant methodology.

Methods for assessing antioxidant stress

Briefly, the radical theory of disease states that, in the absence of adequate antioxidant protection, reactive oxygen species (ROS) cause damage to key biological molecules — proteins and DNA — resulting in disruption of cellular structure and metabolic control and eventually leading to pathological change. ROS may cause these changes directly or, more usually, by initiating peroxidation of polyunsaturated fatty acids in biological membranes or plasma lipoproteins. The evidence for pathological changes resulting from lipid peroxidation will be considered in a later section. The question here is how do we demonstrate that lipid peroxidation is taking place in the living body and how do we quantify it?

Systems for study

The main focus of interest is human disease. Because of difficulties in studying human tissue directly, numerous studies have been conducted with small laboratory animals. Thus, rats and mice have been given diets depleted in antioxidant vitamins (for example vitamins E and C) and/or given diets containing severely peroxidized lipids (2). Another model for oxidative stress is the prematurely born guinea pig. These animals are used to simulate the condition of premature babies whose need for ventilation and additional oxygen can lead to injury to the lungs and subsequent chronic lung disease. These animal models are essential if measurements are to be made of oxidation in inaccessible tissues such as liver, heart, lung and brain. In man, there is the opportunity to study samples of diseased tissue taken at surgery. However, information obtained is limited to a particular disease and problems of interpretation arise as to whether such changes are causal or whether they arise as a result of the disease.

More recently, there has been a strong move towards measurements in tissues of healthy human subjects but these are currently limited to blood components: isolated plasma lipoproteins and white blood cells. Biopsy samples of adipose tissue and muscle are also feasible and another development is the study of isolated colonic cells voided in the faeces. There has also been considerable development of cultured human cell lines derived from different tissues.

What to measure?

It is clear from Figure 3.1 that lipid peroxidation proceeds in several well-defined stages. There are opportunities for measuring products of lipid peroxidation at each stage as indicators of the degree of peroxidation. Thus,

intermediate products such as lipid peroxides, hydroperoxides, hydroxyacids and conjugated dienes have all been measured in human plasma and tissue samples by physical and chemical methods.

An example was a study by Abbey *et al.* (5) who asked the questions: how does dietary fat composition affect the 'oxidizability' of human low-density lipoprotein (LDL) and what degree of variability is observed between subjects and between analytical determinations on the same plasma sample? They, like many other research workers, chose to measure the production of conjugated dienes as their indicator of peroxidation because they are simple to measure spectrophotometrically as a consequence of their characteristic absorption in the ultraviolet (UV) spectrum. The experimental system consisted of freshly isolated LDL incubated with cupric ions to catalyse peroxidation. Diene formation was followed spectrophotometrically at 234 nm. It proceeds in three distinct stages: a lag phase of approximately 40 minutes in which no dienes are formed. This equates with the utilization of endogenous reserves of antioxidant. Recording the lag phase, therefore, tells us something about the antioxidant status of the tissue. When these reserves are depleted, diene formation is rapid and occurs at a roughly linear rate for about 20 minutes before reaching a plateau when formation is balanced by decomposition.

The authors first established that the coefficient of variation of their determinations was about 6%, most of which was accounted for by methodological error rather than intra-individual variability. The 'oxidizability' of LDL measured either as the rate of conjugated diene formation or the total amount of conjugated diene formed, increased in a roughly linear fashion with the concentration of linoleic acid in the LDL, which was in turn a function of the linoleic acid content of the diet.

Interpretation of methods involving the measurement of concentrations, or rates of formation, of intermediate products of lipid peroxidation requires care. Because these products are undergoing rapid turnover, determination of their concentration at a single arbitrary time may give a distorted picture of the degree of oxidation; clearly continuous measurement over a period of time, as in the experiment of Abbey *et al.* (5) is an advantage. Even so, measurements, such as UV absorption, chemiluminescence or total peroxide or carbonyl value are non-specific and may include compounds that have little to do with the process of lipid oxidation. Halliwell (6) regarded many of the techniques as unreliable for human tissues and urged the development of methods that identify specific structures originating from peroxidation of lipids. Thus, separation methods such as high-performance liquid chromatography (HPLC)

or gas chromatography (GC) in combination with mass spectroscopy will be methods of choice in the future.

Similar reservations apply to measurement of end-products of lipid peroxidation. Probably the most widely used test of the extent of lipid peroxidation is the colorimetric determination of malondialdehyde and other carbonyl compounds by reaction with thiobarbituric acid. This test, while simple and rapid, has long been recognized as non-specific and unreliable. It measures a range of substances that react with thiobarbituric acid, some of which may have arisen by pathways unconnected with lipid peroxidation. Coupling the assay with an HPLC separation can avoid many of the artifacts associated with the classical thiobarbituric acid test. More recently, a number of compounds (isoprostanoids) resembling prostaglandins have been detected in human urine and plasma (7). Since they appear to have originated from lipid peroxidation *in vivo*, they have been used as markers of this process. A major problem with all such methods is that they assume that what is measured is representative of overall lipid peroxidation in the body. However, certain end products may arise only from certain types of precursors (for example, specifically from *n*–3 polyunsaturated fatty acids rather than unsaturated fatty acids in general) or from constituents of the diet and so cannot be regarded as proxies for the general process of lipid peroxidation *in vivo*.

Mass spectrometry requires considerable time and skill for sample hydrolysis, derivatization, analysis and interpretation. It is essential, however, that simpler routine methods be first validated against more rigorous methods.

Lipid peroxidation in the body is as important for the secondary damage that can be inflicted upon proteins and DNA as for the lipid modifications themselves. Various assays for protein modification involving whole proteins (for example reaction of protein carbonyl groups with dintrophenylhydrazine) or modified amino acids (8) have been developed. Like the lipid methods, they are being improved by development of specific separation and detection methods. Assessment of 'physiological damage' to specific proteins is yet another approach. For example, the increase in opacity of the lens of the eye in cataract is thought to be due largely to damage to lens crystallin initiated by radicals.

Radical damage to DNA is potentially very serious in that it can modify the genome, with the risk of inducing cancerous growth. Assays for various end-products of DNA damage (for example 8-hydroxydeoxyguanosine, 8-hydroxyguanosine and 8-hydroxyguanine) in urine and plasma are now well established. Again, identification of specific structures is important (9).

An alternative method for assessing DNA damage has become popular. The 'comet assay' relies on the fact that DNA damage often results in breakage of DNA strands and the uncoiling of parts of the molecules. When the sample is subjected to gel electrophoresis, the uncoiled parts are pulled towards the anode. Analysis of the resulting comet-shaped electrophoretic band can give a semi-quantitative estimate of DNA damage. Quantitation is now being improved by treatment of the damaged DNA with specific enzymes and identification of the pattern of hydrolysis products.

Antioxidant Status

The foregoing has been concerned with the methodology for assessment of chemical changes in biological tissues resulting from free radical damage. Radical reactions are taking place continuously as a result of oxidative metabolism and a certain level of oxidation products can be regarded as normal. A level of oxidation products equated with 'damage' will arise when protective antioxidant concentrations fall below a certain threshold. Knowledge of the required local concentrations of antioxidants or of the relative importance of the different natural antioxidants or antioxidant systems in specific tissues, cells or membranes is rudimentary. There is, however, much evidence that antioxidant nutrients act synergistically, a combination offering more efficient protection than the sum of the parts. Water-soluble ascorbic acid may act to regenerate the reduced form of lipid soluble vitamin E.

It is important, therefore, to be able to measure normal concentrations of antioxidant nutrients in biological tissues as well as changes in concentrations that may be predictive of increased risk of the occurrence of oxidative damage. HPLC is now generally the method of choice for determining the principal compounds: the tocopherols, carotenoids and ascorbate. A major problem is that, while it is relatively simple to monitor plasma concentrations of these compounds, the relevance of such measurements to antioxidant status in individual tissues is questionable. Another important question concerns the range of antioxidants that should be measured. In addition to those listed above, should other substances with known antioxidant properties — glutathione, uric acid, the tocotrienols and minor carotenoids — also be measured? Measurement of the activities of antioxidant enzymes, for example, superoxide dismutase, glutathione peroxidase and catalase, as well as the adequacy of supply of nutrient cofactors such as Zn, Cu and Se is also recommended. One research group has adopted the approach of measuring 'total antioxidant activity' as distinct from concentrations of individual compounds. The method measures spectrophotometrically the ability of

antioxidant substances to scavenge 2,2'-azinobis(3-ethyl-benzothiazoline-6-sulphonate) ($ABTS^{\bullet+}$) radicals. It has the potential advantage that it should be able to distinguish between additive and synergistic effects. The method has been patented (10).

Because of the difficulties in assessing antioxidant status of human tissues, the measurement of antioxidant content and activity of foods and diets will undoubtedly continue to be important. However, little or nothing is known about the bioavailability of antioxidant nutrients, i.e. how much of what is consumed in the diet actually finds its way to sites in the body in which it is needed. Progress towards this objective is being made by the use of deuterium-labelled tocopherols to assess the absorption and metabolism of vitamin E in man (11). Further developments with this and other antioxidant nutrients, using deuterated and ^{13}C-labelled compounds can be expected in the future providing much needed background information for understanding the role of dietary antioxidants in protection against free radical damage.

Research into links between diet, oxidant stress and disease

In general there are three main ways in which we may pick up clues about how dietary components may be associated with disease: epidemiological (observational) studies; experiments with animals and dietary intervention studies with human subjects. Frequently, epidemiological observations may provide statistical associations that generate hypotheses which can be tested by experiment. However, the converse may apply when animal experiments or clinical observations suggest links which can be further tested by setting up a controlled epidemiological study — usually with a case-control or a prospective design. Concepts of how radicals are involved in chronic disease have developed by interactions between these three methods as will be illustrated below.

Biological targets for attack by reactive oxygen species and their associated diseases

There may be many sites of attack by ROS in the body. Which ones are particularly vulnerable will depend on local factors that may result in injury at a specific site, or on the integrity of the antioxidant defence at that site. Probably the most intensively studied systems have been arterial damage in relation to atherosclerosis and epithelial cells in relation to cancer (12).

Arterial wall damage: atherosclerosis

In atherosclerotic plaques, a characteristic finding is a large number of cells (called 'foam cells' from their appearance) engorged with droplets of lipid, mainly cholesteryl esters. Foam cells originate from macrophages whose main task is to engulf anything that can be regarded as a 'foreign body'. It has long been recognized that LDL are the main sources of the cholesteryl esters of foam cells. Normally LDL enters cells by first interacting with receptors on the cell surface, whereupon receptor and LDL particle are sucked into the cell. It came as a surprise to cell biologists to find, however, that macrophages in culture were not very adept at taking up 'pure' ('native') LDL and this turned out to be because they did not have normal LDL-receptors on their surfaces. Instead they have scavenger receptors and it was soon learned that, while not recognizing native LDL, these scavenger receptors recognized LDL that had been modified in some way. One of the first biological clues that lipid peroxidation might be involved in atherosclerosis was the finding that peroxidized LDL (in which the apoprotein moiety is modified by interaction with peroxidation products) were avidly taken up by macrophages via the scavenger receptor to become the foam cells characteristic of the atherosclerotic plaque (12).

Whereas the oxidation of LDL and uptake of modified particles by macrophages can be routinely demonstrated *in vitro*, evidence for the involvement of this sequence of events in human atherosclerosis is indirect. Oxidation of LDL *in vitro* is delayed by a number of antioxidants, principally vitamin E, and such antioxidants also inhibit the development of 'fatty streaks' (thought to be the forerunners of atherosclerotic plaques) in the arteries of experimental animals. Oxidized lipids, and indeed modified LDL, are found in human atherosclerotic plaques and antibodies to modified LDL have been detected in human plasma. A prospective epidemiological study in Finland found that the concentration of autoantibodies against oxidized LDL was an independent predictor of the rate of progression of atherosclerosis in the carotid artery. High concentrations of pentane, which is one of the end products of lipid peroxidation, have been detected in the breath of patients with acute myocardial infarction.

Other indirect epidemiological evidence comes from studies of antioxidant vitamins. Gey and his colleagues (13) found a highly significant inverse correlation between CHD mortality in 16 European communities and plasma concentration of vitamin E. In a case-control study in Edinburgh, patients with angina were found to have significantly lower plasma concentrations of vitamin E than healthy controls. In epidemiological studies involving over

120 000 people in the USA, high intakes of vitamin E (from supplements) were associated with significantly reduced risk of CHD.

Current research is focusing on the search for evidence that LDL oxidation and the uptake of modified LDL into atherosclerotic plaques occur in the human body and on the causes and mechanisms of the initiation of the peroxidation.

Platelets: thrombosis

Compared with research into the role of lipid peroxidation in atherosclerosis, little work has been done to examine its role, if any, in the thrombotic phase of cardiovascular disease. The events leading to the formation of a thrombus are exceedingly complex but the aggregation of blood platelets plays a contributory role. Platelet aggregation is stimulated by thromboxanes derived from arachidonic acid via the cyclo-oxygenase pathway (see Chapter 4).

There are several reports that the antioxidant nutrients, vitamins C and E, inhibit platelet aggregation *in vitro*, that animals depleted of vitamin E produce less thromboxane from arachidonic acid and that production is restored by vitamin E repletion (14). Platelet aggregation can be enhanced in the presence of LDL and this effect is amplified if the LDL are oxidized. Research is now looking in a more systematic way at the effects of antioxidant nutrients on platelet aggregation of human subjects *in vivo* and exploring the mechanisms involved.

Epithelial cells: cancer

The view that cancerous growth may arise after a permanent change in the structure of DNA (mutation) is now gaining wide acceptance. DNA damage can be caused by ROS and evidence for this can be found in increased excretion of various degradation products, for example 8-hydroxyguanosine (15). There is also some evidence that ROS can act in an alternative way by modifying the expression of genes involved in the regulation of cell differentiation and growth. Normally, in a given cell, most of the DNA is not expressed; therefore, most mutations have little or no effect on cell function. Moreover, in addition to protection against ROS afforded by primary and secondary antioxidants, the body is well supplied with enzymes designed to repair DNA. However, it has been discovered that people with a reduced ability to effect DNA repair are more susceptible to cancer. Among the best studied cancers in which diet is thought to play a role is colon cancer. Human studies are feasible since it is possible to isolate fully functioning colonic cells that have been voided in faeces.

The lungs: chronic lung disease

Newborn infants are particularly susceptible to oxidative stress because birth represents a rather abrupt exposure to an oxygen-rich environment. Premature infants are especially vulnerable since they often need to be treated with a high oxygen tension to compensate for breathing difficulties due to poorly functioning lungs. To add to the problems, premature infants are frequently found to be deficient in vitamin E and other antioxidants. The resulting oxidative stress gives rise to the condition of chronic lung disease. Current research aims to unravel the mechanisms by which this condition arises by studying premature guinea pigs which seem to represent good models for human infants (16).

Joint fluid and connective tissue: arthritis and systemic lupus erythematosus

Damage to lipids and proteins arising from ROS attack has been detected in the joint fluid of patients with arthritis and increased numbers of chromosome breaks are found in patients with systemic lupus erythematosus, an inflammatory autoimmune disease characterized by alterations in cellular and humoral immunity. It has been proposed that increased concentrations of ROS seen in the serum and lymphocytes of these patients may inactivate so-called 'suppressor T cells' which normally damp down unwanted immune reactions to the body's own proteins. Lymphocytes from patients seem to be more susceptible to damage by ROS than those of healthy people. Serum concentrations of antioxidant nutrients are low in systemic lupus erythematosus patients and in the joint fluids of arthritic patients. These observations provide circumstantial evidence for a role for ROS in these autoimmune inflammatory diseases (12) and therapeutic trials have been proposed to test the efficacy of increased consumption of antioxidant nutrients on the course of the diseases.

Lens proteins: cataract

A feature of cataract is the aggregation of lens proteins such as crystallin, leading to light scattering and opacity. Experimentally, cataract can be produced by oxidants such as hydrogen peroxide. The antioxidant glutathione plays a crucial role in protecting against cataract by preventing the formation of sulphide bridges between protein molecules, maintaining membrane thiol groups in a reduced state, and reducing concentrations of potential oxidants such as hydrogen peroxide (17). A characteristic feature of developing cataract is a reduction in lens glutathione concentrations. Taken together, these findings suggest a role for ROS in cataract development and possible therapeutic benefits of dietary antioxidants which need to be tested more rigorously.

Diabetes

Glucose in solution or conjugated to proteins can generate oxidants in the presence of transition metal ions. In patients with diabetes mellitus, a metabolic disorder involving poor control of glucose metabolism, there is evidence for elevated plasma concentrations of lipid peroxidation products and decreased concentrations of antioxidant nutrients (18). However, atherosclerosis and increased risk for cardiovascular disease are features of diabetes and it is therefore difficult to assess whether apparent involvement of oxidative stress in diabetes is primary or secondary.

Kwashiorkor

Kwashiorkor is one of many diseases of children in which malnutrition plays a major role. It is characterized by oedema (swelling caused by abnormal accumulation of water in tissues) and fatty liver. Its origins are still not resolved but a current theory is that many features result from radical damage (19). Malnourished children are particularly susceptible to infection and associated attack by toxins. Both the toxins themselves and the body's defences against them give rise to an increased flux of ROS. The child's antioxidant defences against ROS are weakened by the malnutrition which results in reduced intakes of antioxidant nutrients and reduced endogenous levels of antioxidant enzymes. Moreover, plasma concentrations of transferrin, the iron-carrying protein, are reduced, resulting in more available free iron to catalyse lipid peroxidation. It is likely, however, that the development of kwashiorkor is more complicated than this and that radical reactions are only a small part of the story.

Future directions

Long before the current explosion of interest in the role of radical reactions in normal biology and their relevance to disease, some people had argued strongly that the process of ageing resulted from decay of cells as a result of continual attack upon their nucleic acids and proteins by radicals (15). Many of the diseases discussed above have been described as 'diseases of ageing' and it is perhaps not surprising that radical mechanisms have been invoked as contributing to their development. A major problem in the future for this area of research will be to decide whether ROS cause the pathological changes or whether ROS play a role on the sidelines simply because they are the products of tissues that are already dying from some other cause.

To be able to resolve this problem, further advances are necessary, particularly in the methods employed for assessing radical damage *in vivo*. The

method for examining oxidative damage to polyunsaturated lipids by measuring various isoprostanes derived from arachidonic, eicosapentaenoic and docosahexaenoic acids is currently being improved. Another promising line of enquiry is to study the ultimate end products of fatty acid peroxidation, namely the hydroxy-fatty acids. The most comprehensive recent review of lipid peroxidation and its association with chronic disease emphasizes the insights to be gained by careful determination of hydroxy-fatty acids in tissues and their use as markers of damaging peroxidative processes (20).

International collaborative research programmes have been established to develop new methods for measuring DNA damage. The study of oxidative damage to proteins is as yet in its infancy and needs intensive and well coordinated research.

There is still much to learn about the role of different antioxidants in foods. Many antioxidants display pro-oxidant activity at high concentrations. This phenomenon may underlie the puzzling finding in some studies that supplements of β-carotene may actually enhance lung cancer rather than retard it, as described by John Stanley in *Lipid Technology* (21). The different effects of some antioxidants when given as supplements of pure synthetic compounds compared with their apparent effects when present in foods need to be defined. Interactions and synergies between antioxidants are likely to be important and although the emphasis has tended to be on 'nutrient antioxidants', the roles of non-nutrient antioxidants present in many plant foods and plant-derived beverages need clarification. Little or nothing is known about the bioavailability of these substances from foods and this needs research effort.

References

1. *Sunday Times*, London, 3 September 1989.
2. Gurr, M.I. (1988) In: Nutritional and Toxicological Aspects of Food Processing, (ed. R.Walker and E.Quattrucci). Taylor and Francis, London.
3. Gurr, M.I. (1993) *Lipid Technology*, 5, 65–68.
4. Halliwell, B. and Gutteridge, J.M.C. (1989) Free Radicals in Biology and Medicine, 2nd ed., Clarendon Press, Oxford.
5. Abbey, M. *et al.* (1993) *American Journal of Clinical Nutrition*, 57, 391–398.
6. Halliwell, B. *et al.* (1992) *Journal of Laboratory and Clinical Medicine*, 119, 598–620.
7. Morrow, J.D. *et al.* (1990). *Proceedings of the National Academy of Sciences, USA*, 87, 9383–9387.
8. Reznick, A. *et al.* (1992) *Biochemical Journal*, 286, 607–611.
9. Halliwell, B. and Aruoma, O.I. (1993) DNA and Free Radicals. Ellis Horwood, Chichester.

10. Rice-Evans, C.A. and Davies, M.J. UK Patent No. 9124272.7.
11. Cheeseman, K.H. *et al*. (1993) *Proceedings of the First Annual Oxygen Society Meeting*, Charleston, NC, USA, November 1993.
12. Strain, J.J. *et al.* (1991) *Journal of Biomedical Science*, 2, 19–24.
13. Gey, K.F. *et al*. (1991) *American Journal of Clinical Nutrition*, 53, 326S–334S.
14. Rice-Evans, C. and Bruckdorfer, K.R. (1992) *Molecular Aspects of Medicine*, 13, 1–112.
15. Ames, B.N. (1989) *Free Radical Research Communications*, 7, 121–128.
16. Kelly, F.J. *et al*. (1991) *International Journal of Biochemistry*, 23, 467–471.
17. Spector, (1984) *Investigative Opthalmology and Visual Sciences*, 25, 130–146.
18. Wolff, S.P. *et al.* (1991) *Free Radicals in Biology and Medicine*, 10, 339–352.
19. Golden, B.E. (1993) In: Human Nutrition and Dietetics, (eds J.S.Garrow and W.P.T. James). Churchill Livingstone, Edinburgh, pp.440–455.
20. Spiteller, G. (1998) *Chemistry and Physics of Lipids*, 95, 105–162.
21. Stanley, J.S. (1999) *Lipid Technology*, 11, 14–16.

Chapter 4

The Nutritional and Biological Properties of the Polyunsaturated Fatty Acids

*Polyunsaturated fatty acids (PUFA) perform many vital functions in biological membranes and as precursors of a variety of lipid regulators of cellular metabolism. They are classified into two main families designated `*n*–6' and '*n*–3'. The parent fatty acid of the *n*–6 family is linoleic acid (c9,c12-18:2) and of the *n*–3 family, α-linolenic acid (c9,c12,c15-18:3). These precursor acids are elaborated into longer-chain more highly unsaturated fatty acids by a series of desaturations and elongations. Some functions are unique to either *n*–3 or *n*–6 PUFA, so that one family cannot always substitute for the other and the members of the *n*–3 and *n*–6 families cannot be interconverted in the human body. Furthermore, linoleic and α-linolenic acids cannot be synthesized in the human body. Therefore, because they perform vital functions, they need to be consumed in the diet from plant sources. They are termed the essential fatty acids.*

Linoleic acid is abundant in several seed oils used in foods. One of its most well-known properties is to maintain a low concentration of cholesterol in the blood and its contributions to 'western' diets has been rising as a result of advice to lower cholesterol as a means of preventing or managing coronary heart disease. Linoleic acid is converted first into γ-linolenic acid (GLA). There is some evidence, though inconsistent, for therapeutic benefits of GLA in diseases that have an inflammatory component and in which there appears to be an impairment of linoleic acid desaturation. Sources of GLA appear to differ in their therapeutic effectiveness and the reasons need further investigation. The last major member of the n–6 family is arachidonic acid, an important membrane component and precursor of eicosanoids.

α-Linolenic acid (ALA) is the main fatty acid of the chloroplast membranes of green plants but the main dietary sources are certain seed oils (eg. rapeseed and soybean oil). Recent evidence provides little support for the conventional pathway for the sequential desaturation and elongation of ALA involving 6-, 5- and 4- desaturases, since the existence of a 4-desaturase has not been demonstrated. An alternative pathway involving a second 6-desaturase and a retroconversion are now proposed. Confirmation of an independent role for ALA in the functioning of the nervous system, in processes affecting vision and in some aspects of behaviour is needed. ALA rich diets may improve some aspects of cardiovascular function but a recently claimed specific role in protecting against heart attacks was not proven.

In the human diet, the major sources of the long-chain n–*3 acids are usually the oils of fatty fish. They are metabolized in the body to a range of oxygenated fatty acids called 'eicosanoids' which are widely involved in metabolic regulation. They influence, among other things, muscle contraction, hormone secretion, blood coagulation and immune function. Dietary intake of* n–*3 PUFA has the potential to change the composition of eicosanoids produced in the body and thus to affect the physiological functions controlled by eicosanoids. This in turn may have implications for a number of clinical disorders that are discussed in later sections.*

Inclusion in the diet of four or more grams per day of n–*3 polyunsaturated fatty acids found in the oils of some fatty fish maintains low blood triacylglycerol concentrations in people with normal levels and significantly reduces their concentration in people with hypertriglyceridaemia. Unlike the* n–*6 family of PUFA, however, there is no consistent effect of* n–*3 PUFA on blood cholesterol. In some individuals,* n–*3 PUFA have even been known to raise blood total cholesterol. There are small and inconsistent effects on high-density lipoprotein (HDL) cholesterol. The triacylglycerol lowering effect is due entirely to changes in plasma very-low-density lipoprotein (VLDL) concentrations brought about by a reduction in VLDL triacylglycerol biosynthesis in the liver and to some extent by reduced clearance and catabolism of VLDL.*

Polyunsaturated fatty acids of the n–*3 family reduce the potential of blood to clot, thus possibly reducing the risk of thrombosis. However, despite numerous publications, the relevance of the findings for practical dietary advice remains uncertain due to limitations in the methodology for measuring thrombus formation and imprecise knowledge of the level of dietary* n–*3 polyunsaturates required to be effective.*

Inclusion of long-chain n–3 polyunsaturated fatty acids characteristic of many fish oils in the diet is quickly reflected in elevated concentrations of these fatty acids in plasma and in the membranes of red and white blood cells. The biochemical effect of an increase in the ratio of n–3/n–6 PUFA in cell membranes is to alter the composition of the eicosanoids formed from these PUFA through the action of the enzymes cyclo-oxygenase and lipoxygenase. The strongly pro-inflammatory eicosanoids from arachidonic acid are reduced and replaced by the weakly inflammatory eicosanoids from eicosapentaenoic and docosahexaenoic acids. This knowledge has formed the basis for dietary treatments for inflammatory diseases, of which rheumatoid arthritis has been most actively researched. Controlled dietary trials have demonstrated modest but potentially beneficial improvements in the severity of rheumatoid arthritis and longer-term studies are now needed to establish whether dietary supplements of n–3 PUFA should be standard recommended treatment.

The two families of polyunsaturates compete for common enzymes involved in their metabolism and their ratio in the diet is therefore crucial to their composition in tissues and their ability to perform their allotted functions. The evidence is discussed that the ratio of n–6 to n–3 polyunsaturates in the diets of developed countries is too high and is responsible for increasing prevalence of various health problems, including some types of cancer, allergies and behavioural disorders.

During 1991, the UK Department of Health published a report on recommended intakes of nutrients to maintain good health, which included values for the essential fatty acids. These recommendations are presented and discussed.

The chapter ends with a more philosophical discussion about what we mean by 'essential' in regard to lipids. An essential nutrient is one that is required by, but cannot be synthesized by, the body and is therefore needed in the diet. An essential metabolite is a substance that is vital for life and can be synthesized in the body. Sometimes, essential metabolites cannot be synthesised in sufficient quantity to meet current demands and may be needed in the diet. In these circumstances they are 'conditionally essential' nutrients. As highlighted here, there is ongoing debate in lipid nutrition about the conditional essentiality of long-chain polyunsaturates derived from metabolism of linoleic and α-linolenic acids. Can the same can be true of saturated fatty acids and cholesterol?

Polyunsaturated and essential fatty acids — the distinction

Fatty acids with more than one double bond in the molecule are classified as 'polyunsaturated', whether the disposition of the double bonds is conjugated or 'methylene interrupted' or whether their geometry is *cis* or *trans*. Naturally-occurring polyunsaturated fatty acids in higher plants and mammals normally have a methylene interrupted sequence of double bonds, with between 2 and 6 double bonds (1). These acids play important roles in the structure of cell membranes, where they contribute to the property of 'membrane fluidity'. They also act as precursors of oxygenated fatty acids ('eicosanoids') which exert powerful local biological activities at very low concentrations. These properties are explained in more detail in a later section. Polyunsaturated fatty acids also influence the metabolism of lipoproteins that carry lipids in the blood, thereby regulating the levels and types of blood lipoproteins. This aspect is touched on briefly later but is discussed in detail in Chapter 1. Recently, roles for polyunsaturated fatty acids in the regulation of gene transcription have also been recognized.

Many tissues of mammals are able to convert saturated into monounsaturated fatty acids through the action of a desaturase that produces a *cis*-9-monoene. Further desaturations are possible on the carboxyl side of the 9-double bond but during the course of evolution the ability to desaturate on the methyl side of the 9-double bond has been lost by animals, including man. Only plants are able to insert double bonds at positions 12 and 15. Nevertheless, polyunsaturated fatty acids with these double bond positions are essential to life and must be provided in the diet. These are the essential fatty acids (EFA). It is now recognized that there are two essential fatty acids, linoleic (*cis*,*cis*-9,12-18:2) and α-linolenic (all-*cis*-9,12,15-18:3). These are the parent acids of two distinct families of polyunsaturated fatty acids formed from linoleic (LA, *n*–6 family) and α-linolenic (ALA, *n*–3 family) acids by a series of alternate desaturations and elongations. A third family (*n*–9) has oleic acid (9-*cis*-18:1) as its parent (**Figure 4.1**).

It has been assumed that longer-chain polyunsaturated fatty acids (LCPUFA) derived from the precursor acids by elongation and desaturation can be formed easily in the human body (see final section on requirements).

In this chapter, I will describe the properties of both precursor acids and also the long-chain derivatives formed from them by desaturation/elongation. I will close the chapter by considering the appropriate dietary balance between the *n*–6 and *n*–3 families and consider the most recent recommendations for the amounts required in the diet.

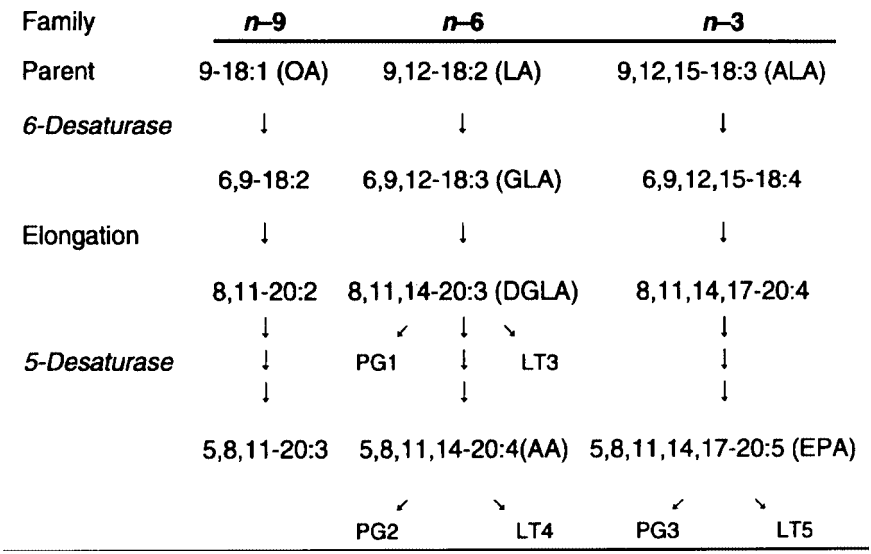

Figure 4.1. Pathways of metabolism in PUFA families.

Linoleic and α-linolenic acids as precursors of the *n*–6 and *n*–3 families of polyunsaturates

Linoleic acid

Fatty acids of the *n*–6 family are characterized by having the last double bond (measured from the carboxyl group) six carbon atoms from the methyl end of the chain. They were originally named `omega-6' fatty acids and, although this naming system is still widely used, the IUB-IUPAC nomenclature prefers *n*–6. Linoleic acid, the parent or precursor fatty acid of the *n*–6 family, is regarded as the primary essential fatty acid. The major sources are seed oils such as sunflower, corn and soybean. Deficiency in the diet is rare but when it does occur, the main early signs are skin lesions with associated 'leakiness' of the skin to water. Linoleic acid deficiency was recorded when infants were given formulas free of linoleic acid (1,2) and in adults who had undergone major bowel surgery resulting in failure to absorb fat (3). No more than about 1% of dietary energy as linoleic acid needs to be consumed to avoid deficiency signs (see final section on dietary requirements).

The main interest in linoleic acid has been because of to its capacity to reduce the concentration of cholesterol in the blood, mainly in the low-density lipoprotein (LDL) fraction (see Chapter 1). Increased dietary intakes have been recommended since at least the 1970s for lowering blood cholesterol as a preventive measure against coronary heart disease (see Chapter 2). As a result intakes have been steadily rising to such a degree that public health bodies are now suggesting upper limits for intake (see later section on recommendations and also the last section on *n*–3/*n*–6 balance).

The requirement to prevent skin lesions is specific to linoleic acid itself. However, linoleic acid is also important as a precursor for longer-chain polyunsaturated fatty acids derived from it by alternate desaturations and elongations, as depicted in Figure 4.1. The separate roles of the longer-chain derivatives are described in a later section.

α-Linolenic acid

Structure and occurrence

The *n*–3 PUFA (sometimes termed `omega-3') are characterized by the location of a double bond three carbon atoms from the methyl end of the fatty acid carbon chain. All fatty acids of nutritional and physiological significance in this family have *cis* double bonds in the methylene-interrupted sequence (1).

The parent, and simplest, member of the *n*–3 family is α-linolenic acid, all-*cis*-9,12,15-octadecatrienoic acid (α-18:3). It is synthesized only in higher plants, algae and phytoplankton. Because of the enormous mass of green plants both on land and in the oceans, and since α-18:3 contributes over half the fatty acids of the lipids of chloroplast membranes, it is probably the predominant fatty acid on the planet. In plants, it is present almost exclusively as a constituent of galactosyldiacylglycerols.

Pathways of metabolism

In Figure 4.1, the elongation and desaturation of ALA to EPA is shown. However, in some tissues, the major end-product is docosahexaenoic acid (DHA). It was always assumed that the conversion of EPA into DHA involved a 4-desaturase. In fact, there is little evidence for the independent existence of a 4-desaturase: it has been invoked simply because its existence would conveniently explain the known *n*–3 metabolites found in membrane phospholipids. If the supposed precursor of DHA, namely 22:5*n*–3 labelled with ^{14}C at carbon 1 is incubated with a rat liver microsomal fraction, no detectable quantities of radiolabelled 22:6*n*–3 are produced. These observations

led Sprecher and his colleagues at Ohio State University (4) to consider an alternative pathway, in which there are two sequential chain elongations after the 5-desaturation, followed by a further 6-desaturation to produce 24:6*n*–3. Retroconversion of this metabolite then results in the formation of DHA, 22:6*n*–3, as illustrated in **Figure 4.2**. Retroconversion is a chain shortening reaction, the reverse of elongation, and is catalysed by enzymes analogous to those of β-oxidation, either in the mitochondria or the peroxisomes of the cell. Sprecher's group provided evidence for this pathway by incubating a rat liver microsomal fraction with [1-^{14}C]22:5, which was converted into [3-^{14}C]24:5 and [3-^{14}C]24:6. Similarly, they showed that [3-^{14}C]-labelled 24:6 was retroconverted into 22:6.

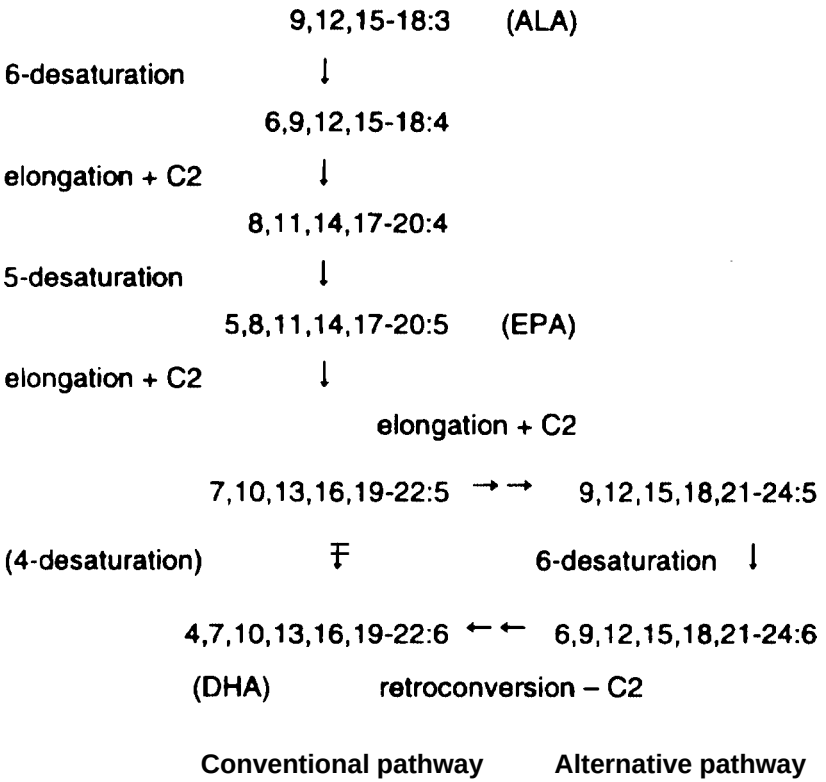

Figure 4.2. Alternative pathways for conversion of α-linolenic acid (ALA) into docosahexaenoic acid (DHA). There is little evidence for the independent existence of a 4-desaturase (see text). EPA = eicosapentaenoic acid.

It is not known whether this pathway occurs in human tissues. It seems that although ALA can be converted into eicosapentaenoic acid (EPA) and DHA in human tissues *in vivo* (cited in 4), the conversion is relatively slow compared with the reaction in rat tissues, so that extrapolation from rat experiments to conclusions about human EFA metabolism should be done with caution.

A puzzling aspect of ALA metabolism is that this fatty acid appears to be more rapidly degraded by β-oxidation than many other fatty acids (5). In view of its supposed essentiality (see below), this is either very wasteful, or the process of oxidation is in some way important metabolically or nutritionally. Possible explanations discussed by Cunnane (5) (breakdown may be necessary to 'detoxify' excess ALA, or to provide materials for synthesis of cholesterol and other lipids) remain unconvincing and more investigation is required.

Essential fatty acid activity of ALA

When the essential fatty acids were discovered over 60 years ago, attention was concentrated on LA and its metabolites in the *n*–6 family. When LA was given a potency of 100, γ-linolenic (GLA) had a potency of 115 and ALA only 9 (6). It might be thought that the presence of the 'essential' double bond in position 12 in ALA would give the compound full EFA activity but perhaps the possession of the additional bond in position 15 gives rise to steric hindrance that does not allow the activity to be expressed.

In a well-designed study, Leat (7) asked the important questions: (i) does ALA have any function that cannot be fulfilled by LA? and (ii) does LA have any function that cannot be fulfilled by ALA? He gave rats or guinea pigs diets that were either completely EFA-deficient or in which an EFA-deficient diet had been supplemented with either LA or ALA. In the rat, there were two functions of LA that could not be replaced by ALA. The final stage of reproduction in females — parturition — was prolonged and impaired in animals given only ALA, probably because of a failure to produce appropriate prostaglandins of the 2-series. In males, spermatogenesis could not take place when the diet contained only ALA. This defect did not occur in male mice and therefore, once more, the extrapolation of findings from a single species to any other should not be undertaken lightly.

Leat was unable to find any function for ALA that could not be replaced either partially or fully by LA. Because of the high content of *n*–3 fatty acids in the retina of the eye, it would seem likely that if there were a specific function for ALA, it might be found in the retina and affect the process of vision. Many authors writing on the subject of *n*–3 essentiality have argued in this way, mainly from a theoretical point of view. In Leat's experiments, it proved

impossible to deplete rat retina sufficiently to test this hypothesis to destruction but guinea pig retinas could be virtually depleted of *n*–3 acids. In those circumstances, electroretinograms appeared normal. The author did concede, however, that it remained possible that ALA and its metabolites were playing a more subtle role in vision that cannot be detected by electroretinography. However, pre-term infants receiving formulas in which the fat component was based on corn oil, with a low content of ALA, were found by Uauy and colleagues (8) to exhibit reduced electroretinogram responses compared with those receiving formulas supplemented with soybean oil or given human milk. Once again, we must be careful when comparing species and there is a clear need for more information on the specific ALA requirements of human beings, especially infants. I referred in reference 1 to circumstantial evidence for the essentiality of ALA in adults but more research is needed before we can be quite confident that it is correct to deem ALA to be absolutely 'essential'.

The main defect in Leat's ALA-depleted guinea pigs was the lack of a startle reflex and further studies on possible behavioural changes due to ALA deficiency would be well worthwhile.

Effects of ALA on coronary heart disease risk factors

Relatively few studies have examined the blood lipid lowering properties of ALA, compared with those that have documented the effects of LA in seed oils or EPA and DHA in fish oils. This is mainly because there are few edible oils that are rich in ALA. Some studies indicate that diets enriched in ALA can lower blood lipids in human subjects with hyperlipidaemia, mainly affecting the triacylglycerol fraction (5).

Other studies have found that ALA inhibits human platelet aggregation, reduces blood pressure in hypertensive patients and that low intakes may be associated with poor glucose tolerance. High intakes were also associated with a suppression of various components of the immune system (5). In none of these phenomena was it certain that the effect was specific for ALA itself and all need further investigation.

Recently, much publicity was given to the finding by a French research team that a diet enriched in ALA dramatically reduced further heart attacks and deaths in patients who had already suffered one heart attack (9). ALA was provided to the 'treated group' in the form of margarine made with rapeseed oil and the use of this oil was also encouraged in cooking and in the preparation of salads and other foods. The results were certainly impressive in that 16 cardiac deaths and 17 non-fatal heart attacks occurred in the control group compared with only 3 cardiac deaths and 5 non-fatal heart attacks in the treated

group. Improvements were apparent so quickly in the treated group that the trial was stopped after 27 months instead of running for the 5 years originally planned.

What is less certain is the cause of the decrease in coronary heart disease. It is impossible to attribute this improvement, as the authors wished to imply, specifically to ALA since, as a result of poor study design, several nutrient changes occurred in the treated group. Whereas the change in ALA was the largest proportional change to occur (a three-fold difference), it represented only a difference of 0.54% of energy due to ALA, or 1 g per day. The change in LA was about 4 g per day or 1.7% of energy. Moreover, these changes were assessed by a combination of 24 hour recall of the diet by the patients, assisted by a dietician, and a food frequency questionnaire, neither of which would be able to discriminate a 1 g change in a single nutrient. There were also differences between groups in their consumption of energy, total fat, cholesterol, vitamin C and vitamin E. There was no indication, either, that other aspects of lifestyle, that might have affected predisposition to heart attacks, were adequately controlled.

It is interesting that the authors 'hedged their bets' by describing their treatment diet as an "ALA-rich Mediterranean diet", implying that if it was not the ALA that was responsible for the dramatic change, then it was perhaps the mere fact of consuming a diet that 'everyone recognizes as healthy'. Although there is no scientific definition of a Mediterranean diet, it has become fashionable among health educators to use this term as if it encapsulated everything that was good in nutrition. While this publication may have demonstrated an undoubtedly dramatic clinical benefit, it has not shown that this was in any way due to nutrition, far less to a specific nutrient, ALA. Unfortunately, it reveals the unscientific nature of much current clinical nutrition research.

Long-chain polyunsaturated fatty acids of the *n*–6 family: γ-linolenic and arachidonic acids

What is γ-linolenic acid?

γ-Linolenic acid has entered the folklore as having health giving properties and it is an opportune time to look at the supporting evidence. The substance was discovered and named in 1919 by two German chemists as a component of evening primrose oil. γ-Linolenic acid is the trivial name for all-*cis*-6,9,12-octadecatrienoic acid, a triunsaturated fatty acid of the *n*–6

family. Since even γ-linolenic is a mouthful, it has now become common practice to use the abbreviation GLA. The positions of its double bonds distinguish it from its isomer, α-linolenic acid, which is all-*cis*-9,12,15-octadecatrienoic acid, the first member or parent fatty acid of the *n*–3 family.

Metabolism of GLA

Each 'family' of unsaturated fatty acids is built up by a series of alternate desaturation and elongation reactions starting with the parent fatty acid. The parent *n*–6 fatty acid is linoleic acid (*cis*,*cis*-9,12-octadecadienoic acid; *c*,*c*-9,12-18:2, LA). As illustrated in Figure 4.1, the first step in the metabolism of LA is the introduction of a double bond between carbon atoms 6 and 7, catalysed by the enzyme 6-desaturase. The product of this desaturation is GLA. The next reaction, a chain elongation, produces the 20-carbon dihomo-γ-linolenic acid (DGLA or sometimes DHLA; *c*,*c*,*c*-8,11,14-20:3) and this, in turn is a substrate for the 5-desaturase whose product is arachidonic acid (*c*,*c*,*c*,*c*-5,8,11,14-20:4, AA). Although further elongation and desaturation are possible, AA is the main product of the *n*–6 family and is the principal *n*–6 polyunsaturated fatty acid in most mammalian cell membranes. GLA, by contrast, is merely an intermediate in the sequence and does not accumulate in mammalian cells. Rather larger quantities of DGLA may be found. Concentrations in human blood platelet phospholipids of GLA and DGLA are around 0.1% and 2% respectively; these may rise to 0.3% and 4% after consumption of a diet rich in GLA (10).

While LA is the principal *n*–6 polyunsaturated fatty acid in most plant cell membranes and in many seed oil triacylglycerols, some plant species accumulate significant amounts of GLA in their seed oils as discussed later.

The main importance of the *n*–6 pathway is to provide a pool of membrane DGLA and AA sufficient continually to maintain a supply of eicosanoids required in metabolic regulation. We can deduce the importance of AA supply from the fact that membrane concentrations of AA are maintained in the face of relatively severe dietary LA restriction and only depleted after a long period of LA deficiency. However, much of what we know about unsaturated fatty acid metabolism comes from studies of laboratory animals, particularly rats. There are large species differences in 6-desaturase activity and it is now generally accepted that the 6-desaturase has low activity in human tissues and is probably the rate-limiting step in the whole sequence from linoleic acid to AA. Furthermore, GLA, the product of the 6-desaturase in the *n*–6 pathway, itself inhibits the desaturation step (11).

The rate limitation of the 6-desaturase in the *n*–6 pathway is important in understanding the claims for health benefits of GLA discussed later. It has been demonstrated both in experiments with cell fractions *in vitro* and by radioactive tracer studies in living rats (11). The studies *in vitro* showed that the 6-desaturase step was the slowest in the pathway, while those *in vivo* showed that labelled GLA was much more extensively converted into DGLA and AA than LA, much of which remained as unmetabolized precursor. There is some indirect evidence that people with low concentrations of DGLA in adipose tissue have intrinsically low 6-desaturase activity. 6-Desaturase activity decreases with age and is also depressed by the so-called 'stress hormones', adrenalin and cortisol, by alcohol, by fatty acids of the *n*–3 family and in the condition of diabetes mellitus.

Essential fatty acid activity

After the discovery of the essential fatty acids in 1929 (1), much energy was devoted to assessing the relative EFA activity of different fatty acids. In the 1960s, bioassays based on water permeability of skin, showed that GLA had higher EFA potency than its precursor, LA. A commonly used biochemical index of EFA deficiency has been the so called triene/tetraene ratio. When the dietary supply of linoleic acid is limited, oleic acid becomes the prime substrate for 6-desaturase. The major product of this (n-9) pathway is the trienoic acid 5,8,11-20:3 and its ratio to AA increases in EFA deficiency and decreases when LA is added back to the diet. The ratio was reduced far more rapidly when GLA was substituted for LA (11).

Hypocholesterolaemic activity

Reports on the blood cholesterol lowering potency of GLA are inconsistent. One publication claimed a large cholesterol lowering effect that was disproportionate to the degree of unsaturation when compared with linoleic acid, while others have found no differences between the effects of linoleic acid and GLA. A major problem of interpretation is that most experiments have used natural oils that contain unusually large quantities of GLA but they also contain linoleic acid and many other fatty acids as well as fat-soluble vitamins and other minor components. Tocotrienols, for example, have some cholesterol-lowering effects. Use of a placebo control, as distinct from an oil that does not contain GLA, also presents interpretational difficulties in view of the complex nature of the natural GLA-containing oils (12).

Eicosanoid formation

Eicosanoids (Greek eicosa = 20), so-called because they are derived from C_{20} polyunsaturated acids are oxygenated products of the cyclo-oxygenase and lipoxygenase pathways forming prostaglandins and leukotrienes respectively (reference 1 and later section). GLA itself is not a precursor for eicosanoids. However, its elongation/desaturation products, DGLA and AA, give rise to series-1 and series-2 prostaglandins, and series-3 and series-4 leukotrienes respectively (Figure 4.1). These substances, which have potent physiological effects at extremely low concentrations, are involved in communication between cells. Most relevant to the therapeutic properties attributed to GLA are the cells of the immune system involved in inflammation (see later section). In brief, the eicosanoids produced from AA have strongly inflammatory and platelet aggregating properties, whereas those produced from DGLA are anti-inflammatory. They also dilate blood vessels and prevent platelet aggregation.

Therapeutic claims

Of prime importance in understanding the claims made for the beneficial health properties of GLA and DGLA are (i) the rate limitation of the 6-desaturase which, if severe, may form a block in the pathway, preventing the formation of sufficiently large quantities of DGLA and AA further along the pathway and (ii) the properties of the eicosanoids formed from DGLA and AA.

The literature is replete with claims for the therapeutic benefits of GLA but disorders for which its efficacy has been tested in controlled clinical trials include: atopic dermatitis, rheumatoid arthritis, diabetic neuropathy, multiple sclerosis, ulcerative colitis, hypertension, premenstrual syndrome, schizophrenia, hyperactivity and several cancers. In almost all categories the results have been inconsistent, some finding improvement in the condition, others none. When this occurs, it is important to examine the design and interpretation of the studies before drawing final conclusions.

In most studies, GLA has been given in the form of evening primrose oil (EPO) but some studies have used borage or blackcurrant oils; their composition differs and each contains many substances other than GLA. The extent to which this may have influenced the outcome is discussed in more detail later. A frequent problem with diseases that have a strong inflammatory component is that their severity may vary over time and the symptoms may differ between individuals. In some trials, the numbers of subjects and the duration of the study may have been inadequate. The nature of the control group is vital.

The control group must receive a placebo — a substance that is judged to be physiologically inactive but whose identity cannot be distinguished from the active substance by the subject — and this may be an inert oil (e.g. a mineral oil) or another seed oil that does not contain GLA.

Some trials are conducted by comparing separate control and treated groups in parallel. Others have a 'crossover' design so that all subjects receive one regimen followed by the other in random order so that they act as their own controls. The two periods are often separated by a 'washout' period in which a different diet is given containing neither GLA nor placebo. The crossover design as used specifically in connection with EPO trials has been severely criticized (13) on the grounds that subjects who receive active treatment in the first period and who notice improvements will have low expectations for the next period while subjects who noticed no improvement in the first period will assume that they were on placebo and will expect an improvement in the next period. These expectations may introduce bias that will affect the measured difference.

There is clearly a need for further work to account for these inconsistencies. Nevertheless, the balance of evidence seems to suggest some benefits from GLA supplementation in diseases with an inflammatory component, which is probably due to the stimulation of prostaglandin E_1 formation from DGLA and the suppression of the corresponding metabolites from AA. A promising avenue of research which needs further study is in connection with diabetic neuropathy. Damage to the nervous system is a long term consequence of diabetes even when blood glucose is well controlled. Some research suggests that impairment of 6-desaturation may be at least partly involved and that a function of GLA could be to by-pass the slow desaturation of LA to GLA. Tissue concentrations of LA are normal or slightly higher than normal in diabetic patients, whereas levels of GLA, DGLA and AA are below normal. Insulin treatment results in the restoration of the GLA, DGLA and AA concentrations. Extremely large intakes of LA can reverse the effects of diabetic neuropathy to some extent, but very much smaller intakes of GLA are required (14).

Against these proposed benefits must be considered the reported risk (13) of increased inflammation, thrombosis and immunosuppression due to a slow accumulation of AA after prolonged use of GLA (more than one year). In the short term, AA does not seem to be elevated by diets rich in GLA (10) and this reinforces the need to consider long term effects of dietary components which, although completely 'natural' are not normally consumed in more than minute amounts.

Sources of GLA and products containing it

The only sources of GLA that are currently readily available are the seed oils of the evening primrose (EPO)and borage (BO) (**Table 4.1**). The seed oil of blackcurrant is also available.

Table 4.1. Composition of seed oils of evening primrose and borage.

Fatty acid		Evening primrose	Borage
16:0	palmitic	7.6	11.9
18:0	stearic	2.0	4.0
18:1*n*–9	oleic	9.7	16.2
18:2*n*–6	linoleic	69.4	36.2
18:3*n*–6	γ-linolenic	9.2	22.8
18:3*n*–3	α-linolenic	0.3	0.3
Others		1.8	8.6[1]

[1]Mainly 20:1+22:1+24:1; source: reference (17).

EPO was the first to be developed but the much higher concentrations of GLA in BO encouraged other manufacturers to market preparations based mainly on BO. One prominent manufacturer of a preparation based on EPO has suggested in its publicity material that BO preparations are less effective than EPO products and may even be harmful. A reason advanced for the greater effectiveness is that GLA is present almost entirely as molecular species of triacylglycerol in which one GLA is combined with two LA molecules: dilinoleoyl-mono-γ-linolenoyl-glycerol (DLMG) (15). The reputed potentially harmful effects of BO seem to be based on a single paper that found increased platelet aggregation and increased formation of thromboxane A_2, a powerful eicosanoid inducer of aggregation from AA, in human subjects given very high levels of BO (16). It was also suggested that the erucic acid content of BO could render the oil toxic, although there is little evidence for this contention. One study at least found no difference in GLA content of tissues or eicosanoid production by aorta when rats were given evening primrose or borage oils at equivalent dietary content of GLA (17). It is clearly important that issues concerning the relative effects of different GLA-containing oils are resolved quickly by further research. Apart from putative effects of DLMG, another possible explanation of the differences between oils is their content of minor components with potent activities. In other words, we may have to question how much of the effects for which claims are made are actually due to GLA at all.

Long-chain polyunsaturated fatty acids of the *n*–3 family

Structure, occurrence and formation

When animals ingest α-18:3, some is incorporated unchanged into lipids, doubtless a little is oxidized, but much is metabolized by a series of desaturations and elongations to longer-chain PUFA (Figure 4.2).

The largest sources of these long-chain PUFA are the oils of fish that eat phytoplankton containing a high proportion of α-18:3 in their lipids (18). In fish, these fatty acids are present in triacylglycerols that are stored in the liver (e.g. cod) or in the flesh (e.g. mackerel, herring). When α-18:3 is ingested by man and other simple-stomached land animals, very little is incorporated into storage triacylglycerols, most being directed into membrane phospholipids. The *n*–3 PUFA are particularly abundant in nervous tissue, especially brain, and in the retina of the eye. Human milk contains small but nutritionally significant amounts of EPA and DHA (19). Ruminants, to whose dietary lipids α-18:3 from pasture makes a large contribution, absorb very little since much is destroyed in the rumen by microbial hydrogenation. However, they manage to conserve sufficient to supply the needs of their nervous tissue membranes.

Metabolism of *n*–3 PUFA to eicosanoids

The *n*–3 PUFA are converted by a process of enzyme-catalysed peroxidation into a diverse series of oxygenated fatty acids termed 'eicosanoids' from their parent C_{20} acids (1). (Strictly, those compounds formed from C_{22} acids should be named 'docosanoids' but the all-embracing term 'eicosanoids' will be used for simplicity.) There are two main pathways, each catalysed by a distinct group of enzymes: the cyclo-oxygenase and lipoxygenase pathways (**Figure 4.3**). The former produces prostaglandins, prostacyclins and thromboxanes; the latter leukotrienes, named from the cells that are their most important source — the leukocytes (white blood cells). All these compounds exert potent biological activities at concentrations down to 10^{-9} g per gram of tissue. So powerful are they that eicosanoids must be produced locally and destroyed as soon as their effect has been exerted. Activities include muscle contraction, regulation of kidney function, modulation of inflammatory and immune reactions, regulation of hormone secretion, bone calcification, reproduction, visual function and electrical activity of the heart. Indeed they appear to function throughout the body as important metabolic regulators (1,19).

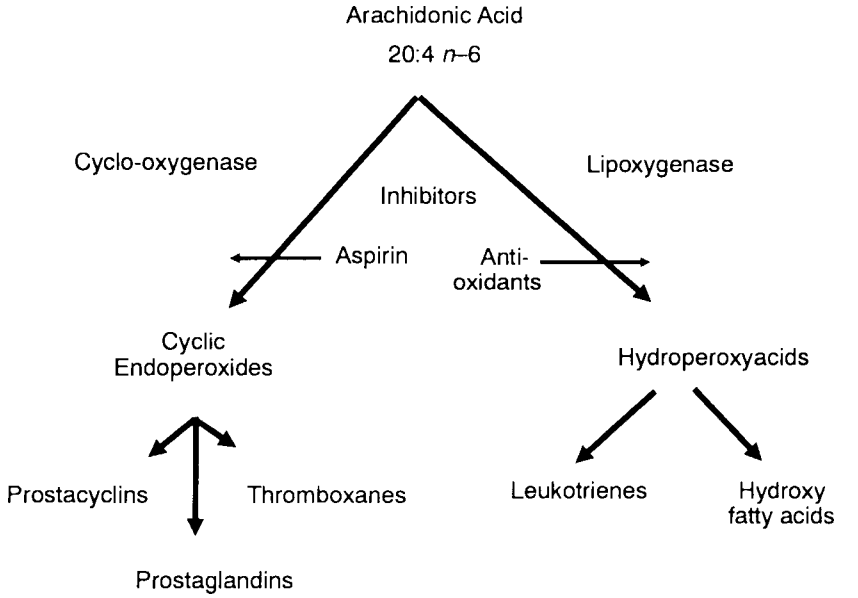

Figure 4.3. Two major pathways of arachidonic acid metabolism to eicosanoids.

The long-chain PUFA substrates for cyclo-oxygenase and lipoxygenase are stored in the phospholipids of cell membranes and released from these lipids by phospholipase A action. Diets common in the UK and similar western countries result in biological membranes in which *n*–6 PUFA predominate. When the diet contains a preponderance of *n*–3 PUFA, these may replace in part at least, the *n*–6 acids (mainly arachidonic acid, all-*cis*- 5,8,11,14-20:4(*n*–6). In these conditions, the *n*–3 PUFA become the preferred substrates for eicosanoid formation. In general, *n*–3 eicosanoids have weaker activities in such processes as platelet aggregation and blood vessel constriction than *n*–6 eicosanoids. An important potential practical consequence is, therefore, that diets rich in *n*–3 PUFA can result in an attenuation of eicosanoid activities. This may be a basis for some of the physiological activities ascribed to *n*–3 PUFA and discussed in succeeding articles in this series.

Dietary requirements for *n*–3 PUFA

Whereas it has been possible to demonstrate by direct experiment that linoleic acid is essential for man, the evidence for essentiality of α-linolenic acid is indirect. Since it cannot be made in the body, yet seems to be important in nervous and visual tissues, its essentiality can be assumed and requirements have been set at about 20% of linoleic acid requirements (see reference 20 and final section). These values are based solely on requirements to prevent EFA deficiency. However, as for linoleic acid, there has been much debate about whether human beings have requirements for α-linolenic acid in excess of those needed to prevent overt EFA deficiency.

Influence on plasma lipids and lipoproteins

Effects on plasma cholesterol: not all polyunsaturated fatty acids are equal

During the past 40 years an enormous amount of research has been devoted to understanding how different types of fatty acids in the diet affect plasma lipids (see Chapter 1) and most people are aware of the generalization that polyunsaturated fatty acids (PUFA) lower plasma cholesterol while saturated fatty acids (SFA) raise it. However, just as not all SFA have a cholesterol-raising effect (see Chapter 2), so not all PUFA have a lowering effect. Thus, PUFA of the *n*–6 family have the cholesterol-lowering effect ascribed by most people to PUFA in general, whereas those of the *n*–3 family seem to have minimal effects, at least when consumed in modest amounts in practical diets. Indeed, there is some evidence to suggest that high dietary intakes of fish oils, rich in *n–3* PUFA, may increase LDL-cholesterol (LDL-C) and apoprotein-B (apoB, the characteristic protein of LDL) concentrations in some people (21).

Recent work has suggested a plausible mechanism (22). When the human diet was supplemented with methyl esters of *n*–3 PUFA derived from certain fish oils, the plasma contained LDL particles enriched with cholesteryl esters esterified with *n*–3 PUFA. When incubated with human liver cancer cells in culture, these particles depressed LDL receptor activity and reduced the abundance of the messenger RNA that codes for the receptor. If this occurs in normal liver cells in the living body, it would provide an explanation for the raised LDL in plasma since the lower receptor activity would result in poor clearance of the particles from plasma.

Effects on triacylglycerols

The most important influence of *n*–3 PUFA is to decrease the concentration of VLDL in plasma. Since these particles are particularly rich in triacylglycerols, the main effect is to reduce blood triacylglycerol concentrations (23). This may have public health significance in view of evidence that triacylglycerol-rich lipoproteins play an important role in cardiovascular disease (24).

The first to describe this phenomenon were Harris and colleagues (25), who compared effects of two diets each providing 40% energy as fat and 500 mg cholesterol per day, one containing PUFA mainly in the form of the *n*–6 family (from vegetable oils), the other rich in the *n*–3 family (from salmon oil). Plasma concentrations of total cholesterol were 174 and 170 mg/dl on the *n*–6 and *n*–3 diets respectively compared with a value of 194 on a 'control' diet in which SFA and oleic acid predominated. However, plasma triacylglycerols were 75 mg/dl on the *n*–6 PUFA diet and 50 mg/dl on the *n*–3 PUFA diet compared with a 'control' value of 76 mg/dl. These differences between *n*–6 and *n*–3 diets were entirely due to changes in the VLDL fraction: LDL and high-density lipoprotein (HDL) were not changed. The amounts of fish required to elicit these effects were quite large, representing intakes of *n–3* PUFA of 20–30 g/day (current UK intakes are about 2 g/day).

Sanders and his colleagues (26) investigated the effects of more realistic amounts of *n*–3 PUFA (4 g/day from cod liver oil). Whereas total cholesterol did not change significantly, triacylglycerol concentrations fell by 23% and there was a significant rise in HDL-C. In another experiment (27), supplements of 8 g/day *n*–3 PUFA resulted in a 40% reduction in triacylglycerol concentration while 1–4 g/day had little effect. A lowering of 54% in plasma triacylglycerols was achieved (28) with 3 g/day *n*–3 PUFA from a 'MaxEPA' fish oil preparation compared with a similar amount of olive oil, with no significant change in total cholesterol or HDL-C.

There is some evidence (23) that dietary *n*–3 PUFA, while having little influence on total cholesterol or LDL-cholesterol, may reduce the size of the LDL particles and increase the ratio of their protein to cholesterol. It has been suggested that the atherogenic nature of LDL particles increases with decrease in particle size (23). The net effect of *n*–3 fatty acids in atherogenesis, therefore, remains unclear, since the effects on VLDL concentration may be construed as beneficial while those on LDL may be adverse.

The studies mentioned so far examined healthy subjects with normal or only moderately raised VLDL triacylglycerols. Several genetic disorders have been described that result in raised triacylglycerol concentrations due to

changes in the genetically determined proportions of different plasma lipoproteins. Thus, in Type IV hyperlipidaemia, only VLDL is raised, while in the Type IIb disorder, there are raised concentrations of VLDL and LDL, in Type V raised VLDL and chylomicrons and in Type III, raised concentrations of chylomicron remnants and VLDL with an abnormal enrichment of cholesterol. All, particularly the latter, are associated with increased risk of atherosclerosis. Many studies (reviewed in 26), have shown that modest intakes of *n*–3 PUFA lower triacylglycerols in these patients as well as in normal subjects. At low levels of intake, there is little effect on total cholesterol, as distinct from triacylglycerols, but at intakes much above 20 g/day, significant lowering of total cholesterol has been achieved in some studies.

In the studies described above, measurements of lipoprotein concentrations were made mainly on fasting blood samples. Recently, interest has turned to effects of dietary PUFA on lipoprotein responses immediately after a meal. A summary of the limited information on this topic can be found at the end of Chapter 1.

Mechanisms

Dietary or drug interventions may reduce plasma lipoprotein concentrations by reducing their synthesis in important tissues like the liver or increasing their uptake from plasma into tissue by receptor mediated mechanisms, thereby increasing their potential to be catabolized. Tracer studies in animals and human subjects have demonstrated effects of *n*–3 PUFA in the rates of synthesis of VLDL triacylglycerols, and at higher intakes, on rates of catabolism (23).

Conclusion

In conclusion, it seems beyond doubt that regular consumption of even a few grams a day of *n*–3 PUFA maintains low concentrations of circulating triacylglycerols. The influence of the *n*–3 PUFA on LDL and HDL seems less clear and there are conflicting reports in the literature. In some experiments higher intakes (over 20 g/day) of *n*–3 PUFA lowered LDL, in others similar amounts had no significant effects and in yet others, the concentration of LDL was even raised. Similarly, some experiments found little effect on HDL at any level of intake, whereas in other experiments HDL concentrations fell somewhat.

One explanation is that different individuals react differently to these dietary fatty acids. However, the possibility of dietary interactions has to be considered. Many of the differences reported in the literature concerning the effects of different SFA and oleic acid can be reconciled by considering

interactions between the SFA and the amounts of linoleic acid in the diet. The effects of any particular SFA depend on the appropriate dietary 'threshold' of linoleic acid (see Chapter 2). Similar considerations may also apply to the effects of the *n*–3 PUFA on plasma lipoproteins.

Dietary effects on blood clotting potential

New interest in thrombosis

In the scientific discussion that led to the formulation of dietary advice to modify fat consumption, the issue of thrombosis has been largely ignored in the preoccupation with atherosclerosis. The balance is now being redressed and thoughtful papers like that of Ulbricht and Southgate (29) have drawn attention to the potential importance of diet in relation to thrombosis. Almost all the recent experimental work linking dietary lipids with thrombotic potential has been concerned with the *n*–3 polyunsaturated fatty acids (PUFA) but before I describe the results of this research, I will briefly outline the physiological events leading to thrombosis since the interpretation of the experimental results demands some understanding of the processes involved and the types of measurements made.

The haemostatic system: blood vessel maintenance

The walls of blood vessels, especially arteries where the pressure is higher, are subject to continual wear and tear, for which a complex system of repair has evolved. Put simply, the blood has to find the resources to plug holes in the vessels to keep the tissue healthy on a minute by minute basis, and on occasion, to prevent uncontrolled bleeding from large wounds. However, if the blood clotting system were too highly primed, the plugs or 'thrombi' would not simply repair the wound but could block the vessels, cutting off blood supply to tissues. This is indeed what happens in a heart attack, when a coronary artery supplying blood to the heart becomes occluded by a thrombus. A balance must therefore be struck between allowing sufficient clotting to occur to repair wounds but guarding against dangerous unchecked thrombus formation. This is termed 'haemostasis'. Three main components of the blood contribute to haemostasis and eicosanoids formed from PUFA play a key role in regulating the behaviour of these components and the interactions between them. Of the three components, blood platelets and the plasma coagulation cascade (**Figure 4.4**) contribute to wound plugging, while the fibrinolytic system ensures that unwanted thrombi are dissolved. At the outset, a distinction has to be made between the continuous normal low level repair of microscopic injuries and the need to repair serious damage that leads to an explosive blood clotting reaction.

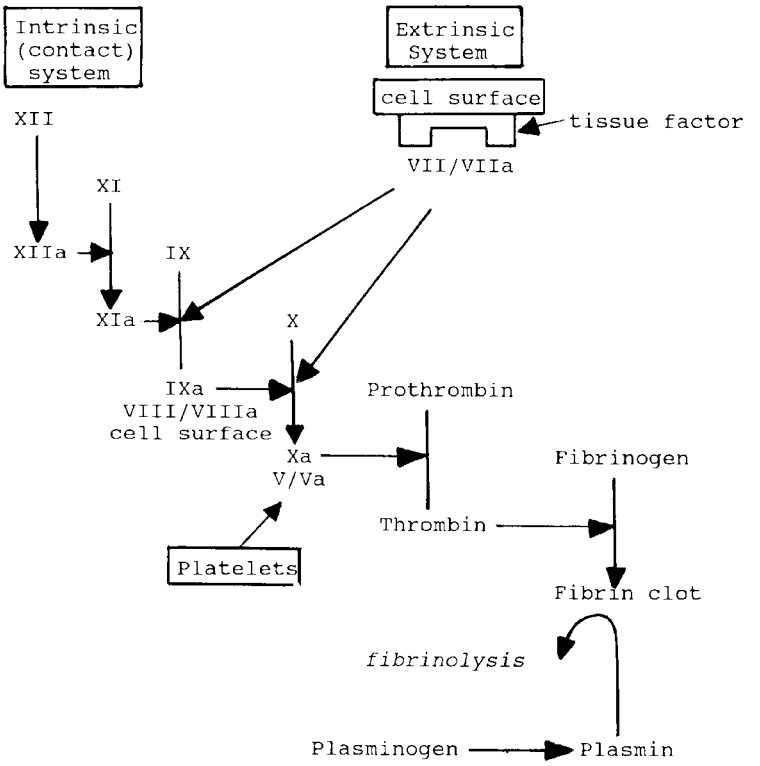

Figure 4.4. The blood coagulation cascade. Coagulation factors are denoted by roman numerals. At each step in the cleavage of inactive to active (denoted by 'a') form, an 'activation peptide' is released.

The clotting system comes into play as soon as the endothelial lining is broken, exposing a 'pro-aggregatory surface'. Firstly, exposure of collagen fibres releases adenosine diphosphate (ADP) which triggers the aggregation of platelets to begin the reaction. 'Activated' platelets cause secretion of various growth factors that organize several types of cells to continue the process of wound healing. Secondly, exposure of a specific cell membrane protein, normally 'hidden' from the blood, permits the binding of a plasma coagulant factor (Factor VII). This triggers a cascade in which inert precursor proteins are converted into active enzymes finally leading to the conversion of fibrinogen into fibrin, a prime component of the repaired wound. These reactions are localized to some extent by requiring a phospholipid surface on

which to take place. Thirdly, thrombus material that is excess to requirements can be dissolved by the enzyme plasmin acting on fibrin as a substrate. This enzyme is normally stored in an inert form as plasminogen and the conversion of inactive to active form is triggered by plasminogen activator. These processes are admirably described in the British Nutrition Foundation's report of its Task Force on Unsaturated Fatty Acids (19).

Two major players in the haemostatic system — the cells of the vessel wall and the blood platelets — produce different types of eicosanoids from the PUFA present in their membranes. Platelets produce mainly thromboxanes (TX) while vessel walls release prostacyclins (PGI) (Figure 4.3). Binding of TX to a specific receptor triggers changes in platelet shape and release of calcium from the cytosol, which in turn releases numerous proteins stored in granules within the platelet. These proteins cause the platelets to aggregate. In the endothelial cells of the vessel wall, by contrast, PGI is produced. This is a potent inhibitor of platelet TX synthesis and counteracts aggregation as well as being a vasodilator. The opposing effects of TX and PGI have led to the concept that the extent of platelet recruitment after vessel wall injury is a function of the ratio of concentrations of opposing factors. Thus, interventions that change this balance are conceived as altering thrombosis risk. While this is almost certainly a gross oversimplification, it provides a useful basis for much experimental work.

Measurement of haemostatic components

As will be appreciated, the entire physiological system regulating blood clotting is enormously complex. If one wishes to investigate the effects of dietary components on the potential for thrombosis to occur, it is usually necessary to make measurements on parts of the system only, especially in human subjects where interventions must be minimally invasive. Thus, it has been common to measure effects on the aggregation of isolated blood platelets *in vitro*, changes in one of the many components of the coagulation cascade, components of the fibrinolytic system or more simply to measure 'bleeding time' (the time taken for bleeding from a cut to stop). Each of these methods has problems with specificity, sensitivity and within-individual variation. Moreover, there is always a danger, when measuring one isolated component of a very complex system *in vitro*, of being misled about the change that would have occurred in the living person.

Experimental results with dietary n–3 PUFA

Citation of original research discussed in the next two sections can be found in references (19) and (30).

Dietary lipids and platelet aggregation

Studies can be grouped according to whether they involved conducting platelet or bleeding time tests in groups of people (i) who were naturally consuming different diets or (ii) whose diets were manipulated experimentally. In the former case one has to accept that other aspects of lifestyle may provide confounding factors, whereas in the latter case subjects can be randomly allocated to diets and there is the possibility for a double-blind placebo-controlled design.

Renewed interest in this subject was stimulated when it was observed that bleeding time was longer and platelet aggregation decreased in 21 Inuit subjects compared with age and sex-matched Danes. In Japan, the concentration of adenosine diphosphate needed to induce 50% of maximum platelet aggregation was higher in inhabitants of a fishing village, whose average fish intake was 250 g/day compared with farming villagers whose fish consumption was 90 g/day. In The Netherlands (Zutphen Study), however, there were no significant differences between bleeding times or platelet aggregation activity between groups consuming 2 or 33 g fish/person/day.

The amount of EPA contained in the 250 g or so of fish consumed daily by Japanese fishing villagers was about 2.5 g. (The same amount is present in about 25 ml cod liver oil or 12.5 g MaxEPA fish oil preparation.) Accordingly, several experimental studies have been conducted to assess the effects of this amount of EPA on platelet function and bleeding time. The latter was prolonged significantly in most studies. Platelet responsiveness was depressed in some, not affected in others or even increased in two.

An overall conclusion from all studies is that fatty fish consumption of the order of 200-300 g/day results in prolonged bleeding times and reduced tendency for platelet aggregation. However, the effects of consumptions of the order of 30 g/day or less that may be practical or acceptable in most 'Western' countries are less conclusive. Experiments involving larger numbers of subjects would be required to reach a firm conclusion.

It is postulated that the dietary factors responsible for the anti-coagulatory effects are the *n*–3 fatty acids present in the marine oils, which replace arachidonic acid in the platelet membrane, giving rise to the less aggregatory thromboxanes and there are several studies that are consistent with this view. However, it is possible to see diet-induced changes in the fatty acid composition of platelet membranes without parallel changes in platelet aggregratory properties as happened in the Zutphen Study. In contrast, adenosine diphosphate-

induced platelet aggregation tendency may decrease in parallel with increases in EPA content of membranes but persist for several weeks after stopping the fish oil diet when the fatty acid composition returned to normal.

In contrast to the effects of *n*–3 fatty acids, observational and dietary intervention studies have shown few significant effects of *n*–6 PUFA or *n*–9 MUFA.

Dietary lipids and the coagulation cascade

It is only recently that properly controlled studies have investigated the effects of dietary fats on some of the individual components of the cascade. Factors studied have been Factor VII, Factor X and fibrinogen and most have used fish oil supplements.

Reduction of total fat intake as a proportion of dietary energy reduced the concentration in plasma of Factor VII_c but the composition of the fatty acids had no influence. Fish oil supplements appeared to have increased Factor X activity in some experiments but not others. Most studies have found fibrinogen concentrations to be unchanged by dietary fish oil supplements but some have observed a reduction and one an increase.

Study of the effects of diet on fibrinolytic factors is in its infancy and again seems to have been concentrated on the effects of fish oils. Some studies have reported an increase in plasminogen activator inhibitor activity after giving dietary fish oil while others have reported no change or a reduction. Likewise, most studies report no change in plasminogen activator concentrations in response to fish oils while some report an increase and one a reduction.

These conflicting results are almost certainly due to technical difficulties in measuring appropriate activity parameters in such a complex system. The coagulation factors circulate in concentrations that are considerably in excess of requirements so that small changes in concentration that may be induced by diet may have no influence on reaction rates. Immunoassays measure all related species of a factor, both active and inactive, and may not, therefore, be particularly useful indicators of coagulant activity *in vivo*. Activation occurs on vessel surfaces rather than in liquid blood. Finally, once the enzymes are removed from their surfaces, they quickly bind to inhibitors so that their activity is neutralized. In current research, these limitations are being overcome increasingly by the measurement of so-called 'activation peptides' which are released into the blood circulation during the conversion of active to inactive enzymes but have no coagulant activity themselves. They may thus act as useful markers of enzyme activity (19,30).

Despite these limitations, there seems little doubt that dietary *n*–3 polyunsaturated fatty acids can influence thrombosis but whether at levels that are practical in most Western diets remains uncertain.

Influence on inflammatory disease

What is inflammation?

The body is vulnerable to injury from a number of sources including attack by microorganisms and physical wounding of tissues. Part of the protective response to such injury is inflammation whose cardinal signs are redness, heat production, swelling and pain. There may be dilation of blood vessels, leakage of fluid from vessels to cause oedema, release of plasma proteinases and of histamine. Inflammatory responses also involve the mobilization of various cells of the immune system, especially macrophages, which engorge microorganisms and cellular debris. The coordination of these cellular activities requires the participation of specific eicosanoids synthesized from membrane polyunsaturated fatty acids. Inflammation is sometimes an acute, short-lived reaction to injury but is often associated with chronic diseases. Among the best documented are rheumatoid arthritis and asthma.

The basis for dietary manipulations with n–3 PUFA in inflammatory disease

Of the various types of cells of the immune system, B-lymphocytes and T-lymphocytes are the main instruments of immune responses to specific antigens. Cells involved in inflammation are mainly polymorphonuclear leukocytes, macrophages and so-called non-specific 'killer' cells. Activation of these cells leads to stimulation of the activity of phospholipase A_2 resulting in the selective release of fatty acids from position 2 in membrane phospholipids. The proportion of *n*–6 to *n*–3 PUFA in cell membranes changes in response to the proportions of these PUFA in the diet and this will, in turn, influence the composition of the products released by the action of phospholipase A_2 (19).

Several pro-inflammatory products are produced by the metabolism of arachidonic acid (AA): prostaglandins and thromboxanes from the cyclo-oxygenase pathway and leukotrienes, lipoxins and various hydroxy-fatty acids from the lipoxygenase pathway (see earlier section, and Figure 4.3). Analogous products are also formed from eicosapentaenoic acid (EPA, a characteristic component of many fish oils). The products from EPA are considerably weaker inflammatory agents than those from AA. Moreover, EPA partially inhibits the formation of both prostaglandins and leukotrienes from AA (**Figure 4.5**). The net effect is that as the proportion of *n*–3 to *n*–6

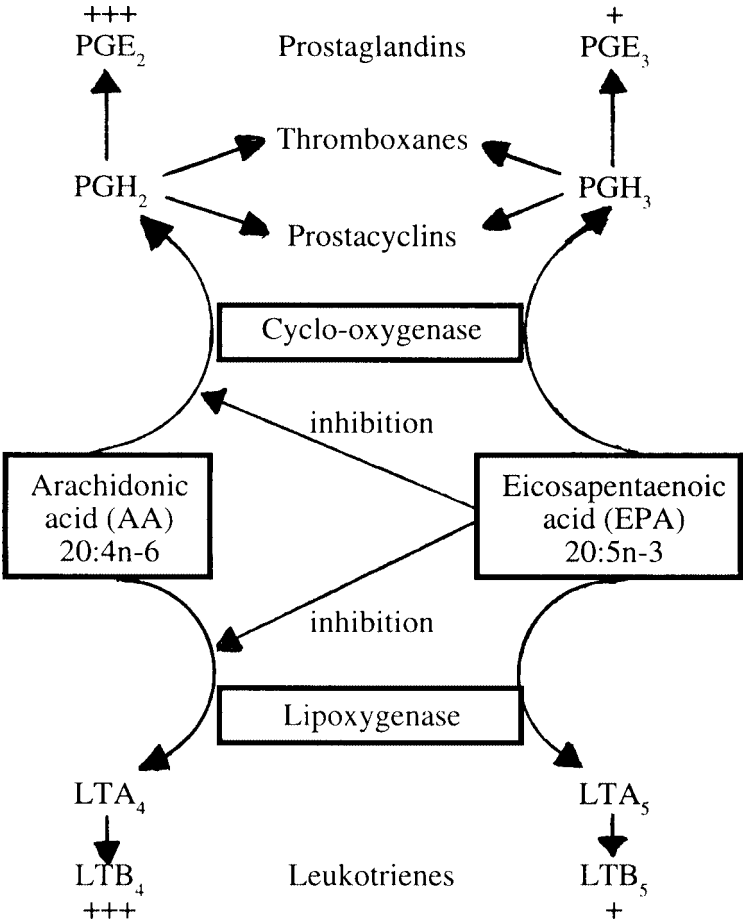

Figure 4.5. Competition between the *n*–3 and *n*–6 PUFA pathways for the production of eicosanoids; +++ signifies strongly inflammatory; + signifies weakly inflammatory.

PUFA in the diet is increased, the composition of cyclo-oxygenase and lipoxygenase products generated in response to injury becomes less inflammatory. This, put simply, is the basis for the hypothesis that changes in dietary lipids may influence the strength of the inflammatory response.

Studies with animals

Increasing the level of *n*–3 PUFA in the diets of experimental animals rapidly results in elevated concentrations of these fatty acids in the plasma lipoproteins and their enrichment in the membranes of blood cells such as erythrocytes and leukocytes. In general, these experiments demonstrate reduced local concentrations of the strongly inflammatory prostaglandins and leukotrienes from AA and suppression of inflammation. The latter was assessed in the living animal by measuring blood concentrations of 'cytokines' — proteins released during inflammatory reactions that activate cells of the immune system. Behaviour of isolated immune cells *in vitro* has also been used to assess the potential to mount an inflammatory response (19).

Rats and mice immunized with a particular mycobacterial peptide develop an arthritic disease closely resembling human rheumatoid arthritis. The severity of the disease can be reduced by giving a diet enriched with EPA. However, in another type of disease model, in which rats were immunized with collagen, dietary fish oil seemed to aggravate the arthritis (19).

Studies of human inflammatory disease

As in laboratory animals, diets rich in *n*–3 PUFA increase the concentration of these fatty acids in the membranes of human leukocytes and reduce the production of strongly inflammatory leukotrienes from AA. They inhibit AA metabolism by suppressing phospholipase A_2 and lipoxygenase activity. They also suppress the synthesis of inflammatory cytokines (19).

The best documented human inflammatory disease in regard to the influence of dietary *n*–3 PUFA is rheumatoid arthritis. In this condition, there is progressive erosion of the cartilage of the joints accompanied by swelling and inflammation. Many trials have been conducted to test the hypothesis that dietary fish oils rich in *n*–3 PUFA will alleviate the condition. Most have demonstrated modest improvement, but the responses have been variable. Problems encountered in this type of research are that:

- even without treatment, the disease is characterized by frequent remissions and exacerbations;
- most studies have been conducted over relatively short periods; and
- not all studies have been well controlled.

Regarding the last-named problem, it is considered important that patients are properly randomized into control and treatment groups to avoid confounding factors. Studies should be conducted double blind so that neither patients or researchers know who has been allocated into control or treatment groups. The control patients should receive a placebo, but it is not easy to decide what the nature of the placebo substance should be. In some experiments it has been liquid paraffin, a supposedly inert mineral oil, but in many it has been olive oil, on the assumption that the fatty acids of this oil are 'neutral' in their influence on inflammation. This may not necessarily be so. In all studies, patients were receiving some kind of drug therapy, such as aspirin or paracetamol, and were maintained on the same treatment during the trials. Thus, although the *n*–3 PUFA supplement was not the only 'treatment', the drug therapy should have been randomized equally between control and treatment groups.

In the short-term experiments that have been conducted thus far, the measurements made to assess the severity of the disease were joint stiffness and pain, recorded either subjectively by the patients or after examination by a rheumatologist. Biochemical tests have usually demonstrated the expected changes in membrane composition and reduced production of inflammatory eicosanoids and cytokines by leukocytes *in vitro*. However, no experiments have been conducted over times sufficiently long to observe effects on cartilage and bone erosion.

A summary of the main results of a selection of studies is presented in **Table 4.2**. The conclusion of many research workers in this field at the present time is that the results of short-term studies of dietary supplementation with fish oils or preparations from fish oils enriched in *n*–3 PUFA are encouraging but that more research is needed before dietary supplements of this nature can be recommended as standard treatment. The efficacy of dietary *n*–3 PUFA treatment for other diseases involving impaired immunity or inflammation, such as asthma, lupus or multiple sclerosis are less certain but subjects of active research.

Dietary *n*–6/*n*–3 polyunsaturates balance: is it important?

A common theme running through this chapter has been that the dietary ratio between *n*–6 and *n*–3 PUFA should greatly influence their mode of action because of competition between PUFA families for enzymes that metabolize them and for sites in membranes where they are stored.

Table 4.2. Effects of dietary n-3 PUFA on the severity of rheumatoid arthritis: summary of clinical trials.

Reference	Details of study[1]	Clinical outcome
31	FO, 20 g/day 12 patients for 6 weeks.	Reduction in severity of some but not all measurements of disease activity.
32	FO, 18 g/day (EPA+DHA, 2.0 + 1.2 g/day) 23 patients for 12 weeks . Placebo: OO, 18 g/day for 12 weeks Double blind.	Significant improvement in objective scores for grip strength and tender joints compared with placebo. Assessments improved in both groups.
33	FO: EPA+DHA: (i) 27+18mg/kg/day (ii) 54+36mg/kg/day 20 patients for 24 weeks. Placebo: OO, 6.8 g/day 12 patients for 24 weeks. Double-blind.	Subjective and objective improvements in joint inflammation especially in higher dose group.
34	FO, EPA+DHA, 2.0 + 1.2 g/day 27 patients for 12 weeks. Placebo: lipids with fatty acid composition similar to Danish diet. Double-blind.	Significant improvement in joint stiffness and tenderness

[1]FO: fish oil; OO: olive oil; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Here I shall summarize several original publications and reviews that have discussed diverse ways in which the *n*–6/*n*–3 ratio in tissues and in the diet might be important and examine the implications for future diets and foods.

Former over-emphasis on linoleic acid

Over 20 years ago an important publication described current knowledge at that time on the role of fats in human nutrition (35). This book appeared soon after the introduction of the new margarines that were rich in PUFA. These products were strongly promoted by the manufacturers and their

associated public relations agencies as useful contributors to the diets of those who wished to control their blood lipid levels as a means of preventing coronary heart disease. The publication was notable in that it regarded 'PUFA' as more or less synonymous with linoleic acid (LA, *n*–6 family) whereas the *n*–3 family was largely ignored. It seemed to be assumed that LA and the *n*–6 family were of major importance to health and that members of the *n*–3 family were mainly of academic interest. The second edition (36) published in 1989 presented a rather different picture, with chapters on early development and on marine lipids, which put more emphasis on the *n*–3 family. Nevertheless, a detailed examination of the consequences of changing the dietary ratio of *n*–6 to *n*–3 PUFA was conspicuously missing and had to wait until the present decade to be given due prominence.

The Japanese perspective

There are wide differences in intakes of *n*–3 PUFA throughout the world. For example, intakes of long-chain (C_{20} and C_{22}) PUFA range from about 4% of dietary energy in Greenland, 1% in Japan, 0.3% in the UK to 0.1% in the USA (37). To examine the potential consequences of changes in the ratio of *n*–6/*n*–3 PUFA, Japan provides one of the best examples, since the changes experienced there have been amongst the highest in the world.

Since the 1940s, Japan has experienced a roughly three-fold increase in intakes of saturated (SFA) and monounsaturated (MUFA) fatty acids and LA, and an increase in the *n*–6/*n*–3 ratio from 2.8 to 4. The current level of total fat consumption (about 60 g/day) is still considerably below that of Western countries (about 90 g/day) but the average intake of high-linoleate oils is comparable. Thus, as a proportion of total fat, the intake of LA is significantly higher in Japan than in Western countries but the intake of *n*–3 PUFA from fish oils has not increased proportionately.

In a detailed review, Okuyama *et al.* (37) associate these changes with certain cancers prevalent in the West, allergic diseases and thrombosis.

Cancer

Diets rich in LA enhance tumour development in laboratory animals dosed with carcinogens, whereas those enriched with *n*–3 PUFA tend to suppress it (38). Tumour cells enriched with *n*–6 PUFA produce larger amounts of a prostaglandin (PGE_2) derived from arachidonic acid (AA, *n*–6), which may have the effect of suppressing the host's immune system. The persistent over-production of inflammatory eicosanoids derived from the *n*–6 PUFA may stimulate proliferation of mutated cells leading to increased chance

of tumour development. Similar effects are not seen in cells enriched with *n*–3 PUFA, because long-chain *n*–3 PUFA, such as eicosapentaenoic acid (EPA), are poorer substrates than AA for the enzymes of eicosanoid production (see earlier section, and Figure 4.4). The metastasis of cancer cells (migration to tissues distant from the site of origin) is stimulated by LA and suppressed by *n*–3 PUFA.

Okuyama and colleagues (37) cite evidence for increases in the incidence of various cancers in Japan and the USA that correlate closely with increases in the dietary *n*–6/*n*–3 ratio. It must be borne in mind that these are merely statistical associations. However, the associations may have some biological plausibility based on the competitive metabolism of the *n*–3 and *n*–6 fatty acid families described above. More research on potential mechanisms is clearly needed.

Allergic hyper-reactivity

There seems no doubt that the incidence of allergic diseases has risen in developed countries in the last few decades and is still rising. Okuyama and colleagues (37) argue that this, too, is associated with an increasing ratio of *n*–6/*n*–3 PUFA. Once again they attribute this to an enhancement of the production of lipid mediators of inflammatory reactions by LA and its suppression by *n*–3 PUFA, citing published work with both experimental animals and human subjects. They also note the low prevalence of asthma among the Innuit peoples, who have among the highest intakes of *n*–3 PUFA in the world.

To keep the issue in perspective, it should be noted that, so complex is the immune system, the results of experiments on the effects of PUFA on immune function in either animals or human beings cannot be interpreted simply. Moreover, other plausible microbiological and immunological mechanisms have been proposed to account for the increased incidence of allergies that have little to do with diet (39). Interestingly, these ideas also involve a shift in the balance between different agents.

The nervous system

The long-chain *n*–3 PUFA, docosahexaenoic acid (DHA), is uniquely important in the membranes of nervous tissue. Failure to provide enough DHA, either as the preformed long-chain fatty acid or its precursor, α-linolenic acid (ALA) results in loss of visual acuity and in diverse behavioural changes. Both Okuyama *et al.* (37) and Connor (40) have discussed how too high a ratio of dietary *n*–6/*n*–3 PUFA can exacerbate the adverse effects of diets

already low in *n*–3 PUFA, partly by the inhibition by the *n*–6 family of conversion of ALA to DHA.

In a wide-ranging review, Hibbeln and Salem (41) discuss their hypothesis that depressive conditions may be due to a relative deficiency (including a high *n*–6/*n*–3 ratio) of *n*–3 PUFA. They propose that this may occur through one or several mechanisms all in some way resulting from a depletion of *n*–3 acids in nervous tissue membranes. They cite evidence for disruption of neurotransmitter systems, defective signal transduction pathways, reduced insulin sensitivity of receptors, enhanced calcium channel activity (resulting in an over-supply of intracellular calcium) and potentiation of a process called 'kindling'. The latter is a situation in which continued stress perpetuates cycles of self-propagating depressive behaviours. The authors mount an impressive list of citations to back their case but concede that the hypothesis "requires a great deal of future experimental work for confirmation".

Where now?

In this brief review I have focused on the three topics of cancer, allergy and behaviour. However there are other important areas of research in which the significance of a high *n*–6/*n*–3 PUFA ratio is being investigated. For example, *n*–3 PUFA lower the plasma triacylglycerol concentration and reduce the postprandial peak of blood triacylglycerols (see earlier section and Chapter 1). The *n*–3 PUFA also increase tissue insulin sensitivity and improve glucose tolerance. There is also some evidence that they reduce blood coagulating potential. All these factors are relevant to the problem of vascular diseases.

Based on his extensive work on the specificities of enzymes that metabolize PUFA, Lands (42) has devised equations that predict the relative concentrations of *n*–3 and *n*–6 PUFA in biological tissues from knowledge of intakes of LA and ALA. Lands is convinced that we need to revise our views on the appropriate dietary balance between *n*–6 and *n*–3 PUFA and suggests that "there has been a conspiracy of silence concerning over-abundance of *n*–6s in the diet" (43).

This research activity is feeding through to producers and consumers and has already stimulated interest in ways of incorporating these highly unsaturated lipids into foods without compromising eating quality. The hunt is on for 'new' seed oils that are relatively stable by virtue of their antioxidant content.

Camelina is one example. I recently discovered that the depot fat of horses contains 17% ALA (44). I cannot imagine that this will be a practical way for many people to increase their *n*–3 PUFA intake, but if we knew how the horse manages to store such a large amount of this fatty acid when few other land species do, we might devise ways to boost the *n*–3 content of animal products.

Dietary requirements for the essential fatty acids

Background

In 1991 the UK Department of Health updated its recommendations for dietary intakes of nutrients, now called 'Dietary Reference Values' (DRV) (20). In this final section I shall discuss the basis for those recommendations and their validity.

EFA are fatty acids that are absolutely needed for life but which cannot be synthesized from other compounds in the body. They must, therefore, be supplied in the diet. The main tasks of nutritionists at the outset are:

- to define which fatty acids are essential; and
- to assess how much is required in the diet.

The Department of Health's DRV Panel set out to assess, from published literature, the range of requirements for EFA, from which estimates of average needs (EAR) and those that would satisfy most of the needs of a specific population group (RNI) could be made. The lowest intakes likely to be adequate for any individual (LRNI) and excessive intakes likely to cause harmful and even overtly toxic effects were also estimated. The same approach was used for all other essential nutrients (vitamins, minerals and amino acids).

Which fatty acids are essential?

Although there is little dispute now that LA and ALA are dietary essentials there is some uncertainty as to whether the longer-chain polyunsaturated fatty acids (LCPUFA) are needed in the diet. Although it is clear that many human tissues can elongate and desaturate the precursor acids, there have been few rigorous studies and the elongation and desaturation activity has often appeared to be very weak. It is possible, therefore, that at times of high demand, the conversion of the precursor acids into LCPUFA might be insufficient. Under these conditions, the LCPUFA could be regarded as 'conditionally essential'. Such a situation occurs in regard to several of the essential amino acids.

There is considerable confusion in the Dietary Reference Values Report about the distinction between EFA and their LCPUFA derivatives. The report quite correctly says that the LCPUFA are not strictly EFA but then goes on to assume that they are. Thus "(EFA) are precursors of prostaglandins, thromboxanes, leukotrienes and of arachidonic, eicosapentaenoic and docosahexaenoic acids" is misleading in that it is the LCPUFA, not the EFA, that are the major precursors of the eicosanoids. The phrase "...most fish are good dietary sources" (of EFA) again blurs the distinction between EFA and LCPUFA, since it is the LCPUFA, not the EFA, that are characteristic of fish. These points may seem trivial.

However, such a report should be educational and is less likely to succeed in this role if it is ambiguous. Moreover, an important research need in EFA at present is to determine the extent to which, under certain conditions, the supply of LCPUFA may be limiting because of inadequate conversion of EFA into LCPUFA. In such circumstances, LCPUFA may become EFA. The Panel does allude to this possibility but this point tends to get lost later because of the EFA/LCPUFA confusion.

The statement: "a specific deficiency arising from inadequate dietary α-linolenic acid has not been demonstrated in healthy humans..." is perplexing as, surely, if specific deficiencies are observed, the subjects are, by definition, not healthy. Also, by defining α-linolenic acid as an EFA in the very first section, then to say deficiency has not been shown merely adds to the confusion. In my view, the Panel has been over-cautious here in omitting mention of the work of both Holman *et al.* (45) and Bjerve *et al.* (46). Neither paper showed unambiguously that the deficiency syndrome observed was due to α-linolenic acid, but the circumstantial evidence was compelling.

How much EFA is required?

The Panel offers a value for linoleic acid requirement of 1% dietary energy which is in line with most other (conservative) recommendations (**Table 4.3**). This seems sensible. However, they reject suggestions of higher values without giving reasons; some brief explanation would have been appropriate, since someone reading the Food & Agriculture Organization (FAO) Document (47) cited may reasonably be quite impressed by the arguments given in that publication for higher values. Also, it is a pity that possible extra needs in pregnancy and lactation are not discussed (if only to dismiss these on the grounds of adequacy of the mother's adipose tissue stores), since this is related to the fulfilment of requirements in infancy through the breast milk. The weakest aspect of this section is the derivation of the value

Table 4.3. Dietary Reference Values (DRV) for fatty acids and total fat.

Fat		Population average intake as % energy[1]	
		including alcohol	excluding alcohol
Fatty acids			
Saturated		10	11
Cis-Monounsaturated		12	13
Cis-Polyunsaturated[2]		6	6.5
including: linoleic	1		
linolenic	0.2		
Trans-unsaturated[3]		2	2
Total		30	32.5
Total fat[4]		33	35

[1]Alcohol is taken to contribute, on average, 5% of energy. [2]The recommended intakes to avoid essential fatty acid deficiency are: linoleic at least 1% and α-linolenic acid at least 0.2% of total dietary energy for infants, children and adults. The figures of 6/6.5 are thought to be optimum intakes of total PUFA to maintain appropriate blood lipid levels. The recommended individual maximum intake is 10% of energy. [3]About 5 g/day. [4]Total fat intake should be calculated from the sum of fatty acid intakes and glycerol, i.e. total fat = SFA + MUFA + PUFA + *trans* + glycerol. An increase in consumption of any fatty acid should be avoided.

for α-linolenic acid. The European Society for Paediatric Gastroenterology and Nutrition (48) is quoted as suggesting a ratio of linoleic/α-linolenic of 4.5:0.5 (9:1), the implication being that this is an average value in human milk. In the absence of more rigorous information, this seems a reasonable approach.

However, the last sentence of section 3.1.5 reads: "The Panel, *therefore*, (my italics) recommended that..... linoleic acid should provide at least 1% and α-linolenic at least 0.2% of total energy". This gives a ratio of 5:1. Whereas this may be entirely reasonable on the basis of current knowledge, it is unsatisfactory in that it appears to be a figure plucked out of the air. The use of the word 'therefore' implies that the figures have been derived through some kind of logical process from what has gone before, but this does not seem to be the case. One can sympathize with the Panel's dilemma in wrestling with inadequate data. However, it should at least be demonstrated that the chosen figure was derived with some kind of logic or else no recommendation should have been made. Otherwise the integrity of the report is in some question.

Harmful effects of lipid peroxidation

The section on lipid peroxidation focuses on the biology of lipid peroxidation *in vivo* and the role of radicals in the progression of degenerative disease and gives a good concise summary. It properly points out that the presence of peroxidized lipids may be a result rather than a cause of disease but does not consider the possibility that both relationships may exist in certain circumstances, i.e. lipid peroxides could have a role in initiating pathological processes as well as being a result of necrosis. The scientific evidence for an initiating role seems to be underplayed.

It is surprising that in a document concerned with appropriate dietary intakes of nutrients, there is no discussion about the effects of consumption of foods containing lipids that have been peroxidized either in food storage or preparation. Significant research has been reported on products of peroxidation of both PUFA and cholesterol in foods (49 and Chapter 3). While there is good evidence that peroxides and hydroperoxides are not absorbed from the gut, there is substantial evidence for the absorption of low molecular weight products and for the cytotoxicity of some of these compounds. Much of this work is in the food science literature and, moreover, a large proportion is from Japanese laboratories, facts that need a certain awareness of the appropriate fields before an adequate assessment of the literature can be undertaken. The discipline of food science has been underrated especially among those with medical backgrounds and this may account for the omission here.

The practical implications are for:

- care over adequate intakes of antioxidant nutrients, which will give added protection against peroxidation products formed both before consumption and *in vivo*; and
- more care in storage and preparation.

The latter is not a concern of the report but the former is alluded to in several sections. Again, there is a slight feeling of statements being plucked out of the air although this may be a result of the need strictly to limit the size of an already long document. Thus in the conclusions, the statement is made that "intake of antioxidants is the major dietary contributor to that balance [between radical activity and antioxidant status]" which suggests an important role for dietary antioxidants, yet earlier the phraseology is more cautious: "it is not established how their [antioxidants] dietary intake modulates their activity in the body". This would have been an appropriate place to cross reference to the work of the Vitamins Working Group, since vitamins C, E, and possibly A and the carotenoids are regarded as having an important role

as antioxidants, one function of which is to limit the peroxidation of PUFA *in vivo*. In the chapter on vitamin E, the Vitamins Working Group could give no precise requirement for vitamin E but remarked that requirements for this nutrient would be determined by PUFA intakes. However, although the need for antioxidant protection is mentioned in the chapter on fat, there is no discussion of quantitative interrelationships and no cross-reference to the chapters on antioxidant vitamins. This may suggest insufficient liaison between the two working groups.

It is most interesting that although the potentially harmful effects of high PUFA intakes are played down ("No evidence exists that high dietary intakes of PUFA have been associated with any human disease"), in the final event, the working group was overcome with caution and was able to recommend that "the dietary intake of polyunsaturated fatty acids by individuals should not exceed 10% of food energy". The reasons for this limitation are not really adequately discussed.

Research into the metabolism of and requirement for the polyunsaturated fatty acids is proceeding quickly now. A revision of the recommendations for dietary intakes of the EFA and their long-chain derivatives will soon be required. This should take into account the importance of the balance between the *n*–3 and *n*–6 families discussed earlier and the possible requirements for the LCPUFA as 'conditionally essential' under certain conditions.

Endpiece: What is 'essential' in regard to lipids?

Essential nutrients and essential metabolites

The concept of 'essential nutrients' is well established in nutrition science. Although most components of cells, tissues and organs have essential roles to play in the body, when applied to nutrients the word 'essential' implies that a substance, which is needed by the body but cannot be adequately provided by the body, is required absolutely in the diet. There is little doubt now that LA and ALA fit this definition of essentiality.

Some difficulty arises when we think about the longer-chain more highly polyunsaturated metabolites of linoleic and α-linolenic acids (LCPUFA). I once heard a colleague describe LCPUFA as essential fatty acids because, he said, without them we would die. This is a biochemist's definition and the term should be 'essential metabolites' as distinct from essential nutrients. As far as we know, human tissues contain the enzymes required for the further desaturation and elongation along the *n*–3 and *n*–6 pathways (1). Cats are interesting in that

they lack the 6-desaturase and cannot synthesize γ-linolenic or arachidonic acids. Cats need arachidonic acid and must obtain it from the diet; hence they are obligate carnivores.

Conditionally essential fatty acids and lipids?

Some have argued that a number of enzymes of LCPUFA metabolism, notably the 6-desaturase, work so slowly in human tissues that insufficient LCPUFA can be formed from the precursors, linoleic and α-linolenic acids for the body's needs (50). Therefore, the LCPUFA can be regarded as essential for all practical purposes. Others would not regard them as essential in all cases but would say that they become essential in certain clinical conditions, for example diabetes, or at certain stages of life, e.g. in foetal or early neonatal life (50). There are parallels here with the amino acids. For example, glycine, which can normally be synthesized quite adequately in the body, is used for the synthesis of glutathione. Sometimes the demands for glutathione are so high that additional glycine is needed from the diet. Under these conditions, it is known as a 'conditionally essential' amino acid. Thus, LCPUFA may, according to some, be 'conditionally essential' fatty acids. In my view, the evidence is not particularly persuasive and the case for conditionally essential fatty acids has certainly not been proven so conclusively as for amino acids.

Cholesterol

There is no doubt that cholesterol is an essential metabolite. It is an obligatory and often major component of animal cell membranes. No other naturally-occurring sterol structure is known to be able to replace cholesterol while still maintaining membrane function. Cholesterol is also an important precursor of bile acids, steroid hormones and cholecalciferol (vitamin D). Many studies that employed isotopic labelling techniques have demonstrated active cholesterol synthesis in human tissues, principally the liver and the gut. It seems therefore that cholesterol could not be regarded even as conditionally essential. However, there may be an exception, namely in newborn bottle-fed babies. At this age large amounts of cholesterol are required for the membranes of the brain and nervous system. Human milk is particularly rich in cholesterol and it is possible that the baby's liver may not be able to synthesize all the cholesterol needed to compensate for its lack in infant formula (see Chapter 6).

Saturated fatty acids

All types of fatty acids, saturated, monounsaturated and polyunsaturated are essential components of biological membranes, using 'essential' in the biochemical rather than the nutritional sense. It seems to have been universally

assumed that a supply of saturated and monounsaturated fatty acids is not absolutely necessary, because they can be synthesized in the body. Evidence for very active fatty acid biosynthesis in human tissues, however, is lacking even when fat makes only a small contribution to dietary energy (51). It could be argued, therefore, that a dietary source of long-chain saturated fatty acids is often necessary for the supply of structural lipids. For example, saturated fatty acids contribute 50% of some membrane phospholipids and 100% of the phospholipid fatty acids of the lung surfactant (52). The need for dietary monounsaturated fatty acids is less certain since there does seem to be a generally high level of 9-desaturase activity in human tissues.

Conclusion

We therefore have the intriguing possibility that the distinction between the classic essential fatty acids and the so-called non-essential fatty acids is not as clear as it once appeared to be. It may be argued that 'essential fatty acid deficiency' signs have not been observed in relation to the saturated fatty acids but given the near impossibility of devising diets depleted of saturated fatty acids and the potential for some endogenous biosynthesis, this is not surprising.

More research is required into the ability of human tissues to synthesize saturated, monounsaturated and long-chain polyunsaturated fatty acids in conditions of high demand for these substances. With improved techniques for the use of stable isotopes as tracers in human metabolism, this should be feasible. It will be difficult and expensive but undoubtedly rewarding.

References

1. Gurr, M.I. and Harwood, J.L. (1991) Lipid Biochemistry: An Introduction, 4th edition, Chapman and Hall, London.
2. Hansen, A.E *et al.* (1958) *Journal of Nutrition*, 66, 565–576.
3. Press, M. *et al.* (1974) *British Medical Journal*, 2, 247–250.
4. Sprecher, H. (1992) in Essential Fatty Acids and Eicosanoids (A.Sinclair and R.Gibson, eds) AOCS Press, Champaign, Illinois, pp.18–22.
5. Cunnane, S.C. (1992) in Essential Fatty Acids and Eicosanoids (A.Sinclair and R.Gibson, eds) AOCS Press, Champaign, Illinois, pp.379–382.
6. Thomasson, H.J. (1962) *Nature*, 194, 973.
7. Leat, W.M.F. (1989) in Dietary Omega–3 and Omega-6 Fatty Acids (C.Galli and A.P.Simopoulos, eds) Plenum Press, New York and London, pp.219–226.
8. Uauy, R.D. *et al*. (1990) *Pediatric Research*, 28, 485–492.
9. de Lorgeril, M. *et al*. (1994) *Lancet*, 343, 1454–1459.
10. Barre, D.E. and Holub, B.L. (1992) *Lipids*, 27, 315–320.

11. Hassam, A.G. (1985) in The Role of Fats in Human Nutrition, (F.B.Padley and J.Podmore, eds) Ellis Horwood, Chichester, pp.84–100.
12. Gurr, M.I. *et al.* (1989) *Nutrition Research Reviews*, 2, 63–86.
13. Kleijnen, J. (1994) *British Medical Journal*, 309, 824–825.
14. Horrobin, D.F. (1992) in Essential Fatty Acids and Eicosanoids (A.Sinclair and R.Gibson, eds) AOCS Press, Champaign, Illinois, pp.367–372.
15. Horrobin, D.F. (1994) *Pharmaceutical Technology Europe*, December 1994, 14–15.
16. Barre, D.E. *et al.* (1993) *Nutrition Research*, 13, 739–751.
17. Raederstorff, D. and Moser, U. (1992) *Lipids*, 27, 1018–1023.
18. Lees, R.S. and Karel, M. (eds) (1990) Omega-3 Fatty Acids in Health and Disease. Marcel Dekker, New York and Basel.
19. British Nutrition Foundation (1992). Report of the Task Force on Unsaturated Fatty Acids. Chapman and Hall, London.
20. Department of Health (1991) Dietary reference values for food energy and nutrients for the United Kingdom. Report on Health and Social Subjects, 41, Her Majesty's Stationery Office, London.
21. Nestel, P. *et al.* (1984) *Journal of Clinical Investigation*, 74, 82–89.
22. Lindsey, S. *et al.* (1992) *Journal of Lipid Research*, 33, 647–658.
23. Illingworth, D.R. and Ullmann, D.(1990) in: Omega-3 Fatty Acids in Health and Disease (Lees, R.S and Karel, M., eds) Marcel Dekker, New York and Basel, pp.39–69.
24. Simons, L.A. *et al.* (1978) *Atherosclerosis*, 54, 75–88.
25. Harris, W.S. *et al.* (1983) *Metabolism*, 32, 179–184.
26. Sanders, T.A.B. *et al.* (1981) *Clinical Science*, 61, 317–324.
27. Bronsgeest-Schoute, H.C. *et al.* (1981) *American Journal of Clinical Nutrition*, 34, 1752–1757.
28. Rogers, S. *et al.* (1987) *Atherosclerosis*, 63, 137–143.
29. Ulbricht, T.L.V and Southgate, D.A.T. (1991) *Lancet*, 338, 985–992.
30. Vorster H.H. *et al.* (1997) *Nutrition Research Reviews*, 10, 115–135.
31. Sperling, R.I. *et al*. (1987) *Arthritis and Rheumatism*, 30, 987–988.
32. Cleland, L.G. *et al.* (1988) *Journal of Rheumatology*, 15, 1471–1475.
33. Kremer, J.M. *et al.* (1990) *Arthritis and Rheumatism*, 33, 810–820.
34. Nielsen, G.L. *et al.* (1992) *European Journal of Clinical Investigation*, 22, 687–691.
35. Vergroesen, A.J. (1975)(ed) The Role of Fats in Human Nutrition, 1st edition, Academic Press, London.
36. Vergroesen, A.J. and Crawford, M.A. (1989)(eds) The Role of Fats in Human Nutrition, 2nd edition, Academic Press, London.
37. Okuyama H. *et al.* (1996) *Progress in Lipid Research*, 35, 409–457.
38. Ip, C. *et al.* (eds) (1986) Dietary Fat and Cancer. Alan R Liss, New York.
39. Bryan, J. (1997) *Biologist*, 44, 445.
40. Connor, W.E. (1991) *Atherosclerosis* Reviews, 23, 191–220.
41. Hibbeln, J.R. and Salem, N. (1995) *American Journal of Clinical Nutrition*, 62, 1–9.

42. Lands, W.E.M. (1995) *American Journal of Clinical Nutrition*, 61, 721S–725S.
43. Health and Nutrition News (1997) *INFORM*, 8, 704.
44. Brooker, E.G. and Shorland, F.B. (1950) *Biochemical Journal*, 46, 80–85.
45. Holman, R.T. *et al.* (1982) *American Journal of Clinical Nutrition*, 35, 617–623.
46. Bjerve, K.S. *et al.* (1987) *American Journal of Clinical Nutrition*, 45, 66–77.
47. Food and Agriculture Organization (1980) FAO Food and Nutrition Series, 20, Rome, Italy.
48. European Society for Paediatric Gastroenterology and Nutrition (1987) *Acta Paediatrica Scandinavica*, Suppl. 336.
49. Gurr, M.I. (1988) In: Nutritional and Toxicological Effects of Food Processing (R.Walker and E.Quattrucci, eds) pp.139–155. Taylor and Francis, London.
50. Sinclair, A and Gibson, R.(eds) (1992) See several papers in: Essential Fatty Acids and Eicosanoids. AOCS Press, Champaign, Illinois.
51. Leitch, C.A. and Jones, P.J.H. (1993) *Journal of Lipid Research*, 34, 157–163.
52. Gunstone, F.D. *et al.* (1994) *The Lipid Handbook*, 2nd ed. Chapman and Hall, London.

Chapter 5

Dietary Fats in Relation to Weight Control

Some epidemiological studies reveal a statistically positive association between the proportion of fat in the diet and the percentage of fat in the body. High-fat diets are associated with appreciable short-term increases in energy intake. The body may attempt to compensate by decreasing food intake but this may be insufficient to overcome the effects of the high energy density of fat. A fat calorie may indeed be more fattening than a carbohydrate calorie because the body seems unable, in the short-term, to increase its oxidation of fat in response to excessive intakes. Instead, the excess is put into stores that are apparently limitless. In contrast, carbohydrate stores are limited and intakes in excess of requirements are readily oxidized. In the longer term, fat oxidation adjusts to maintain a balance between intake of fuels and their oxidation, resulting in a new but higher stable weight.

Tailor-made triacylglycerols containing mixtures of long-chain fatty acids that are relatively poorly digested and absorbed and short-chain and medium-chain acids with intrinsically lower energy value have a metabolizable energy value of 5 rather than 9 kcal/g.

Several compounds have been tested as non-digestible fat substitutes but only one, a sucrose polyester, has performed adequately. The Procter & Gamble sucrose polyester, olestra (Olean), has been given US Food & Drug Administration (FDA) approval for limited food use. The ester bonds of sucrose polyester are not digested by pancreatic lipase and so the fatty acids are not absorbed and the substance has no energy value. Of major concern to nutritionists has been the reduction in absorption of several fat-soluble vitamins during the consumption of sucrose polyester, and olestra is fortified with vitamins A, D, E and K. Nevertheless some concern remains about the effects on fat-soluble vitamin status of continued consumption of relatively high levels of sucrose polyester and the effect on absorption of other fat-soluble substances, such as carotenoids, that may not be fortified. There is no evidence

that sucrose polyester can be fermented in the large bowel and extensive safety testing has not revealed significant problems, although some nutritionists still have reservations about possible longer-term effects in man.

The prevalence of obesity appears to be increasing in the UK and many other industrialized countries, despite decreases or little change in total energy intakes. Current advice about weight maintenance and weight loss is to reduce the proportion of fat in the diet. The variety of low-fat alternatives to more traditional foods has increased in recent years. It seems clear that the use of lower fat foods can reduce fat intakes but not everyone who adopts this course of action achieves reduced energy intake. Everyone replaces some fat energy with carbohydrate; some make a full energy compensation, while others do not fully compensate and therefore reduce energy intake. The long-term influences on body weight need further evaluation in free-living, as distinct from experimental, conditions.

Energy nutrients and body weight control

Problems with dietary fat

Some fat in the diet is almost inevitable. All biological tissues contain lipids to varying extents. Moreover, two fatty acids, linoleic and α-linolenic, are essential in the diet (see Chapter 4) and without them we would die. The amounts required are very small, however, and it is quite difficult to devise natural diets that are deficient in essential fatty acids. Nevertheless, people in industrial countries have developed a taste for relatively large amounts of fat and there are many (e.g. see reference 1) who believe that we would be well advised to reduce the amount considerably. Excessive amounts of dietary fat have been associated with many ills, including cardiovascular disease, cancer, diabetes, and obesity. Of these, the most obvious direct link might seem to be between fat intake and obesity because (i) fat has weight-for-weight over twice the energy of carbohydrates and (ii) fat contributes to palatability and might, therefore, be expected to encourage us to eat more food.

Many have argued that what matters in regard to the potential for gaining excessive weight is total energy intake, rather than whether the calories are contributed by fat or carbohydrate. In other words, they have assumed that the 'fattening potential' of one calorie of fat that is excess to requirements is equal to the fattening value of one calorie of carbohydrate. This view has been challenged mainly by two research groups, those of Tremblay and his colleagues in Canada (2) and of Flatt and colleagues in the USA and Switzerland (3).

Metabolic basis of body weight control

Before we can examine the new concepts developed by these researchers, it is important to understand the basics of how the body regulates its size and composition. The structure of the body relies mainly on proteins, the bone minerals, and the structural lipids found in membranes. To maintain this structure and sustain life, fuels, mainly glucose and the fatty acids of triacylglycerols, have to be burned to supply metabolic energy. These fuels are derived, ultimately, from the foods we eat but are held in storage as glycogen (carbohydrate) and adipose tissue (lipid). The biochemistry involved in the accretion of body tissues and fuel stores and the utilization of fuels from these stores to supply metabolic energy is collectively known as 'energy metabolism'.

It is clear that the body weight of adults (even if it is above what might be regarded as 'ideal') remains relatively stable over long periods of time despite considerable short-term fluctuations. The relative proportions of the major body constituents, protein, carbohydrate and fat remain relatively constant during these periods even though the food we eat every day may vary widely in composition. It is apparent that there is a balance between the accretion of body constituents and the oxidation of protein, carbohydrate and fat that acts to correct any major perturbations from what the body regards as normal.

Metabolism adapts so that the disposal of protein, carbohydrate and fat occurs at rates that, on average, correspond to their relative proportions in the diet. The correspondence is not exact because there is some conversion of amino acids and triacylglycerol-glycerol into glucose and some conversion of carbohydrate into fat, although the latter is not an important process in human beings. There is a complex interplay between the composition of the diet and of the body stores that results in a minute to minute supply of circulating substrates and hormones to regulate the composition of the fuel mix that is oxidized.

Protein oxidation is efficiently controlled, responding precisely to the amount of protein consumed in the diet, so that the deposition of structural protein and the production and maintenance of key enzyme systems is precisely regulated (**Table 5.1**). Carbohydrates and fats are the main fuels giving rise to the production of adenosine triphosphate (ATP), which is the main energy-rich molecule used by the body to provide the power for synthetic biochemical reactions. Most tissues, of which muscle is the principal user of fuel, use both fats and carbohydrates as sources of energy. The brain is unique in not being able to oxidize fat. It relies almost entirely on glucose as a fuel. This is the reason why the body maintains blood glucose concentrations within

Table 5.1. Principal concepts underlying the regulation of body weight.

• Body energy stores are stable only when total energy intake is equivalent to total energy expenditure.
• Adjustment of protein oxidation to intake is effectively achieved and indeed is a first priority in the regulation of the body's metabolism (8).
• The oxidation of carbohydrate must be attuned to intake because the carbohydrate store (glycogen) is small and never appears to expand (8).
• Fat oxidation cannot adapt quickly to excessive fat intakes.
• In the longer term, fat oxidation adapts to maintain equilibrium between the mix of fuels consumed and their oxidation, maintaining body fat stores at a new stable level.
• Individuals differ markedly in their ability to increase fat oxidation in response to exercise.
• High-fat diets are associated with appreciable short term increases in energy intake.

narrow limits and, so that this can be achieved, carbohydrate stores in the form of the glucose polymer, glycogen, are also maintained within strict limits.

One thing is certain: the body cannot thwart the laws of thermodynamics (Table 5.1). Body energy stores can only remain stable when energy intake is equivalent to energy expenditure over an extended period. Energy intake is equivalent to the sum of the energy contents of the proteins, carbohydrates and fats in the diet. Energy expenditure is more complex. A large proportion is accounted for by the 'basal metabolic rate'. This represents the heat generated by all the bodily functions that continue even when we are asleep: the pumping of the heart, the process of breathing and all the biochemical reactions in all tissues and organs. In our waking hours, each of us expends various amounts of energy in physical activity. After each meal, too, there is an increment in heat production over and above the basal metabolic rate due to the metabolism of the foods ingested. This is known as diet-induced thermogenesis. During the metabolism of food constituents, oxygen is consumed and carbon dioxide generated. The ratio of the carbon dioxide produced to oxygen consumed is called the respiratory quotient (RQ, **Figure 5.1**). Measurement of RQ gives a good measure of the fuel mix being burned in the body at that time.

RQ	=	carbon dioxide produced/oxygen consumed for each fuel
RQ, carbohydrate	=	0.7
RQ, protein	=	0.8
RQ, fat	=	1.0
FQ	=	([EI as protein/total EI] × RQ protein) + ([EI as lipid/total EI] × RQ lipid) + ([EI as carbohydrate/total EI] × RQ carbohydrate)
where EI	=	energy intake

Figure 5.1. The relationship between Respiratory Quotient (RQ) and Food Quotient (FQ).

Some early concepts of how body weight is maintained

If indeed the weight of most people does remain stable for long periods, then there must be some mechanism by which the body is able to adjust either its intake of energy as food or its expenditure of energy by oxidizing the components of that food, or both. Is there some body constituent that has some 'set point' above which adaptive mechanisms come into play so as to bring that constituent back to its set point? Which constituent? What are the mechanisms? When, as often occurs, weight increases and then settles down to a new set point, what triggers this change and what determines the new set point?

Clearly, candidates for regulated body components are the energy stores: glycogen (small — about 1.5–2 kg) or fat in adipose tissue (large — potentially two orders of magnitude larger than glycogen stores). One school of thought held that the regulation of energy intake was controlled by signals originating from body fat stores, working through detectors in the brain. This was called the lipostatic theory. When Tremblay and colleagues (4) induced fat loss in obese subjects by means of increased physical activity, they found that there was a point beyond which they could lose no further fat. This coincided with an inability of their fat cells to increase the rate at which they hydrolysed triacylglycerols compared with the fat cells of lean subjects. These observations are consistent with some kind of 'lipostatic' control mechanism.

During the past decade, there has been an explosion of interest in a substance called leptin. This protein, produced in the adipose tissue, appears to be involved in some way in providing signals to the part of the brain that is involved in eating behaviour, about the state of the fat stores. It was discovered that a strain of genetically obese mouse does not produce leptin and that this

is indeed the main gene defect. When obese people have been examined, it has been found that they produce no less leptin than lean people and it is now speculated that obesity may in part be a condition of 'leptin insensitivity'. This means that, whereas the body produces enough leptin, it is unable to send the appropriate signals, perhaps because of a defect in its receptor. This would have parallels with non-insulin-dependent diabetes (maturity onset or type 2 diabetes) in which the body produces insulin but the tissues are insensitive to its action. Recent knowledge of leptin has been comprehensively reviewed (5) and provides further support for a 'lipostatic' mechanism of control of feeding behaviour.

An alternative to the lipostatic theory is a 'glucostatic' mechanism. Since variations in glucose metabolism are known to affect food intake, another school of thought proposed the presence of brain receptors that were sensitive to differences in glucose concentration between the venous and arterial blood and stimulated according to the rate of glucose utilization. In support of this idea, there is evidence that depriving cells of glucose increases food intake and also that carbohydrate intakes are more stable than fat intakes in human beings (6).

These ideas, developed in the 1950s and 60s, put emphasis on controls over food intake. In the 1970s and 80s, the pendulum swung more towards concepts that individuals who are prone to obesity would be characterized by defects in energy expenditure. These would favour a positive energy balance, whereas individuals who remained lean could adapt their energy expenditure according to fluctuations in food intake or to changes in body stores. Although such adaptive changes in energy expenditure have been well characterized in animals, there is little evidence for their importance in man. The merits of the recent concepts of Flatt and of Tremblay and others is that they strive to integrate and unify concepts of control of food intake, carbohydrate and lipid stores and substrate oxidation.

Relative values of fats and carbohydrates as fuels: is a fat calorie more fattening than a carbohydrate calorie?

The basic concepts are summarized in Table 5.1 and newer concepts described in detail below.

- Equivalence of total energy intake and expenditure is not sufficient in itself to maintain energy balance. There must also be an equilibrium between the intake and oxidation of each macronutrient: protein, carbohydrate and fat. This follows from the characteristics of oxidation and storage of each macronutrient as outlined below.

- As explained earlier, the respiratory quotient (RQ) is used by physiologists to give information about the efficiency of utilization of different fuels (macronutrients). Flatt (7) developed the concept of food quotient (FQ, Figure 5.1) recognizing that we do not eat nutrients but real foods. FQ reflects the proportions of the fuels in the dietary mix and thus the macronutrient composition of the diet. Since FQ is related to RQ, foods of low FQ will have a relatively high proportion of fat and those with high FQ a relatively low proportion of fat. Flatt (7) found that weight maintenance in mice was only possible when the composition of the fuel mix oxidized was equal, on average, to the composition of the diet, i.e. when FQ = RQ.
- It is possible to define an individual's 'carbohydrate need'. The rate of oxidation of carbohydrate is made to match carbohydrate intake quickly enough to prevent excessive exhaustion or accumulation of carbohydrate reserves.
- The same is not true of fat oxidation. It appears that there is little or no ability to increase fat oxidation in response to excessive fat intakes in the short-term and since the fat stores are virtually unlimited, fat storage increases more readily in response to excessive consumption of fat than to carbohydrate. This was demonstrated by Flatt and colleagues (8) who gave volunteers experimental meals with different proportions of fat and carbohydrate and measured rates of protein, carbohydrate and fat oxidation by indirect calorimetry (measurement of oxygen consumed and carbon dioxide expired). The amounts of each fuel oxidized were similar and rates of oxidation of fat and carbohydrate were uninfluenced by the fat content of the meal. In the longer term, fat oxidation adapts so that the oxidation of the fuel mix is appropriate to the new body composition, bringing about a new equilibrium between intake and oxidation of fuels and resulting in a new, higher, stable weight (2,3).
- Physical activity may either increase or attenuate food intake in people consuming high-fat diets, depending on each individual's inherited ability to increase fat oxidation in response to exercise. This interesting observation was made by Tremblay and colleagues (6) when they measured the voluntary consumption of various high-fat foods before and after a vigorous exercise session and compared their subjects' ability to oxidize fuels with those of a control group that did not exercise. The mean RQ of the exercise group was significantly reduced compared with that of the control group. This means that there was a change in the fuel mix that they were oxidizing in the direction of greater utilization of fat. Since there had been no significant change in the ratio of fat to carbohydrate in the foods consumed, this must mean that the exercise group were burning more of their own body fat. Although the average intakes of fat and carbohydrate ingested by the exercise group were

not significantly different from those of the control (non-exercised) group, there were huge difference between individuals in their responses to exercise. Thus, one individual decreased his energy intake by 540 kcal while another increased his by about the same amount. There was a highly significant correlation between the change in energy intake and the RQ; that is to say, those subjects who decreased their energy intake were those who burned most body fat. Important conclusions from these experiments are that: (i) there is no short-term reduction in carbohydrate requirements when a person has access to a high-fat diet, probably because of an inability of the body rapidly to increase fat oxidation in response to an increase in fat consumption; (ii) energy intake after exercise is influenced by the degree to which a person can adapt his or her metabolism to oxidize lipids, an ability that may be genetically determined.

- High-fat diets are associated with appreciable short-term increases in energy intake. Tremblay *et al.* (6) showed that their experimental subjects, when given foods with a high ratio of fat to carbohydrate, tended to consume sufficient carbohydrate to fulfil the expected daily carbohydrate need (see above). This led to an increase in energy from fat. There was some tendency for total food intake to be reduced (suggesting that some kind of compensation mechanism was at work) but, because of the high energy density of the foods, the body was not able fully to compensate and energy intake increased. Some epidemiological studies seem to confirm that there is a positive statistical association between percent body fat and proportion of fat in the diet (6).

Conclusions

The answer to the question posed in the title: "Is a fat calorie more fattening than a carbohydrate calorie?" appears, from recent research, to be `Yes'. The reasons are complex but the main cause is the body's inability to increase fat oxidation rapidly in response to increases in fat consumption. Instead, fat is put into stores, which are, to all intents and purposes, limitless. By contrast, a certain level of daily carbohydrate oxidation seems essential and when intake exceeds this, the excess is readily oxidized. However, in the longer term, fat oxidation adjusts to the higher body fat stores bringing intake and oxidation into equilibrium and resulting in a new stable weight. These concepts provide a more scientifically based rationale for advice to limit dietary fat than do those concerned with hyperlipidaemias and heart disease.

Whereas a decade or so ago, the different views about the regulation of body weight seemed to be in conflict, the various observations described here can now be integrated into a model that goes some way to demonstrating how

the body manages to maintain constancy of composition for relatively long periods, despite large fluctuations in intake and in physical activity (2,3).

Fats and fattening: can we fool the system?

Can we have our fat and eat it?

In the preceding section, I discussed recent research suggesting that, calorie for calorie, over-consumption of fat was more likely to lead to overweight than over-consumption of carbohydrate. Fat has also been in the firing line from the point of view of coronary heart disease and diabetes, as well as obesity, so it not surprising that the search for fats with no energy value has been hotting up. To paraphrase an old saying: can we 'have our fat and eat it?'.

The liking for a fat rich diet seems to be deep seated, a conclusion that is supported by the observation that as a society gets richer, the proportion of its food energy provided by fat increases. Moreover, once it has become the norm for fat to provide about 40% of food energy, it seems quite difficult to make reductions, as witnessed in the UK over the past 20 years or so. Statistically, average fat intakes are associated with the prevalence of obesity in populations. It is not surprising that food scientists have explored, with some ingenuity, ways for people to 'have their fat and eat it'.

In the remainder of this chapter, I shall describe the search for food components that have all the textural and sensory properties of fat, yet do not deliver as much useful energy to the body as real fat. First, I shall describe reduced energy fats, that have about half the energy of normal fats. Then I shall focus on 'fat analogues': artificial or synthetic fats that are not substrates for pancreatic lipase and therefore effectively have zero energy. Finally, I shall ask whether these fat substitutes actually work: does their consumption really help to decrease total fat and energy intake or is the system not that easily fooled?

Reduced energy fats

How might we devise digestible fats (thereby avoiding problems, real or imagined, of having undigested fat in the gut), which have intrinsically lower metabolizable energies than 'normal' fats, contradicting the 'golden rule' that all fats provide 9 kcal (38 kJ) per gram? It has long been recognized that the shorter the chain length of a fatty acid (and therefore, the lower its molecular mass), the lower its gross energy per gram. Palmitic acid (16:0) consists of 75% carbon, whereas carbon constitutes 67% in capric acid (8:0) and 55% in butyric (4:0) acid and the heats of combustion decrease accordingly.

The metabolizable energy per gram also decreases with diminishing molecular mass since during the β-oxidation of fatty acids, the first committed step utilizes ATP to form the coenzyme A thiolester. This consumes more ATP per gram of short-chain than of long-chain fatty acids. Subsequently, ATP yield from the oxidation of fatty acids mainly depends on the utilization of NADH produced during β-oxidation in the mitochondrial electron transport chain. Less NADH is produced per gram of shorter-chain than of longer-chain fatty acids, making a further contribution to the smaller metabolizable energy values of shorter chain length fatty acids. Furthermore, the contribution of glycerol (with a metabolizable energy value of 4.3 kcal/g) to the metabolizable energy of triacylglycerols becomes relatively more significant as the proportion of short-chain fatty acids increases and the molecular mass of the triacylglycerol decreases.

The first such fats to attract interest were the medium-chain triacylglycerols (9). These fats are fractionated from coconut oil and have a fatty acid composition that mainly comprises caprylic (8:0) and capric (10:0) acids. However, medium-chain triacylglycerols have not become widely used as 'low calorie fats'. Their long-term effectiveness has never been scientifically demonstrated; they are expensive and the melting point is rather too low for many food applications. Their main application has been as components of therapeutic diets for people who are unable to digest and absorb fat. The principle is that the medium-chain fatty acids are more rapidly hydrolysed by lipases than long-chain fatty acids and are absorbed rapidly into the portal blood and are delivered within minutes to the liver, where they are substrates for oxidative metabolism.

It was logical, therefore, to think in terms of mixed acid triacylglycerols containing short and long-chain fatty acids. In 1991, Peters and colleagues (10) described 'caprenin', a mixed acid glyceride of one long-chain acid (behenic acid, 22:0) and two medium-chain acids (capric, 10:0, and caprylic, 8:0) and in three papers (11–13), published during 1994, another triacylglycerol having similar attributes and named 'salatrim' was described. The authors have cleverly combined the advantages of the reduced energy value of the short-chain fatty acids with the poorer absorption of stearic acid (18:0) to produce a triacylglycerol with significantly reduced energy value (5 kcal/g; 21 kJ/g) compared with conventional fats (9 kcal/g; 38 kJ/g).

An important point emerging from the study was that the actual absorption of 18:0 decreased as the proportion of 18:0 to short-chain fatty acids increased and was also lower when it was esterified in positions 1 or 3 than in position 2. In a human clinical trial in which 17 subjects received a diet containing 22%

of energy from salatrim for 7 days, a metabolizable energy value of salatrim of 4.9 kcal/g and absorption of stearic acid ranging from 63 to 70% were reported (13).

Salatrim is produced by interesterifying triacylglycerols containing short-chain fatty acids (eg tributyrin) with long-chain saturated triacylglycerols (e.g. hydrogenated rapeseed oil). It is, therefore, simple to produce by methods that are standard in the fats and oils processing industry. This material has the potential to reduce substantially the energy value of foods into which it is incorporated, while still retaining desirable textural properties. Whether it turns out to be a useful aid to weight maintenance remains to be seen.

Its effectiveness will need to be examined over extended periods under carefully controlled conditions. No one product is likely to be the answer to slimmers' prayers.

Non-digestible fats

Fat digestion

Dietary fat consists mainly of triacylglycerols (**Figure 5.2a**). Because these molecules are, for the most part, not absorbed in the small intestine, they must first be partially digested. This is accomplished by the enzyme lipase secreted from the pancreas. Unlike many enzymes, which catalyse the biochemical transformation of molecules dispersed in aqueous solution, pancreatic lipase is active upon the surface of large particles of emulsified triacylglycerols. The enzyme cleaves preferentially the fatty acids in positions 1 and 3 of the triacylglycerols, so that the absorbed fat digestion products are mainly fatty acids and 2-monoacylglycerols. Immediately prior to absorption, these digestion products are components of mixed micelles, which also contain phosphoglycerides, lysophosphoglycerides, cholesterol, bile salts, fat-soluble vitamins and other minor fat-soluble dietary components and some undigested triacylglycerols (14). Thus, small quantities of triacylglycerols may be absorbed intact.

It follows that synthetic molecules with fatty properties, but whose fatty acid components were linked by chemical bonds that were not susceptible to attack by pancreatic lipase, would not be absorbed to any great extent. Such molecules would therefore not contribute usable energy to the body.

Early examples of attempts to fabricate non-digestible fats (9) were esters of fatty acids and polyglycerol (**Figure 5.2b**) and so-called 'retrofats', in which fatty alcohols were linked with citric acid (**Figure 5.2c**). For a number of reasons these compounds did not come into commercial use.

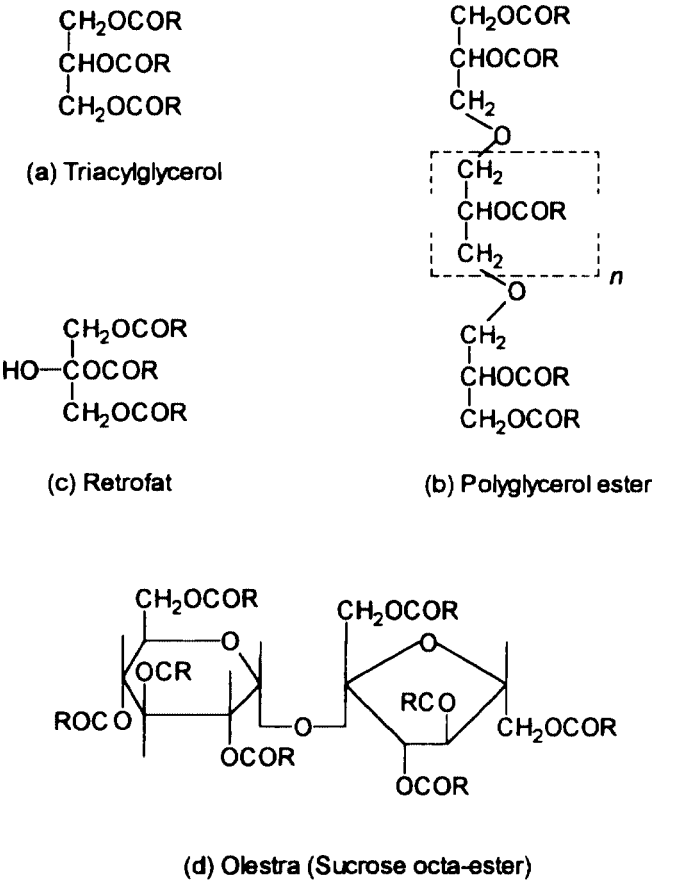

Figure 5.2. Digestible and non-digestible fats. 'R' represents the hydrocarbon chain of a long-chain fatty acid (a,b,d) or fatty alcohol (c). In a triacylglycerol (a), glycerol hydroxyl groups are esterified with long-chain fatty acids. The same types of bonds occur in polyglycerol esters (b). In retrofats (c), the ester bonds are formed between long-chain fatty alcohols and the carboxylic acid groups of citric acid. In the sucrose polyester olestra (d), between 6 and 8 of the sucrose hydroxyl groups are esterified with long-chain fatty acids.

The focus has been almost entirely on olestra (**Figure 5.2d**), a sucrose polyester product of the Procter & Gamble company that has recently received US FDA approval for food use (15). This approval has come after 25 years of testing in about 8000 people.

Properties of sucrose polyester

The backbone of olestra is sucrose, a disaccharide with 8 available hydroxyl groups with which fatty acids can be esterified. A summary of its chemistry, physical properties, method of preparation and safety aspects can be found in the September/October 1992 issue of *Lipid Technology* (16) and its nutritional properties have also been reviewed (17).

The physical properties of sucrose polyester can be tailored by changing the number of fatty acids esterified in the molecule (the commercial product normally is a mixture of hexa-, hepta- and octa-esters) and by changing the ratio of saturated to unsaturated acids (16). Thus, like triacylglycerols, olestra has a high melting point when the proportion of saturated fatty acids is high and becomes progressively more liquid as the unsaturated fatty acid content increases.

Of particular interest nutritionally are: (i) the extent to which the substance is truly non-absorbed; (ii) any interactions with other nutrients in the gut; (iii) potential for fermentation by bacteria in the colon; (iv) long-term toxicity and (v) influence on spontaneous intake of energy from other components of the diet.

Absorption

When olestra is labelled with a radioactive tag and given to rats, the amount of radioactive lipid in the tissues is extremely small indicating that less than 0.001% is absorbed. There is some evidence, however, that molecular species with fewer than six fatty acids may be absorbed to a greater extent than the fully esterified molecule. This may account for an 'oily material' found in lymph nodes in some animal studies and needs further research.

Interactions with fat-soluble vitamins

Fat-soluble vitamins are consumed in milligram or microgram quantities and require a certain amount of fat in the food to be efficiently absorbed. They become incorporated into the oily core of the 'mixed micelles' during fat absorption and are carried by the fat into the cells lining the small intestine and are subsequently absorbed into the blood. It is well recognized that olestra reduces the absorption of the fat-soluble vitamins A, D, E and K.

The extent of the reduction depends on the conditions of the study but a reductions of 15–20% have not been uncommon and a recent study with human subjects recorded a 17% reduction in absorption of β-carotene (18). The manufacturers have recognized this fact and the product is fortified with vitamins A, D, E and K.

Some nutritionists argue that fortification may not be an entirely satisfactory solution for several reasons. Unlike the water-soluble vitamins, vitamins A and D are toxic above a certain level in the diet. It may not be a simple matter to control intakes of these compounds as the range of fortified products increases. Although at this stage limited use is envisaged in the USA (savoury snacks, crisps, tortilla chips and cheese puffs), it is likely, many argue, that pressures will increase to extend its use. Another argument is that the range of fat-soluble micronutrients thought to have important health-giving properties has increased considerably as nutritional knowledge has expanded. Fortification with vitamins A, D, E, and K will not compensate for these other compounds which include the carotenoids and tocotrienols.

Fate in the colon

It is now well known that carbohydrates that are undigested in the small intestine travel to the colon where they are fermented by anaerobic microorganisms, producing gases (hydrogen, methane, carbon dioxide), short-chain fatty acids and other organic acids. This group of carbohydrates includes lactose, various other disaccharides and sugar alcohols used as sweeteners or bulking agents and non-starch polysaccharides otherwise known as 'dietary fibre'. It could be envisaged that continual exposure of the colonic microorganisms to undigested fatty substrates, such as sucrose polyester, might result in some species 'learning' to digest and ferment these substrates.

So far there has been no evidence for this. A recent study demonstrated no significant effects of olestra on the concentration of hydrogen or methane in the breath when healthy subjects consumed 24 g olestra daily for 36 days (19). The olestra did not significantly alter the gut microflora, interfere with normal fermentation of dietary fibre or adversely affect health in any sense. Although of reasonably long duration for a human dietary study, 36 days may nevertheless be a short time in terms of the adaptation required to ferment this substrate and it will be interesting to see whether subsequent work detects fermentative activity after extended periods of consumption of olestra or similar compounds.

It is interesting that the above mentioned study found no interference with gut function in view of widely expressed concerns about a tendency to

diarrhoea. This is likely to vary widely between individuals and some people may adapt in the longer term to the presence of undigested material in the gut.

Long-term toxicity

During the 25 years that olestra has been tested, numerous studies have failed to demonstrate significant toxic effects. Most, of course, have been the routine type of laboratory animal tests and many argue that these do not guarantee safety in human beings who consume unknown quantities over decades. The animal tests may be appropriate, it is argued, for the food additives to which we have become accustomed but olestra will be consumed on a scale that distinguishes it from 'additives' even if the range of foods in which it is allowed to be used is limited.

Readers should be aware that the only food use approval so far is in the USA. Other countries like the UK have not immediately followed. Whether this is the result of normal inertia or whether nutritional and toxicological concerns are greater outside the USA is unclear and the reaction of the European authorities will be watched with interest.

Do fat substitutes work?

The problem of obesity

There is no question that obesity is a major problem in the UK and many other industrialized countries now. According to figures published annually by the UK Ministry of Agriculture, Fisheries & Food (MAFF) (20), total daily energy intakes of British people have been falling slowly but steadily year by year, whereas other surveys indicate that the prevalence of overweight and obesity (assessed by measurement of the body mass index [BMI]) is rising. BMI is calculated by dividing weight in kilograms by the square of the height in metres. The normal range is taken to be 20–25; overweight refers to a BMI of 25–30 and obese to a BMI in excess of 30. The apparent paradox might be explained if the fall in energy intake is an artefact of the methods used to estimate it. MAFF data have been supplied for food consumed in the home; food eaten out of the home may have compensated for reductions in household food consumption. The general view, however, is that the energy expended in physical activity has declined more than the energy consumed as food: we have become a more sedentary society.

Dietary guidelines are always prefaced with the exhortation to match energy expenditure with energy intake to avoid obesity. Mounting evidence is that calorie for calorie, fat is more fattening than carbohydrate (see earlier

sections). Therefore, reduction of dietary fat intake as a means of losing or maintaining weight tends to have been emphasized in recent years. One result, as described earlier, has been an increasing interest in either products that have a reduced fat content compared with conventional ones or substitute fats that have intrinsically lower or even zero energy value.

Regulation of energy intake

It is assumed that routine replacement of conventional fat products with reduced-fat versions will inevitably result in lower overall fat, and therefore energy, intakes and this will certainly be true in individual meals. In the longer term (days, months, years) it would not be true if the body detects what it considers to be a 'deficit' in its normal fat energy intake and adjusts subsequent intake so as to maintain total energy intake constant.

Research on physiological and metabolic factors controlling eating behaviour has been based mainly on the principle that absorbed nutrients and their metabolites are detected by a centre in the brain (the hypothalamus). These metabolic signals are processed by the hypothalamus, which then relays to other parts of the brain messages that we interpret as hunger or satiety (21). A problem with a purely metabolic approach is that time is needed for foods to be digested and the nutrients absorbed and detected. How does the body detect what is to be its last mouthful of food? Satiety is now known to be conditioned by previous experiences of the satiating effects of different foods. Thus cognitive factors (preconceptions or beliefs about a food) play an important role in determining food intake: an important point when we consider the extent to which we are bombarded by different messages about the fat content of foods.

Satiety is also related to the sensory qualities of the food. We may become satiated to sausages but can still tackle some ice cream! Thus novelty and variety in food makes it more difficult for the body to regulate intake. Timescale is also important. There may be an immediate impact of a sensory stimulus on energy intake during the same day, yet experiments have shown that in many people, complete responsiveness to sensory cues may need several days to become fully effective. The relative amounts of the main energy yielding nutrients also determine the degree of satiety and how long it takes to develop. It is common to be told that, contrary to expectation, fat is the least satiating of the macronutrients but it has been demonstrated quite clearly that the satiety effect depends on the quantity of fat consumed earlier in the day.

A matter of 'compensation'?

The question then is: if we are accustomed to a certain daily intake of fat and then begin to reduce it, does the body detect the 'deficit' and subconsciously adjust fat (or total energy) intake over a longer period of time? This phenomenon is referred to as compensation. As you might expect, the answer to this question is 'yes and no'!

Experiments to investigate compensation (22) usually take the following form. Subjects participating in the experiment are given a small meal of known nutritional composition, usually but not always, at mid-morning. In the jargon of the trade, this is called a 'pre-load'. The proportions of fat, carbohydrate and protein in the pre-load can be adjusted. Depending on the aim of the study, subjects may either be aware or unaware of whether it is a 'high-fat' or 'low-fat' meal. In the latter case, a certain amount of ingenuity is needed by the experimenters to disguise the meal composition. Before consuming the pre-load, subjects complete a standardized questionnaire in which they rate their feelings of hunger and they complete another immediately after eating the pre-load. Then follows an interval of time which can be varied considerably before the next meal. Subjects are told that they can eat as much or as little as they wish from a selection of foods offered. Again, they complete a questionnaire about hunger, fullness and so on, before and after the meal. Thus, the pre-load is a predetermined and obligatory meal, whereas the later meal is free-choice. The idea is to measure the extent to which the composition of the 'pre-load' has affected the quantity and type of foods and nutrients freely selected at a later time.

An overview of all the studies of this kind (23) published to date reveals that:

- reducing the proportion of fat in the diet is usually accompanied by an immediate decrease in energy intake followed later by adaptation that tends towards restoring the original energy intake;
- in some studies adaptation has been complete, in others far from complete; and
- in most studies, there has been a net decrease in fat but net increases in protein and carbohydrate intakes.

The extent to which the 'fat deficit' is replaced by carbohydrate to restore energy intake completely depends on a number of factors, not least the previous history of the subjects and the type of people they are. In one study (24), a group of young or early middle age men who were unconcerned about their body weight and what they ate, accurately compensated for the energy in

the pre-loads regardless of their nutrient composition. However, men who maintained normal weight but were constantly concerned about eating and body weight, did not show such precise energy compensation. (In the jargon of the trade, people who are unconcerned about eating and body weight are called 'unrestrained eaters' as distinct from 'restrained eaters'). The same lack of precision in compensating for changes in energy density of foods applied to women whether they were restrained or unrestrained, normal weight, or obese.

Another study by the same research group (25) tested the hypothesis that the perceived fat content of the pre-load would influence subsequent food and energy intake, irrespective of its actual fat content. Twenty-four normal weight restrained women and the same number of unrestrained women were given yoghurt pre-loads followed by a free-choice meal 4.5 hours later. Half the subjects received accurate information (on a label) about the *fat* content of the yoghurt (which did not necessarily correspond to the energy content); the remainder received no information. Women who were given information on food labels consumed more energy in the free-choice meal after eating a pre-load labelled 'low-fat' than after eating one with a similar energy content but labelled 'high-fat'. The opposite response was observed in women given no information. Thus, messages about the fat content of a food can markedly influence subsequent energy intakes.

There have been several studies on the extent to which compensation occurs after consuming olestra, which has no energy value. In several studies (23) substitution of conventional fat by olestra in single meals resulted in a significant reduction in the day's energy derived from fat with a reciprocal increase in carbohydrate intake, without affecting the day's total energy intake or feelings of hunger and fullness. In others, the compensation was far from complete, with the result that total fat and energy intakes were reduced.

In conclusion, the use of reduced fat foods, including those that contain calorie-free fat substitutes, usually results in lower daily fat intakes and may or may not result in overall energy reduction. Individuals 'compensate' for the reduction in fat by consuming more carbohydrate to different extents depending on their previous history and personality. While it has been well demonstrated that diets composed of a free choice of low-fat foods lead to significant weight loss under controlled experimental conditions, the long-term effectiveness of such diets in 'real life' is far from clear. This review (and indeed most studies on low-fat foods) has considered only energy intake and neglected energy expenditure, changes in which may also affect long-term weight maintenance.

References

1. Department of Health (1992) Dietary reference values for food energy and nutrients for the United Kingdom. Reports on Health and Social Subjects, 41, Her Majesty's Stationery Office, London.
2. Tremblay, A. (1992) *International Journal of Obesity*, 16, 953–957.
3. Flatt, J.P. (1988) *Diabetes/Metabolism Reviews*, 4, 571–581.
4. Tremblay, A. *et al*. (1984) *International Journal of Obesity*, 8, 641–648.
5. Andrews, J.F. *et al.* (1998) *Proceedings of the Nutrition Society*, 57, 409–485.
6. Tremblay, A. *et al*. (1989) *American Journal of Clinical Nutrition*, 49, 799–805.
7. Flatt, J.P. (1987) *American Journal of Clinical Nutrition*, 45, 296–306.
8. Flatt, J.P. *et al*. (1985) *Journal of Clinical Investigation*, 76, 1019–1024.
9. Babayan, V.K. (1974) *Journal of the American Oil Chemists' Society*, 51, 260–264.
10. Peters, J.C. *et al*. (1991) *Journal of the American College of Toxicology*, 10, 357–367.
11. Hayes, J.R. *et al*. (1994) *Journal of Agricultural and Food Chemistry*, 42, 474–483.
12. Klemann, L.P. *et al*. (1994) *Journal of Agricultural and Food Chemistry*, 42, 484–488.
13. Finley, J.W. *et al*. (1994) *Journal of Agricultural and Food Chemistry*, 42, 495–499.
14. Gurr, M.I. and Harwood, J.L. (1991) Lipid Biochemistry: An Introduction (4th edition). Chapman and Hall, London.
15. Jones, J.M. (1996) *Lipid Technology Newsletter*, 2, 1–2.
16. Lawson, K. and Kester, J. (1992) *Lipid Technology*, 4, 115–118.
17. Miller, K.W. and Allgood, G.S. (1993). Nutritional assessment of olestra, a non-calorific fat substitute. *International Journal of Food Science and Nutrition*, 44 (Suppl.1) S77–S82.
18. Weststrate, J. and van het Hof, K.H. (1995) *American Journal of Clinical Nutrition*, 62, 591–597.
19. Eastwood, M.A. and Allgood, G.S. (1995) *European Journal of Clinical Nutrition*, 49, 627–639.
20. Ministry of Agriculture, Fisheries and Food. Annual Reports of the National Food Survey Committee. Her Majesty's Stationery Office, London.
21. Human Nutrition and Dietetics (1993) (J.S Garrow and W.P.T James, eds). 9th edition, Churchill Livingstone, Edinburgh.
22. Rolls, B.J. and Hammer, V.A. (1995) *American Journal of Clinical Nutrition*, 62 (Suppl), 1086S-1095S.
23. Gershoff, S.N. (1995) *Nutrition Reviews*, 53, 305–313.
24. Rolls, B.J. *et al*. (1994). *American Journal of Clinical Nutrition*, 60, 476–487.
25. Shide, D.J. and Rolls, B.J. (1995) *Journal of the American Dietetic Association*, 95, 993–998.

Chapter 6

Lipids in Foods and Raw Materials

This chapter discusses the attributes of the lipids in meats, milk, infant feeds and in the oil palm. Specific properties of the short-chain and medium-chain fatty acids, 'conjugated linoleic acid' and plant sterols are also described.

Meat lipids are present in the phospholipids of the membranes of the meat tissue (structural lipids) and in the storage fat (adipose tissue) associated with the meat. Some storage fat is located within the muscle tissue (marbling). The structural lipids represent a small proportion of total lipid but contribute a large proportion of meat polyunsaturated fatty acids. They contribute significant intakes of arachidonic acid for people who eat meat. The storage fats are generally present in larger amounts but have a higher proportion of saturated and monounsaturated fatty acids. Whereas meat is not indispensable in the diet, it does contribute significant amounts of several essential minerals, (particularly iron and zinc) and vitamins (particularly B_{12}). If people elect to reduce meat intake to lower-fat consumption, care needs to be taken to maintain a balanced diet.

In dietary recommendations to reduce intakes of total fat, and especially saturated fatty acids, milk and milk products are often cited as foods to reduce, to avoid, or to take as low-fat varieties. However, whereas milk fat itself has a cholesterol-raising effect, full-fat milk products do not. Concern about blood cholesterol is not a reason to avoid whole milk products. The evidence is discussed.

The fatty acid composition of food fats is frequently regarded as some kind of 'index of healthiness'. Such a simplistic approach ignores the enormous complexity of food fats. Milk fat provides a good example of a food fat whose minor components are increasingly subjects of intensive research. As well as several types of fatty acids, this section reviews current research into the properties of phospholipids present in the milk fat globule membrane and of some of their degradation products.

Conjugated linoleic acid (CLA) is the term given to a mixture of cis/trans *isomers of linoleic acid. The highest concentrations are found in milk fat and other products containing milk fat, followed by ruminant meats. One of the components of CLA,* cis-*9,*trans-*11-18:2 is an anti-cancer agent in laboratory animals and in cell cultures. The mechanism by which CLA inhibits cancer is unknown. Research in the next few years is likely to discover this mechanism and may pave the way for the application of CLA in cancer therapy. Preliminary observations suggest that CLA reduces blood cholesterol in rabbits given a high-fat diet and may even reduce the progression of atherosclerosis but these results need confirmation. The active anti-cancer compound is the most abundant component of CLA in dairy products. With further intensive research, full-fat dairy foods may yet become 'functional foods'.*

The lipid composition of human breast milk is generally taken as 'the gold standard' when designing commercial infant formulas. However, determining the appropriate lipid composition of a formula is not straightforward because the composition of human milk changes during a lactation, during a day, and even during a single feed. Moreover it varies with the mother's diet. The fat in cow's milk, traditionally the basis for infant formulas in industrialized countries, has a less than ideal composition for human babies. At first, emphasis was placed on the linoleic content of the formula. More recently, issues such as the linoleic/α-linolenic acid ratio and the ratio of C_{18} essential fatty acids to long-chain C_{20} and C_{22} polyunsaturates have become more important. The appropriate inclusions of cholesterol and the fat-soluble micronutrients are still debated.

The short and medium-chain fatty acids (SMCFA) that are present in significant quantities in dietary fats are saturated acids with chain lengths less than C_{12}. In the general enthusiasm to label saturated fatty acids as toxic, it is often forgotten, or not realized, that the short and medium-chain fatty acids do not raise plasma cholesterol and are not deposited in significant amounts in adipose tissue. This is partly because they are absorbed rapidly as free acids into the blood vessels feeding the liver and are immediately metabolized. They have applications in diets for people unable to absorb long-chain fatty acids and there would be merit in investigating other applications and other sources of supply.

In a sustained campaign against 'tropical oils' in the USA, palm oil has been grouped with the lauric oils palm kernel and coconut and categorized as a 'cholesterol-raising saturated fat'. The truth is quite otherwise and the evidence is discussed in this section.

Plant sterols may contribute nearly as much as cholesterol to sterol intakes of people eating mixed diets and even more to those of vegetarians and vegans. They are not themselves absorbed but inhibit the absorption of cholesterol. There is some evidence that naturally occurring plant sterols reduce blood cholesterol to a small degree. Sitostanol, the product formed by the chemical reduction of sitosterol causes a much larger reduction in blood cholesterol, however. A 1995 study demonstrated a 10% reduction in cholesterol in 102 Finnish people with mild hypercholesterolaemia when sitostanol was consumed regularly as a component of a margarine. The amounts required were much larger than the normal daily consumption of natural plant sterols.

Meat lipids

Meat in the diet

In the UK today, meat provides about 14% of energy, 23% of fat and 18% of iron intakes. Meat currently supplies 9% of dietary saturates and 2.5% of polyunsaturates (1). Because of its relatively high contribution to fat intakes and its perceived high content of saturated fatty acids, meat has sometimes been singled out as a food whose consumption might need to be reduced. As always this simplistic approach to nutrition has its pitfalls since the perceived benefits may be offset by other less favourable nutritional changes. It is therefore worthwhile examining the contribution of lipids by meat to the diet in a little more detail. I have limited the range of meats to be discussed here to those generally consumed in the industrialized countries of western Europe, mainly the muscle meats from ruminants (cow, sheep, goat) and monogastric animals (pigs, poultry, game) with brief consideration of offal meats (liver, kidney, brain etc).

Types of lipids in meat

Two distinct types of lipids can be identified in meat depending on their location. The muscle tissue that is the main component of meat is richly endowed with membranes whose principal lipids are phosphoglycerides and cholesterol. These will be called ‘structural fats’. The phosphoglycerides contain a limited range of fatty acids in which polyunsaturated acids of the *n*–6 family predominate (**Table 6.1**). Because biological membranes operate optimally within a limited range of ‘fluidity’ and because fluidity is in part, but not wholly, influenced by fatty acid composition, the latter is conservative and not extensively influenced by diet or other environmental factors. Some

Table 6.1. Fatty acid composition of the structural lipids of several meats (g/100 g total fatty acids).

Fatty acid	Beef		Lamb		Chicken		Pig
	A	B	C	D	E	F	G
16:0	16	14	22	22	23	25	19
16:1	2	2	2	1	6	3	2
18:0	11	14	13	18	12	17	12
18:1*n*–9	20	5	30	28	33	26	19
18:2*n*–6	26	47	18	1	18	15	26
18:3*n*–3	1	1	4	0	1	1	0
20:4*n*–6	13	11	7	4	6	6	8
20:5*n*–3	tr	tr	tr	1	tr	tr	tr
22:5*n*–3	tr	tr	tr	3	tr	tr	tr
22:6*n*–3	tr	tr	tr	10	tr	5	tr
Others	11	6	4	12	1	1	14

Adapted from Table 3.7 in Gurr, M.I., *Role of Fats in Food and Nutrition* (2nd edn), Elsevier, London, 1992. A, muscle, cattle given low-fat diet; B, muscle, cattle given 'protected' safflower oil diet; C, muscle; D, brain; E, muscle; F, liver; tr = trace. Others: generally the sum of a large number of minor components each present at low concentration; in the ruminant lipids, these will include numerous odd-chain and branched-chain components, as well as *cis* and *trans* positional isomers. In pig liver, these probably include several long-chain polyunsaturated components.

changes in fatty acid composition can be brought about by dietary fat modification but such dietary changes need to be very substantial (Table 6.1).

Muscle meats may be associated with variable amounts of adipose tissue depending on the degree of fatness of the animal and on the way the meat was butchered. The fatty acid composition of these 'storage' fats is generally quite different from that of the 'structural' fats, being richer in saturated and monounsaturated fatty acids with relatively small concentrations of polyunsaturated fatty acids (**Table 6.2**). The latter statement needs to be qualified when discussing monogastric animals, whose adipose tissue fatty acid composition, unlike ruminants, responds markedly to diet (Table 6.2). Thus, pigs or poultry given diets rich in linoleic acid contain high concentrations of this fatty acid in their adipose tissue. The same is true of certain other fatty acids including the *trans* unsaturated fatty acids. Monogastric animals given diets rich in hydrogenated fats contain a high proportion of *trans* unsaturated fatty acids in their storage lipids.

Table 6.2. Fatty acid composition of the storage lipids of several meats (g/100 g total fatty acids).

Fatty acid	Pig (lard)			Poultry		Beef (suet)		Lamb	
	A	B	C	D	E	F	G	H	I
14:0	1	1	1	1	1	3	3	3	4
16:0	29	21	21	27	22	26	20	21	19
16:1	3	3	4	9	5	5	4	9	6
18:0	15	12	17	7	6	8	10	20	16
18:1	43	46	54	45	27	45	33	41	37
18:2	9	16	3	11	35	2	23	5	12
Others	0	1	0	0	0	4	7	6	6

Adapted from Table 3.2 in Gurr, M.I., *Role of Fats in Food and Nutrition* (2nd edn), Elsevier, London, 1992. A, pigs given low-fat cereal-based diet; B, pigs given high-fat diet containing soybean oil; C, pigs given high-fat diet containing beef tallow; D, poultry given low-fat cereal-based diet; E, poultry given high-fat diet containing soybean oil; F, cattle given diet based on hay; G, cattle given diet containing 'protected' safflower oil; H, lambs given cereal-based concentrate diet; I, lambs given diet containing 'protected' safflower oil.

Storage fat is often found within muscle tissue as a result of 'marbling'. Its fatty acid composition resembles that of the adipose tissue. Therefore the fatty acid composition provided by muscle meat will depend on the extent of marbling. Inadvertent inclusion of marbling fat with muscle when sampling for analysis will tend to give a misleading view of the structural fat composition, diluting the proportion of polyunsaturated fatty acids (2).

Offal meats, which are rich in membranes, contribute to intakes of 'structural lipids' (Table 6.1) but the overall contribution will clearly depend on the amounts of adipose tissue associated with them.

Meat as a source of long-chain polyunsaturates

The two primary essential fatty acids, linoleic (*n*–6) and α-linolenic (*n*–3) acids are normally supplied by plant foods and meat is usually thought of as merely adding to the intake of saturates and monounsaturates. Meat is about the only significant source of arachidonic acid (*n*–6) as well as supplying small quantities of linoleic acid. In the UK, meat may supply up to about 1 g arachidonic acid daily. Strictly, arachidonic acid is not essential if it is assumed that human tissues have adequate capacity to desaturate and chain elongate dietary linoleic acid (see Chapter 4). However, there is evidence that some individuals may either have limited ability to convert linoleic into arachidonic

acid or the conversion occurs only very slowly. For such people, arachidonic acid may be a 'conditionally essential fatty acid' and meat will supply useful quantities in the diet. It is of interest to note that for obligate carnivores such as cats, arachidonic acid is a 'true' essential fatty acid since these animals totally lack the 6-desaturase.

Muscle meat also supplies very small quantities of *n*–3 polyunsaturates but those people who like to consume offal, including brains, will receive significant quantities of the long-chain *n*–3 polyunsaturated fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids.

Lean meat, because of its relatively high proportion of polyunsaturated fatty acids, can make a useful contribution to diets aimed at reducing plasma lipid concentrations (3).

Modification of meat lipid composition

Increasing the unsaturated fatty acid content of pig and poultry feeds results in increased levels of these acids in storage fat (Table 6.2). There is little influence on structural fats with normal feeding regimens, although inclusion of fish oils can increase the ratio of *n*–3/*n*–6 polyunsaturated fatty acids compared with what is normally found (2). The motivation for effecting this type of change would be to increase the range of meat products with a higher unsaturated/saturated fatty acid ratio, which might be regarded by some as 'healthier'. However, as the fat texture becomes softer, the eating quality becomes less desirable to the consumer and the shelf-life may also be reduced appreciably due to oxidative rancidity (2,4). Similar problems occurred with soft backfat produced by giving pigs diets enriched with copper. The mechanism may be stimulation of the 9-desaturase by copper or influences on the structure of triacylglycerols in the subcutaneous adipose tissue (2). Such manipulations, therefore, have to be undertaken with care.

Increasing the polyunsaturated fatty acid content of ruminant meats is more difficult because of the extensive degree of biohydrogenation of dietary fatty acids by rumen microorganisms. Giving 'protected' vegetable oils to cattle increases the linoleic acid content of the milk, the storage fat and even to some extent the muscle (5) (Tables 6.1, 6.2). The principle is that fat globules protected by a coat of protein, that has been cross-linked by formaldehyde treatment, escape hydrogenation in the neutral pH of the rumen but become available for digestion and absorption after passing through the acid pH of the abomasum. Such products have not become established in the market place mainly because of short shelf-life of products due to oxidative rancidity.

Giving high-fat diets in which the fat is not protected also results in significant although less substantial changes in fatty acid composition. This is because large quantities of fat in the rumen inhibit biohydrogenation and the result is an increase mainly in oleic, rather than linoleic acid, which is less susceptible to oxidative deterioration.

Another type of modification of meat fat has been the reduction of cholesterol content by treatment with enzymes, or extraction with cyclodextrins or supercritical carbon dioxide (6). In this reviewer's opinion, such manipulations are unnecessary and scientifically unjustified in view of the minimal effects of dietary cholesterol on blood cholesterol (7).

Meat lipids are also modified by cooking. Although lengthy heating at high temperatures may tend to reduce the polyunsaturates which are more susceptible to oxidation, many controlled studies have found that the polyunsaturated to saturated fatty acid ratio actually increases after cooking. This may be because the more vulnerable polyunsaturates are protected in the membranes of the lean tissue, whereas the storage triacylglycerols, containing relatively more of the saturates may melt and run off when cooking lean meat (2).

Minor lipid components in meat

Meat fat supplies all the fat soluble vitamins A, D, E and K but only liver supplies one of these, vitamin A, in substantial quantity to make a significant contribution to daily intakes. Recent research has focused on conjugated linoleic acid (CLA), present mainly in foods of ruminant origin, as providing potential protection against cancer (see later section). Of particular relevance to the meat industry is its ability to prevent growth suppression associated with immune stimulation in growing chicks, to improve growth efficiency and to increase lean/fat ratio (8). If these results are confirmed, this compound may yet find application in the animal feeds industry and the human sports or slimming industries. A more detailed discussion of the properties of CLA will be found in a later section.

Consequences of meat reduction

Human beings have evolved to be able to survive on a wide range of different foods. No one food, or even food group, is indispensable. It is clearly feasible to survive without meat and to obtain the nutrients which meat supplies in abundance, such as protein, iron, zinc and vitamin B_{12}, from other foods. Nevertheless, if meat is to be omitted from the diet, then care needs to be taken

to ensure that the diet contains sufficient variety to be able to cover the nutrients that would have been supplied by meat. This is especially important in people who are particularly susceptible to anaemia.

Several epidemiological studies have implicated meat consumption as a risk factor for some forms of cancer and coronary heart disease. It has frequently been assumed that the fat component of meat is the main contributor to the increased risk. However, the contribution of other constituents of meat (for example heterocyclic compounds generated during cooking) have also been discussed. This is having its effect (together with other worries such as bovine spongiform encephalopathy, BSE) on public attitudes to meat and meat fat. In this section I have tried to indicate that meat fat has positive contributions to play in the diet and in health and that avoidance of meat for 'health' reasons is not based on sound science.

Lipids of milk

Worries about milk fat

Concerns about the amount and type of fat in the diet are widespread and may relate to associations of high-fat diets with obesity or of saturated fatty acids with risk of coronary heart disease or both. Because people need to translate nutrients like 'fat' and 'saturated fatty acids' into foods, there has been a tendency to earmark certain foods as 'fatty' or 'rich in saturates' and milk and dairy products have frequently been given this label. Such over-simplifications are readily taken up by the media, from which many people derive their nutritional knowledge and attitudes (9) but have been reinforced and given scientific credibility by well-respected reports such as that of the UK Department of Health COMA Panel on Diet and Cardiovascular Disease which specifically referred to the fat content of milk, cheese and cream (10). These products have acquired an image of 'high in fat and saturated fatty acids' and 'cholesterol-raising'.

Milk fat and plasma cholesterol

Surprisingly few studies have investigated the cholesterolaemic effects of milk fat or butter fat specifically. Much of the work that gave rise to current understanding of the hypercholesterolaemic effects of saturated fatty acids and the hypocholesterolaemic effects of polyunsaturated fatty acids (now known to be grossly over-simplified) was done by Ancel Keys and his colleagues in the late 1950s (see Chapter 1). It involved painstaking studies with men under

carefully controlled conditions in metabolic units, in which they received diets containing fat between 10 and 40% of energy provided by corn, soybean, sunflower seed, rapeseed, safflower, cottonseed, coconut, olive, pilchard and menhaden oils and butter fat. A diet containing 38% of energy as fat, all of which was butterfat (P/S = 0.12) showed an increase of plasma cholesterol of 1% compared with a control diet with the same fat level (P/S = 0.18) formulated to resemble a 'typical American diet'. By contrast, diets containing a similar level of cottonseed, sunflower or safflower (P/S = 1.23) lowered cholesterol by about 13% as did diets containing 11% of their energy as fat (P/S = 0.27). Several other studies gave similar results, albeit with rather larger increases due to butter fat (reviewed in 11).

A study carried out under more 'domestic' as distinct from 'laboratory' conditions also achieved similar results (12). These authors replaced much of the fat (mainly olive oil) in a typical southern Italian diet with butter, cream and meat fat. They observed a 14% rise in plasma total cholesterol in both men and women. In men this was largely due to a rise in low-density lipoprotein cholesterol (LDL-cholesterol) but in women, the high-density lipoprotein cholesterol (HDL-cholesterol) also rose significantly, which might be interpreted as a beneficial effect. Unfortunately, the authors allowed both the type of fat and the amount to change. Therefore, one cannot be certain whether the rise in cholesterol was due specifically to the contribution of dairy fat or to a rise in the total amount of fat.

From these studies, there seems little doubt that consumption of milk fat results, on average, in a rise in plasma total cholesterol when it replaces more unsaturated fats in the diet under controlled conditions. However, these average values obscure the fact the individuals may sometimes respond with a fall in blood cholesterol (see Chapter 1).

Skimmed or whole milk and plasma cholesterol

Interest in the influence of milk, as distinct from milk fat, probably began with the observations of Mann and his colleagues in 1977 that the Masai tribe of East Africa apparently consumed large quantities of milk (mainly fermented) and other animal products, yet maintained a very low plasma cholesterol. Howard and Marks compared two groups of human subjects given four pints of milk daily for three weeks, one given whole milk, the other skimmed milk. There were significant falls in plasma cholesterol in both groups, rather larger for skimmed milk (10%) than whole milk (5%) as compared with a rise (17%) in a group receiving an equivalent amount of butter fat alone as a supplement. One other study showed a similar significant fall in cholesterol due to skimmed

milk and three others (including the one that was best designed, controlled and conducted) found no effect. One other study found a small but significant fall due to whole milk and four studies found no difference. Many of the studies were poorly controlled but none of the studies conducted either with skimmed or whole milk showed any indication of a rise in plasma cholesterol (reviewed in 13).

The conclusion from the available evidence is that the inclusion of whole milk in diets does not have a cholesterol raising effect, despite its content of potentially hypercholesterolaemic fat. Skimmed milk may have a slight hypocholesterolaemic effect.

Cultured and culture-containing products

Mann's work with the Masai and then with American students led him to propose that yogurt specifically contained a 'factor' that resulted in blood cholesterol lowering. Subsequently his own experiments with rats and the results of three other studies (reviewed in 13) indicated that the effect of yogurt was no different from that of skimmed milk. None of the experiments was entirely satisfactory and some were so bad that sensible conclusions are not possible. Yet, there is a certain consistency to the results that suggests that, at best, yogurt may be mildly hypocholesterolaemic. Some research, published since my 1989 review (13) suggested that there was significant cholesterol lowering when the cultured product was fermented with specific strains of *Enterococcus faecium* and *Streptococcus thermophilus* (14) but the authors were unable to confirm this in the longer term (15). Perhaps more importantly, there is nothing in any study to suggest that the inclusion of cultured products in the diet, even those containing milk fat, raises plasma total cholesterol or LDL-cholesterol. Various claims for an elevation of HDL-cholesterol, which may be beneficial, need further investigation and verification.

Culture-containing milk products are those in which live organisms have been added to the milk but not allowed to ferment. It is well documented that certain of the gut microflora can metabolize cholesterol and also influence its metabolism in the body. Canadian researchers gave formulas containing *Lactobacillus acidophilus* to newborn babies and found that blood cholesterol had dropped significantly by the eighth day of feeding (reviewed in 13). The same effects were seen in babies given bicarbonate implying that any change leading to increased colonization of *Lactobacilli*, whether ingestion of organisms or manipulation of gastrointestinal pH, can modify cholesterol metabolism.

Gilliland (16) has screened strains of *Lactobacillus acidophilus* from both pig and human gut for ability to assimilate cholesterol from the culture medium. Only those pig strains that were able to assimilate cholesterol caused a lowering of cholesterol when given as a dietary supplement to pigs. Similar human experiments have not yet been reported.

In summary, very limited evidence suggests that ingestion of certain strains of *L. acidophilus* added to milks in the form of 'culture-containing products' may lead to small reductions in plasma cholesterol. The effects are observable only with strains that are able to survive in the gut and to metabolize cholesterol. Failure to demonstrate effects with some culture-containing products and, indeed, some cultured products may be due to use of inappropriate strains, but this point remains to be firmly established.

Is there a cholesterol-lowering 'factor' in milk?

Gibney and Burstyn (17) argued that the relatively low plasma cholesterol concentration of the Masai was quite likely to be due to their low overall energy intake coupled with an inherited capacity to regulate plasma cholesterol particularly precisely. Others have suggested that changes in blood cholesterol they observed during their experiments could be explained by concomitant changes in intakes of total fat and cholesterol (11). However, the data in the experiments in question are so poor as completely to preclude any conclusion on this point. Howard has argued that there must be a 'factor' in the non-fat portion of milk which counteracts, and even overrides, the effect of the fat, but that it is not specific to fermented milk (reviewed in 13). Calcium, lactose, orotic acid, hydroxymethylglutarate and other substances have been suggested but with little evidence for any of them. The Gilliland school of thought (16) argues for an effect due to specific strains of culture organisms. This would not, however, explain the hypocholesterolaemic or at least cholesterostatic effects of non-fermented milk in most experiments nor the experiments in which pasteurization of yoghurt (and thus destruction of the metabolic activities of the organisms) did not abolish the cholesterol-lowering activity of the yogurt (reviewed in 13).

Despite some rather poor research on this topic, there seems little doubt that there is a mysterious phenomenon that requires explanation by further research. The whole saga illustrates the folly of assuming, because a food contains a fat component that in itself raises blood cholesterol, that the food taken in the context of a normal mixed diet will necessarily result in a high blood cholesterol concentration.

Biological properties of some cow's milk fat components

Fat contributes energy, essential fatty acids and fat-soluble micronutrients to the diet. It also improves flavour perception and imparts a pleasing texture to foods, thereby improving palatability. Many have attributed the high prevalence of coronary heart disease in industrialized countries to high intakes of saturated fatty acids (18). Because of its high proportion of saturated and low proportion of polyunsaturated fatty acids, milk fat has acquired an adverse image in health terms. This has occurred despite the availability and popularity of low-fat dairy products. Nevertheless, much research is in progress that reveals some interesting and potentially beneficial biological properties of other milk fat components. Much of this research is still in its early stages and this article will assess recent progress (**Table 6.3**).

Flavour components

Milk fat contains components that contribute to its characteristic flavour, that has not been satisfactorily reproduced artificially, and to other sensory properties (19). This is a subject in its own right and will not be considered further here.

Long-chain saturated fatty acids

Long-chain saturated fatty acids are normally thought of as merely contributing to raised blood cholesterol and therefore to be avoided. It is worth remembering that saturated fatty acids are major components of the phospholipids of cell membranes (20) accounting for between 15 and 50% of membrane fatty acids. The phospholipids of lung surfactant are entirely esterified with palmitic acid, without which the lungs would collapse. As a class, long-chain saturated fatty acids are not essential in the diet because they can be made in the body. However, it is now generally accepted that fatty acid biosynthesis proceeds at a very low rate in human beings even when the diet contains little fat. Saturated fatty acids can certainly be regarded as essential components of membranes and it could be that under certain conditions, they are conditionally essential nutrients (see Chapter 4).

Short-chain fatty acids

Butyric and caproic acids are unique to milk fat. About one-third of milk fat triacylglycerols contain one butyric acid molecule (21). Butyric acid is now recognized as playing a role in the regulation of cellular metabolism as described in more detail in a later section.

Table 6.3. Some milk fat components of special biological interest.

Short-chain fatty acids

- Regulation of colonic cell growth
- Modulation of cell differentiation, programmed cell death, gene expression

Medium-chain fatty acids

- Lower energy value per mole than long-chain fatty acids
- Minimal contribution to plasma or adipose tissue lipids: rapid oxidation in liver
- Antimicrobial properties

Conjugated linoleic acid

- Cancer inhibitor
- Influences cholesterol metabolism
- Influences growth efficiency
- Antioxidant (?)

Phospholipids

- *General:* protect against ulceration of stomach/intestine
- *Sphingomyelin:* involved in cell signalling, growth regulation and membrane permeability
- *Alkyl lipids:* cancer inhibitors, influence cell differentiation, programmed cell death, cell signalling, activate platelets

Fat-soluble vitamins

- *Vitamin A:* visual processes, cell differentiation
- *Carotenoids:* antioxidants, metabolic regulation
- *Vitamin D:* Ca and P metabolism, cancer inhibitor (?)
- *Vitamin E:* antioxidant, cell growth regulation

Medium-chain fatty acids

Caprylic and capric acids (and the shorter chain length acids) are more rapidly hydrolysed from dietary triacylglycerols and are absorbed directly into the blood supplying the liver, rather than being incorporated into chylomicrons. They are therefore metabolized differently and have distinctive properties from the long-chain fatty acids as described in more detail in a later section.

Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a general term for a mixture of isomers of linoleic acid that are formed during the process of biohydrogenation in the cow's rumen (21,22). Although CLA occurs in other fats, sometimes as a result of high-temperature cooking, milk fat and ruminant meat fats have the highest concentrations of all food fats. The most abundant isomer and the one with the greatest biological activity is *cis*-9, *trans*-11-octadecadienoic acid. It has been shown to inhibit the development of mammary cancers in laboratory rodents at a level of about 1% in the diet. This and other properties are discussed in more detail in a later section.

Phospholipids

Milk phospholipids are located mainly in the milk fat globule membrane. Diverse beneficial health effects have been ascribed to the whole phospholipid mixture as well as to specific lipid components. A protective effect of milk against ulceration has been reported in rats and more recently in human subjects (23). The effect may be due to a specific phospholipid that coats the gastric or duodenal mucosa, not unlike the action of the lung surfactant. However, certain limitations in the design of these studies limit the interpretation of the results and more research is needed to resolve this important question. One paper has described a protective effect of milk phospholipids on tooth enamel (24).

Sphingomyelin is an important milk fat membrane constituent. It is a choline-containing phospholipid, like phosphatidylcholine but based on sphingosine rather than glycerol. The fatty acid is linked through an N-amide rather than an O-ester bond (20). The ratio of sphingomyelin to phosphatidylcholine is an important determinant of membrane permeability. In addition, sphingomyelin is involved in trans-membrane signalling processes (21). Cleavage of the phosphocholine moiety yields ceramide which can act as a 'second messenger', cell growth regulator, inducer of apoptosis and a regulator of gene expression. Cleavage of the fatty acid yields sphingosine which also has several regulatory properties including inhibition of

protein kinase C, stimulation of γ-interferon secretion and inhibition of colon cancer development.

Ether (alkyl) lipids are also present in the membrane. They may be cytotoxic or cytostatic to tumour cells, inducers of differentiation and apoptosis, and activators of platelets. They are also involved in various cell signalling mechanisms (21).

Fat-soluble vitamins

In many European countries, milk and milk products are major suppliers of vitamin A and its precursor β-carotene. In the UK, for example, about 20% of vitamin A activity in the diet is supplied by milk and milk products. Vitamin A is essential for vision and for the regulation of epithelial cell differentiation. Various derivatives have been implicated in the suppression of tumour development. As well as its pro-vitamin A properties, β-carotene has a number of biological properties that are independent of its capacity to form vitamin A. It is an antioxidant and may also play a role in tumour suppression. Milk is not normally an important source of vitamin D (except in countries, such as USA, that routinely fortify dairy products with the vitamin) but milk products may assume importance as sources of vitamin D in special circumstances when for example, sunlight is limiting (the elderly and dark-skinned immigrants to northern countries etc). It is involved in calcium and phosphorus metabolism and more recently has been implicated in the protection against colon cancer.

Health implications of milk fat composition

Despite this long list of potentially beneficial components, it is impossible to state categorically that their inclusion in the diet will in fact improve health or that their exclusion will cause health to deteriorate. Possible exceptions are the classical fat-soluble vitamins but even then, the overall contribution and importance of the milk vitamins will be crucially dependent on the composition of the rest of the diet.

One interesting study that tested the extent to which single foods could support growth and health of rats (25) found that whole milk was very much superior to skimmed milk even when the latter was supplemented with fat-soluble vitamins. The difference is likely to have been the contribution of milk fat to essential fatty acid requirements, despite their low concentration in cow's milk (**Table 6.4**).

Limitations in how to interpret published data in terms of applicability to human health are mainly concerned with the appropriateness of animal

Table 6.4. The ability of different foods to support growth and tissue development in young rats.

Food	Nutritional index
Laboratory diet	100
Eggs, cooked	78
Whole milk (vitamin D added)	63
White bread, enriched	57
Cooked hamburger	52
Potatoes, cooked	48
Canned tuna	44
Polished rice	29
Skimmed milk (vitamins A and D added)	12

From reference (25). The index was derived by assessment of the growth of the whole body and the development of the internal organs when rats were given each food as the sole component of the diet in comparison with the growth and development in rats given a diet specifically formulated to provide optimum growth and nutrition.

models, the accessibility of the milk components received from the diet to target tissues and the doses required to be effective. Thus, much of the work on the anti-tumour effects of these fat-soluble substances in milk has been done with laboratory animals given large doses of a chemical carcinogen or in tissue culture. The extent to which either of these models is translatable to human cancer is unknown. Little is known about the fate of the substances when they are ingested, especially intact lipids such as sphingomyelin. Will they actually be delivered to sites where they could be effective? Will the amounts present in milk products have any biological significance in practical conditions? Parodi (22) has calculated that the amounts of CLA consumed in Australia are not unrealistic in relation to the amounts that have been shown experimentally to be effective in animals.

Despite these uncertainties, milk consumption has been associated in some (but not all) epidemiological studies with reduced prevalence of heart disease, stroke, breast cancer and other cancers. If these effects are real, they should be explainable in terms of the biological effects of milk components which may include some of those described above. This provides a challenge for research to demonstrate the validity of these mechanisms and for the industry to exploit the research findings in terms either of novel products or promotional activity within the bounds of what is legally permissible.

A *trans* fatty acid that is good to eat? Conjugated linoleic acid

What is CLA?

During the process of the biohydrogenation of polyunsaturated fatty acids, catalysed by enzymes present in anaerobic microorganisms in the rumen of ruminant animals, the original methylene-interrupted sequence of *cis*-double bonds is disrupted (26). *Cis-trans* isomerization occurs as well as migration of double bonds along the chain and the total number of double bonds per molecule is reduced. An early event, before any reduction occurs, is the formation of a *cis-trans* conjugated double bond sequence (**Figure 6.1**).

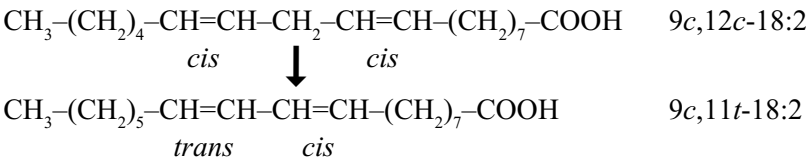

Figure 6.1. Formation of a *cis–trans* conjugated double bond sequence during biohydrogenation of polyunsaturated fatty acids.

Modern sensitive techniques for separation and analysis have demonstrated the presence of several conjugated diene isomers with double bonds in different positions. Collectively, these have been given the name conjugated linoleic acid, now generally shortened to CLA. Isomers with double bond positions at 9 and 11, 10 and 12 and 11 and 13 are present (22).

Where is CLA found?

CLA is present in a range of food fats but milk fat and dairy products containing milk fat have the highest concentrations. The proportion of the different isomers in CLA varies but in dairy products, the 9*c*,11*t*-isomer contributes about 90% of the mixture. Reports that processing of dairy products can alter the CLA content significantly must be treated with caution since there may be greater variation according to the season in which the milk was collected. However, the presence of whey protein during processing seems to result in an increase in CLA content. For example, cheeses are the richest sources of CLA in the diet, natural cheeses containing between

0.6 mg/g fat (blue cheese) to 1.9 mg/g fat (Parmesan). However, processed cheeses in which whey protein concentrate was added contained up to 8.8 mg/g fat (22).

The second most important source of CLA is in non-dairy ruminant products (e.g. lamb and beef fat). Vegetable fats as well as margarines and other products made from them contain little CLA. This is true even for industrially hydrogenated fats (27) perhaps because modern techniques result mainly in the production of monoene isomers.

Detailed analyses of the CLA content of a wide variety of foods including meats, seafood, milks, cheeses, canned foods and infant foods have been presented by Chin and colleagues (28). Data are presented as total CLA isomers in mg/g fat and, for certain foods, the proportion of the isomers present as 9*c*,11*t*-18:2 is given. A surprising omission was information on margarines and other spreads and there is clearly a need for further analyses of these products.

Anti-cancer properties of CLA

Although there are relatively few publications, the evidence for anti-carcinogenic properties of CLA is steadily increasing. Such evidence comes from cell culture studies (including human cells) and from studies with live animals, mainly rats and mice (22,29). The activity was originally discovered when Pariza and Hargraves (30) found that crude extracts from fried ground beef inhibited mutagenesis in bacteria and carcinogenesis in mice. Purified fractions, containing the CLA compounds, and synthetic CLA, inhibited tumour development induced in mice by the carcinogen dimethylbenzanthracene (DMBA) by about 50% when compared with control animals given linoleic acid or solvent (31). However, these were not dietary experiments: the CLA was applied to the skin in the area where the carcinogen was administered.

Later work by Ip and colleagues (29) administered CLA as a dietary component and demonstrated a significant reduction of mammary tumours induced by DMBA in rats when the diet contained as little as 0.1% by weight of CLA. These and other authors also demonstrated the incorporation of CLA into the phospholipids of cell membranes as well as showing that when incorporated into 'model membranes', CLA acted as an antioxidant, inhibiting the peroxidation of polyunsaturated fatty acids. Cell division in human cancer cells grown in culture is inhibited in the presence of CLA (22).

The mechanism by which CLA exerts its anti-carcinogenic effect is still unknown, although several clues are now emerging. Since oxidative reactions

involving radical attack may be involved in the initiation of cancer, CLA may be acting as an antioxidant, although this remains controversial. Eicosanoid synthesis from polyunsaturated fatty acids (which is an enzymic reaction involving free radicals) may also be modified by CLA. There is also some evidence that CLA may suppress cancer growth by inhibiting the biosynthesis of proteins and/or polynucleotides (22).

The influence of dietary CLA on CLA in the body

There is abundant evidence that CLA isomers from the diet enter many body tissues. CLA has been detected in human adipose tissue, milk, and in the blood, where it is associated with cholesteryl esters, triacylglycerols and phospholipids. Although it is probable that most of this originates from the diet, there are several ways in which small amounts might be formed in the human body. For example, the human colon contains anaerobic bacteria, similar to some present in ruminants, that are capable of forming CLA from linoleic acid (26). It is also possible that 9*c*,11*t*-18:2 could be formed from 11*t*-18:1 (present in the diet) by the action of fatty acid Δ-9 desaturase. Very small amounts might also be formed non-enzymically by autoxidation of linoleic acid.

Several studies have now reported that CLA levels in the body can be changed by dietary means (22). Thus, regular consumption of 112 g Cheddar cheese (providing 179 mg CLA) per day for 4 weeks resulted in a significant elevation of CLA in the blood: from 7.1 to 9.6 micromoles/litre (32). Within a month of returning to the original diet, levels had returned to baseline again. It seems that, if the anti-cancer properties of CLA in man are proved beyond doubt, cheese will provide a good source of this compound. Parodi (22) calculates that Australians probably consume about a third of the dose of CLA found to be effective against rat mammary cancer (i.e. from 0.5 to 1.5 g CLA-day). This is probably not too different from the amounts consumed in many European countries.

Other properties of CLA

If CLA acts mainly as an antioxidant, it should prove beneficial in other disease processes in which radical catalysed oxidation is thought to contribute. Many believe that atherosclerosis is one such process. A report has appeared showing that when rabbits were given 0.5 g CLA per day, atherosclerosis induced by a high-fat, high cholesterol diet was reduced, although the reduction did not reach statistical significance (33). There was also, surprisingly, a fall in blood cholesterol. It remains to be established

whether CLA really does have the potential to reduce atherosclerosis and further research will be awaited with interest.

CLA was also found to protect against the growth suppression associated with immune stimulation in chicks, rats and mice. An important finding of this research was that CLA improved growth efficiency.

Lipids in infant nutrition

The need for artificial feeds

The amount and composition of lipids that should be included in commercial infant feeds poses a challenging problem in practical nutrition. It also illustrates some of the most important current topics in fundamental nutritional biochemistry, particularly the optimal ratio between the *n*–6 and *n*–3 families of polyunsaturated fatty acids (34,35).

All medical practitioners agree that human milk is almost always the most appropriate food for human babies born at term; the baby born prematurely poses a set of nutritional problems that need special consideration. Because some mothers either cannot or do not wish to breast feed, the need for infant formulas has arisen. The aim has generally been to try to mimic the composition of human milk as far as possible, although several biological facts militate against ever fully achieving this goal (35,36). Human milk contains several proteins, whose role is to protect against infection, which would be technically difficult or costly to include in a formula. There are also present many live cells of the immune system which could not be incorporated into a formula. Regarding the lipids, the individual components are well characterized but the change in amount and composition of breast milk fat during lactation and even during a single feed are facets that are not easily replicated.

Cow's and human milks compared

Because of its ready availability and recognized nutritive value, cow's milk has long formed the basis for most commercial infant formulas used in industrialized countries. Nevertheless, because of the different physiologies of calf and human infant and their relative state of development at birth, unmodifed cow's milk is inappropriate for the human infant. It was very early recognized that the protein and sodium concentrations in cow's milk were far too high for the baby's immature digestive system and kidneys. Only relatively recently has the need radically to modify the fat composition of cow's milk for use in formula been realized.

A comparison of the fatty acid compositions of cow's and human milks is given in **Table 6.5**. It is also noteworthy that the cholesterol contents of these milks are significantly different, being 14 mg/100 g for cow's milk and 16 mg/100 g for human milk.

Table 6.5. Comparison of the fatty acid composition of cow's and human milk fats (g/100 g total fatty acids).

Fatty acid	Cow	Human
Saturated		
4:0	3.1	0.2
6:0	2.0	0.2
8:0	1.2	0.5
10:0	2.6	1.0
12:0	3.1	4.4
14:0	10.8	6.3
16:0	29.6	22.0
18:0	10.9	8.1
Monounsaturated		
14:1*c*	0.9	0.4
16:1*c*	1.1	3.3
18:1*c*	24.7	31.3
18:1*t*	1.0	2.7
Polyunsaturated		
18:2*cc*, *n*–6	1.1	10.9
18:3*ccc*, *n*–3	0.3	1.0
20:4*cccc*, *n*–6	–	0.5
20:5all*c*, *n*–3	–	0.1
22:6all*c*, *n*–3	–	0.3
t isomers	2.0	1.2
Others	5.6	5.8

c = *cis*; *t* = *trans*; others: mixture of odd-carbon-numbered and branched-chain fatty acids.

The main difficulty in making such comparisons is that the fatty acid composition of human milk is much more influenced by the composition of the maternal diet than that of cow's milk. As a woman increases her intake of

polyunsaturated fatty acids, for example, so the proportion in her milk increases (34,37). The same is true of the *trans* unsaturated fatty acids. Nevertheless, even when human diets are relatively low in polyunsaturates, the proportion of total polyunsaturated fatty acids in the milk is considerably higher than in ruminant milk. Moreover, the long-chain polyunsaturates of the *n*–3 family, virtually absent from cow's milk, make a small but highly significant contribution to human milk.

Requirements of infants for lipids

The human infant is born relatively immature and requires an energy-rich diet compared with the more mature calf. Lipids are prime suppliers of metabolic energy in human milk.

The immaturity of organ systems in the human baby also puts great demand on raw materials for membrane synthesis. Foremost among these are the polyunsaturated fatty acids and cholesterol. The fat soluble vitamins are also crucial in this early stage of development.

A large part of the demand for membrane lipids in the newborn baby is for the development of the brain and nervous system (35,38). Human foetal brain experiences a rapid growth spurt towards the end of pregnancy which continues into post-natal life. Brain and nervous system phospholipids contain a high proportion of arachidonic (20:4*n*–6) and docosahexaenoic (22:6*n*–3) acids and low concentrations of their precursors, linoleic (18:2*n*–6) and α-linolenic (18:3*n*–3) acids. The photoreceptors in the retina of the eye also contain a high proportion of phospholipids containing two docosahexaenoic acid moieties.

The infant's biosynthetic capacity

What is required in the diet is intimately linked with the baby's capacity to synthesize its own materials. During life as a human foetus, the principal fuel is glucose, supplied in the maternal blood. At this stage, therefore, the enzymes for converting sugar into lipid are fully developed. The fact that lipid synthesis from carbohydrate is an extremely active process in the human foetus is amply illustrated by the relatively large amount of body fat present in a newborn human baby.

Similarly, the enzymes of cholesterol synthesis are also fully developed at this stage to provide for the needs of membranes, particularly in the rapidly developing nervous system. The rate of synthesis of cholesterol by the human baby, as measured by isotopic incorporation from deuterated water (39), is regulated by the concentration of cholesterol in the milk. In one such study (39)

endogenous cholesterol biosynthesis rates were inversely proportional to the cholesterol concentration in the feed: highest with soy formula which contained no cholesterol, intermediate with cow's milk based formula and lowest with breast milk. Despite lower cholesterol synthesis in the breast-fed babies, these infants had the highest concentration of blood LDL-cholesterol, which is the main vehicle delivering cholesterol to tissues.

The primary essential fatty acids, linoleic acid and α-linolenic acid, need to be supplied from the mother's diet through her circulation since neither mother nor baby can synthesize them. The elongation and further desaturation of these precursors into longer-chain more highly unsaturated derivatives is a slow and inefficient process in placenta and in foetal and newborn infant tissues and the selective incorporation of pre-formed long-chain polyunsaturates is thought to be the main contributor to the accumulation of these fatty acids in nervous tissue (34,38).

Requirement for lipids in infant formula

Digestibility of triacylglycerols

The newborn infant's digestive capacity is immature owing to limited pancreatic and biliary secretions. Human milk lipids are more efficiently digested partly because of their higher degree of unsaturation and partly as a result of the structure of the triacylglycerol molecules. In human milk a much larger proportion of palmitic acid (16:0) is located in position 2 than in cow's milk triacylglycerols. As a result of lipase digestion most palmitic acid is liberated as monopalmitin from human milk fat and as free fatty acid from cow's milk fat. Palmitic acid is more efficiently absorbed as monopalmitin than as free fatty acid (40). There is scope, therefore, for the use of 'structured lipids' in infant formulas (36) and, for example, a fat blend (Betapol) has been developed by Unilever Research, which has high levels of 16:0 esterified to the *sn*–2 position of the triacylglycerols (41). It is also noteworthy that taurine conjugates of bile acids are important in fat emulsification in the human infant's small intestine. Taurine occurs abundantly in the non-protein nitrogen fraction of human milk and is hardly present in cow's milk. Taurine is now added to nearly all infant formulas (36).

Cholesterol

Infant formulas containing cow's milk fat contain cholesterol but at a lower concentration than in human milk. When vegetable oils are used in infant formulas, the product contains little or no cholesterol. Cholesterol is not normally added to formulas, despite the knowledge that human milk is rich in

this component, because of fears that such a step may inadvertently encourage high LDL-cholesterol in early life and increase the risk of cardiovascular disease later. There is dispute between experts on this point (36) which seems set to continue. As pointed out in an earlier section, it is normal for breast-fed babies to have a high circulating concentration of LDL, which may be required to deliver cholesterol and long-chain polyunsaturates to the brain.

Polyunsaturated fatty acids

There is little dispute that linoleic acid and α-linolenic acid, the primary essential fatty acids, should be included in infant formula although there is surprisingly little guidance on the actual amounts. During the 1970s there was a vogue for incorporating large amounts of linoleic acid-rich vegetable oils into formulas that resulted in extremely high concentrations of linoleic acid in the infants' adipose tissue (42). This practice has now been abandoned in favour of more modest inclusions. Van Aerde and Clandinin, in a first class discussion of current understanding of the needs for polyunsaturates by newborn infants (34) estimate that formulas should contain 5–6% of energy as linoleic acid (12–16% of milk fatty acids) and 1% of energy as α-linolenic acid (2.25–3% of milk fatty acids). This would result in a ratio of linoleic acid/α-linolenic acid of 4:1–6:1 which is much lower than has been commonly found in traditional formulas (15:1–20:1).

Concern about the poor conversion of the precursor essential fatty acids into long-chain derivatives has led to the introduction of arachidonic acid and docosahexaenoic acid into formulas for pre-term infants and it is likely that the same will apply to formulas for term infants in due course (34-36). Giving formula enriched with fish oil improves docosahexaenoic acid status and visual function in pre-term babies but their arachidonic acid status and growth may deteriorate (34,43). The optimal amounts of precursor and product polyunsaturates and the optimal ratios between *n*–3 and *n*–6 families remains a crucial topic for nutrition research (34–36,38,43). On the basis of current information, the recommendation (34) is for long-chain polyunsaturates of the *n*–3 family to represent 0.25–0.50% of energy (0.5–1.0% of milk fatty acids) and those of the *n*–6 family to provide 0.35–0.7% of energy (0.7–1.4% milk fatty acids) giving an *n*–6/*n*–3 ratio between 1.1:1 and 1.4:1. Improvements in the polyunsaturated fatty acid content of infant feeds may be assisted by an ever-widening range of sources of polyunsaturates from plants and microorganisms. For example, the fungus *Mortierella alpina* is a good source of arachidonic acid and several fungi, algae and marine bacteria yield eicosapentaenoic acid. These developments have been reviewed by Gunstone in *Lipid Technology* (1997, Vol.9, pp.91–94).

Fat-soluble vitamins

Little basic information exists to give guidance for appropriate levels of the fat-soluble vitamins in infant feeds. The practice of using vegetable oils to supply linoleic acid should ensure that adequate vitamin E is present because most oils rich in linoleic acid are also good sources of vitamin E. Human milk, particularly colostrum, contains considerable amounts of β-carotene. This results in rapid increases in the blood concentration of β-carotene in breast-fed infants that does not normally occur in those who are bottle fed. β-Carotene can be converted into retinal in the gut of human babies (36) and thus may provide a source of vitamin A. Carotene may also provide an important part of the infant's defence against radicals (36). Much research is still needed to provide a firm scientific basis for the design of infant formulas and the idea of human milk as a 'gold standard' may need to be abandoned as more information on the functional requirements for different fatty acids becomes available.

Short-chain and medium-chain fatty acids in nutrition and metabolism

In this and other chapters, I have discussed aspects of fatty acids in human nutrition that have gained prominence in terms of health. These aspects are also well summarized in Chapter 3 of the UK Department of Health's booklet 'Dietary Reference Values for Food Energy and Nutrients for the United Kingdom' (44). However, short and medium-chain fatty acids are not an 'issue' and are not given a passing mention in this publication, so why do I choose to highlight them here? The reason is precisely that they tend to be forgotten and because they have interesting metabolic properties that are distinctly different from those of the longer-chain fatty acids. I estimate that intakes in the UK may be on average about 3 g/day and it is pertinent to ask whether we should not give more attention to their effects on nutrition and health?

Structure, properties and occurrence

Short-chain fatty acids can be defined as having chain lengths from C_2 to C_6 and medium-chain acids from C_8 to C_{10}. Such a definition is somewhat arbitrary, but has some basis in physical and metabolic properties. Some may argue that acetate (2:0) is not a fatty acid, but its inclusion is justified since it is found in some naturally-occurring lipids and is intimately bound up in fatty acid metabolism. Most of the acids considered here have an even number of carbon atoms but propionate (3:0) is also important in metabolism. Some may

regard the C_{12} acid, lauric acid, as a 'medium-chain acid' but for metabolic reasons described later, I will define it as a 'long-chain fatty acid'. Some short and medium-chain fatty acids may contain double bonds or chain branches but those of quantitative significance in human diets are all saturated.

The short-chain fatty acids are apparently water-soluble but in fact they are associated together in micelles and do not exist as single molecules. They are often referred to as 'volatile fatty acids' (VFA). After C_8 the length of the hydrocarbon chain limits water solubility (20).

In foods, the short-chain fatty acids are present in small quantities, generally as components of flavour volatiles or in milks. Medium chain acids are present mainly in milks or in certain seed oils.

Ruminant milk fat normally contains about 5 g/100 g of 4:0 plus 6:0 and similar amounts of 8:0 plus 10:0. Giving the animals rations with high inclusions of fat tends to reduce the proportion of these acids with a concomitant increase in longer-chain fatty acids. Human milk fat normally has no short-chain and only 1 or 2% by weight of medium-chain fatty acids in societies in which fat intakes are relatively high, but these may be significantly higher in the milk of women consuming diets with a very low fat content (20). The medium-chain fatty acids are not distributed randomly in milk triacylglycerols and tend to be located predominantly on position 3 (45).

The most abundant sources of medium-chain fatty acids used in the preparation of human foods are coconut and palm kernel oils which contain respectively 15% and 8% by weight of 8:0 + 10:0. The most abundant and characteristic acid in these oils is 12:0, lauric acid, which comprises nearly 50% of the total fatty acids. Seed oils of *Cuphea* species also contain medium-chain fatty acids and could become another potential food source (46).

Metabolism

The origin and metabolism of the volatile and medium-chain fatty acids is quite different and needs to be described separately.

Short-chain fatty acids

In ruminants, acetic, propionic and butyric acids are formed by microbial fermentation of polysaccharides in the rumen. They are absorbed directly from the rumen and metabolized mainly in the liver for the production of glucose and long-chain fatty acids. The importance of fermentative production of short-chain fatty acids in man and other simple-stomached animals like pigs, has been

realized only relatively recently. The site of production is the colon, which has a large microbial population, and the substrates are mainly the non-starch polysaccharide components of the diet (dietary fibre) but also undigested starch that has reached the large intestine. Butyric acid is thought to be metabolized mainly by the epithelial cells of the colon and may have a special role to play in the maintenance of mucosal integrity. It also plays a role in the regulation of cellular growth, especially in the colon, inhibits cell proliferation, induces differentiation and apoptosis (programmed cell death) (21) and modulates the expression of genes in several types of cells. These activities suggest that it may be useful in suppressing tumour development and indeed it has already found clinical use against leukaemia. Acetic and propionic acids are absorbed through the colon and experiments with pigs demonstrate that they may become substrates for long-chain lipids that are probably synthesized in the liver. It is inferred that this may also happen in man. Thus, so-called indigestible carbohydrates may be significant energy sources, although the extra energy provided may be balanced by a slight loss in digestibility of fat and protein caused by high-fibre diets (47).

Medium-chain fatty acids

In man and other animals, the production of medium-chain fatty acids occurs almost exclusively in the mammary gland, hence their characteristic occurrence in milk fat. In most tissues that synthesize fatty acids, there is an enzyme called thioesterase, which is part of the complex of enzymes known as fatty acid synthetase. This enzyme releases fatty acids from the complex and is specific for long-chain fatty acids. Therefore, medium-chain fatty acids which are intermediates in the growing fatty acid chains never escape from the complex. In mammary gland, however, there is a thioesterase specific for the medium-chain fatty acids, releasing them and making them available for incorporation into milk fat triacylglycerols (20).

Short and medium-chain fatty acids in milk fat triacylglycerols are hydrolysed particularly rapidly by lipases. In human babies this process probably begins in the mouth as a result of the action of a salivary lipase or a lipase present in human milk. The most extensive digestion, however, occurs in the stomach as a result of the action of lingual lipase, which is produced in glands around the tongue and is carried into the stomach where it acts with a pH optimum in the acid range. There is some evidence for direct absorption from the stomach. This mode of digestion is particularly important for new-born babies, in whom pancreatic secretions, which contain the lipase that catalyses most of the lipid digestion in the duodenum of older children and adults, are not fully developed (20).

Fatty acids with chain lengths of C_{14} and longer are absorbed as monoacylglycerol and free fatty acid components of 'mixed micelles' into the small intestinal epithelial cells, where they are reincorporated into triacylglycerols that are then exported into the bloodstream as lipoproteins known as chylomicrons (20). Chylomicrons are cleared from the plasma mainly by the adipose tissue. Therefore, immediately following a meal containing fat, the fatty acid composition of plasma lipoproteins and adipose tissue reflects, to some extent, the long-chain fatty acid profile of the diet.

Fatty acids with chain lengths up to C_{10} have quite a different fate. They are absorbed as free fatty acids and enter the blood flow in the portal vein which directly feeds the liver. This is a rapid process. In the liver, they are rapidly broken down by the process of β-oxidation which removes two carbon atoms at a time and, to a small extent, ω-oxidation. The resulting acetate (from even-numbered chain length fatty acids) may enter a number of metabolic pathways including resynthesis of long-chain fatty acids. Propionate, which is the end-product of breakdown of odd-numbered chain length fatty acids, can be directed into metabolic pathways resulting in glucose synthesis. Thus, odd chain acids and propionate have metabolic effects more akin to sugars than lipids (48).

Lauric acid, 12:0, is in an intermediate position. Some is absorbed through the portal vein while some is incorporated into chylomicrons. However, the balance of evidence suggests that the predominant route is through chylomicrons, hence my decision to classify lauric acid as a 'long-chain fatty acid'. A reflection of the intermediate position of lauric acid is the uncertainty about its cholesterol-raising activity. Some research has placed it amongst the most hypercholesterolaemic fatty acids, while other publications have found only a small effect (49). It is entirely probable that some people absorb most of their lauric acid through the portal route while others handle it as a long-chain fatty acid.

The implications of the mode of absorption of short-chain and medium-chain fatty acids are: (i) they do not contribute significantly to plasma lipoproteins and, therefore, have no 'cholesterol-raising activity'; (ii) they are not deposited in adipose tissue; and (iii) they are rapidly absorbed in conditions in which the absorption of long-chain fatty acids may be impaired. Failure to digest lipids may occur as a result of diseases resulting in insufficient lipase or bile salt; malabsorption is generally due to damage to the mucosal surfaces of the small intestine. Absorption may also 'back up' due to congenital failure to produce specific apoproteins which are obligatory components of plasma lipoproteins. Patients with malabsorption are at risk of deficiencies in energy,

fat-soluble vitamins and essential fatty acids but can usually absorb medium-chain fatty acids (50).

Medium-chain fatty acids also have antimicrobial properties and this may be one reason why milk contains a relatively high concentration, as a protection for the newborn. One paper has described a protective effect of milk fat on human tooth decay which the authors ascribed to adsorption of milk lipids onto the enamel surface (24). The effect may also have been due, in part, to the known antimicrobial effects of milk fatty acids and this whole area deserves more research.

Food uses: now and in the future

For patients who are unable to absorb fat, normal dietary fats may be replaced by medium-chain triacylglycerols (MCT) (50). This product is composed mainly of triacylglycerols, fractionated from coconut oil, containing 8:0 and 10:0 and can be purchased as cooking oils or fat spreads.

MCT have also found applications as a source of energy in the enteral feeding of pre-term babies.

Molecular species containing short and medium-chain fatty acids can be fractionated from milk fat which is surplus to demand but the cost of the starting material and the process would add up to a very expensive product. New seed oils, like those from *Cuphea* may in the future provide alternatives to coconut oil.

An alternative application which has been much discussed is in reduced-calorie diets and diets that will not elevate plasma cholesterol because medium-chain fatty acids are not deposited in adipose tissue and do not contribute to plasma lipoproteins (51). However, to the authors's knowledge, convincing evidence for their long-term effectiveness in man is yet to be published. Their effectiveness would need to be substantial in view of the cost of the product and competition from other low calorie fat substitutes now being developed.

Oil palm lipids: edible oils with interesting nutritional attributes

Background

It is common but misleading to characterize particular oils as 'saturated' or 'polyunsaturated' or the like. First, natural oils do not tend to contain triacylglycerol molecules in which only one type of fatty acid is esterified and

if they do it is only in nutritionally insignificant amounts. In general, all are mixtures of saturated and unsaturated fatty acids. How much of one particular class needs to be present for the fat to be designated 'saturated' or 'unsaturated'? It is only the fatty acids that should be so designated, not the oils themselves. Second, because of the preoccupation that there has been with the influence of dietary fatty acids on plasma cholesterol concentration (see Chapter 1), many people now would assume that if an oil is called a 'saturated' oil, its consumption will inevitably lead to higher plasma cholesterol concentrations.

Palm oil is a good example of a natural edible oil that illustrates the fallacy of the latter assumption as well as the difficulty of applying the classification saturated or unsaturated. Another reason why I have chosen this topic is because the term saturated has been used as a term of abuse in an orchestrated campaign in the USA against so-called tropical oils and well illustrates the misuse of nutrition research for commercial or ideological ends.

Several organizations in the USA have been waging a sustained campaign against 'tropical oils' (coconut, palm kernel and palm oil) which culminated in late 1988 with full page advertisements in the *New York Times* and the *Washington Times* headed "The Poisoning of America". These advertisements came from the most emotive and vociferous of these groups: The National Heart Savers Association. The advertisements, which mentioned several common American foods, urged consumers to eliminate the use of saturated fats in the diet and condemned the food industry for continuing to use these products in the manufacture of various foods. They stressed that half the American population had cholesterol levels that were too high, that eating saturated fats raises one's plasma cholesterol concentration and that high cholesterol concentrations lead to heart attacks.

Although the American Council on Science and Health denounced the advertisements as a disservice to the US consumer, it was not before many US food manufacturers had replaced these three oils with other raw materials. In 1986, a consumer organization petitioned the Food and Drug Administration to require that the name of any of the three tropical oils on a food label must be followed by the phrase: "a saturated fat". In fact, palm oil contributed less than 2% of the saturated fatty acids in the US diet, much less than many other fats and oils for which this specification was not required. In early 1987, an almost identical petition was submitted by the American Soybean Association. Both received the response that the petition would be considered within labelling regulations being considered for 'low cholesterol' claims. The USA is a major exporter of oils and fats and its main product is soybean oil. This trade is now under extreme competitive pressure from South American soybeans,

European Union and Canadian rapeseed as well as palm oil from Malaysia, Indonesia and Africa. Health concerns and commercial competition are clearly becoming interwoven.

It is not my intention here to argue the case whether there is a strong link between the consumption of saturated fatty acids and coronary heart disease: that topic is the subject of Chapter 2. The following will be concerned with the nutritional attributes of palm oil to illustrate the dangers of making generalizations based on the assumption that an oil is 'saturated'.

Palm oil as a food raw material

Palm oil is one of the sixteen edible oils possessing an FAO/WHO Food Standard under the Codex Alimentarius Commission Programme and has a long history of food use dating back over 5000 years. It is now consumed worldwide as a cooking oil as well as being a raw material for the manufacture of margarines and shortenings. It is incorporated into many industrial fat blends and a wide variety of food products.

Palm oil is obtained from the fleshy mesocarp of the oil palm fruit and should be clearly distinguished from palm kernel oil. The fatty acid compositions of these two oils are quite different (46), palm oil containing about 50% saturated fatty acids and 50% unsaturated, whereas the kernel oil is a 'lauric oil' similar to coconut, containing 83% saturated fatty acids (**Table 6.6**). Unrefined palm oil is rich in carotenoids, tocopherols and tocotrienols. Refining unfortunately removes much of the carotenoids but the refined oil retains a high concentration of compounds with vitamin E activity. Palm oil contains about 18 ppm cholesterol and palm kernel oil 17 ppm. These values are typical of many vegetable oils and contrast with, for example, lard, which contains 3500 ppm cholesterol.

Because of the physical properties of palm oil, due to the saturated fatty acid composition and the structure of the triacylglycerols, it can be included in products with minimal hydrogenation, giving product qualities which could only be achieved using more unsaturated oils if they were extensively hydrogenated. Those who are persuaded of the need to keep the concentration of *trans* fatty acids to a minimum will clearly find this property of great benefit.

Metabolism of palm oil

Palm oil contains no very-long-chain or unusual fatty acids and is readily digested, absorbed and used as a source of energy. The medium-chain fatty acids (8:0, 10:0) in palm kernel oil are absorbed by a different route from the

Table 6.6. Composition of the so-called 'tropical oils'.

Fatty acid[1]	Palm oil	Palm kernel	Coconut
8:0	0	4	8
10:0	0	4	7
12:0	tr	45	48
14:0	1	18	16
16:0	42	9	9
16:1	tr	0	tr
18:0	4	3	2
18:1	43	15	7
18:2n-6	8	2	2
18:3n-3	tr	0	0
Others	2	0	1

[1]In the shorthand notation for fatty acids, the number before the colon represents the number of carbon atoms and the number after the colon, the number of double bonds; *n*–3 and *n*–6 denote the number of carbons between the last double bond and the methyl end of the chain. tr = trace.

longer-chain fatty acids (see reference 20 and an earlier section). Instead of being transported in plasma lipoproteins, they pass into the blood vessels supplying the liver as albumin-bound non-esterified fatty acids and are rapidly metabolized in the liver.

Influence on blood cholesterol

The saturated fatty acids, lauric, myristic and palmitic acids are generally regarded as causing a rise in plasma cholesterol when they replace unsaturated fatty acids in the diet. Since palm oil contains equal proportions of saturated and unsaturated fatty acids, its effect on blood cholesterol is difficult to predict. Considering the position of palm oil on world edible oil markets (second only to soybean) it is surprising that palm oil has not more often been included in systematic work to establish the relative effects of different fatty acids and different oils on plasma lipoprotein concentrations.

Several recent controlled human studies in Europe, USA, Australia and Asia (52–55) have confirmed that there is no rise in blood total cholesterol concentration when palm oil, providing most of the dietary fat, is used as an alternative to other fats in a traditional diet. In several of these studies, the concentration of HDL-cholesterol, regarded as beneficial to health, was significantly enhanced. The content of lipoprotein[a], a potent risk indicator

for coronary heart disease, was significantly reduced when palm oil provided most of the dietary fat (56). In most of these studies, the plasma cholesterol concentrations were lower at the end of the experiment in the subjects given palm oil than at the beginning, when they had been consuming their normal diets. This might have been due to a 'placebo' effect. It could, however, have been due to the oleic and linoleic acids in the palm oil. Monounsaturated fatty acids are now regarded as being similar in their cholesterolaemic effects to polyunsaturated fatty acids (see Chapter 4). Yet another possibility is that the effect is due to some of the minor non-saponifiable compounds in palm oil. For example, the tocotrienols have been found, in animal experiments, to be powerful inhibitors of HMG-CoA reductase, the enzyme that catalyses the rate-limiting step in cholesterol biosynthesis (57). These results with palm oil do emphasize the need, when discussing 'cholesterol raising' or 'lowering' effects, to state clearly the standard against which raising or lowering is being measured.

It has frequently been assumed by research workers comparing the influence of two dietary oils on plasma cholesterol that such influences must necessarily be a result of differences in fatty acid composition, whereas other components, low in concentration, might have relatively large biological effects.

Influence on blood coagulation

There is much current interest in the possible influences of dietary lipids on factors that regulate the potential of the blood to clot in the process of wound healing. Such phenomena as the aggregation of blood platelets and the formation of fibrin clots (thrombi), involving a whole cascade of clotting factors and enzymes, as well as the dissolution of unwanted clots (fibrinolysis) may involve lipids in some way and may, therefore, be modified by fat from the diet (58). One of the major difficulties is how to measure these complex processes in a way that is meaningful in terms of what happens in the living blood. Most techniques have been 'test-tube' experiments which may be so artificial that they are not relevant to 'real life'. Hornstra (59) developed an animal model to study the potential for thrombi to form in blood. A small loop of translucent plastic is introduced into the aorta of a rat with part of the tube exteriorized so that the blood flow can be observed. The damage caused to the blood vessel by this procedure causes a thrombus to form and the time taken for it to grow to a size when it completely blocks the artery (obstruction time) is measured. This moment can be observed readily because the blood in the tube turns from bright red to black.

The obstruction times of rats given various fats and oils are shown in **Table 6.7**. While it is not certain how well the technique represents the true behaviour of the blood in a living person, it should nevertheless give a fairly good rank order of dietary oils in terms of their influence on the tendency for thrombosis to occur. As can be seen in Table 6.7, the obstruction time diminishes (i.e. thrombosis tendency increases) with the proportion of long-chain saturated fatty acids in the oil. However, palm oil clearly does not behave as expected from its fatty acid composition. Other results from this laboratory suggest that the effect may be due to a compound in the unsaponifiable fraction, possibly a tocopherol or related substance.

Table 6.7. Dietary fats and oils ranked in order of their tendency to induce thrombosis.

Dietary fat	OT (hours)[1]	% 14:0 + 16:0
Palm	195	43
Rapeseed	190	8
Linseed	185	13
Sunflower	170	11
Olive	145	16
Coconut	130	24
Hydrogenated soybean	125	23
Whale	110	28
Hydrogenated coconut	90	30

[1]OT = obstruction time, the time taken for blood to clot.

Influence on cancer

The scientific debate about whether the risk of cancer is influenced by the amount or type of fat in the diet continues without resolution. The epidemiological evidence linking fat with most human cancers is weak, though less so for colon cancer. For experimental evidence we have to turn to animal experiments which may not be directly relevant to man. There are several publications concerning the effects of palm oil specifically. For example, Sylvester *et al.* (60) found that rats given diets containing 20%

palm or corn oils prior to administration of a carcinogen had no more tumours than animals given a low-fat diet (5% corn oil) whereas in those given 20% beef fat or lard, mammary tumour development was enhanced. Another study (61) found that, compared with several other edible oils, dietary palm oil reduced the number of chemically-induced tumours in rats.

On current evidence, it appears that the inclusion of palm oil in diets at a level similar to the total fat intake in industrialized countries should carry no particular risk for cancer development. However, we should have caution in extrapolating results obtained in animals to man. It is worth noting that two important reports published in 1997 and 1998 concluded that there is little good scientific evidence for a role for dietary fats in the development of human cancers. I have discussed this in detail in an article in *Lipid Technology* (62).

Postscript

The moral of this tale is that the nutritional and metabolic effects of an edible oil cannot be judged from its fatty acid composition alone. Nutritionists should in future investigate the properties of components of fats and oils, which although quantitatively minor, may yet have important metabolic effects. Use of the simplistic terms 'saturated fat' and 'unsaturated fat' should be abandoned and many previous assumptions should be re-evaluated.

Plant sterols in the diet

Sterols in plant lipids

When nutritionists discuss dietary sterols, the emphasis is nearly always on cholesterol, the major sterol of the animal kingdom. It is often forgotten that plant lipids also contain sterols. The major plant sterols are β-sitosterol, campesterol, stigmasterol and avenasterol; only traces of cholesterol are present (46). Rapeseed oil contains a small amount of brassicasterol. Some examples of sterol compositions of seed oils important in human diets are presented in **Table 6.8**.

Dietary intakes

Little information is available on intakes of plant sterols. One Finnish publication has indicated that the average amounts consumed — 160–360 mg/day (63) — may not be much lower than intakes of cholesterol, which is some 300–400 mg/day in UK adults (64). Strict vegetarians may consume amounts of plant sterols that far exceed their intakes of cholesterol.

Table 6.8. Sterol composition of some common edible plant oils (mg/kg).

Oil	Cholesterol	Campesterol		β-Sitosterol	Δ-7-Stigmasterol	
		Brassicasterol	Stigmasterol		Δ-5-Avenasterol	
Cocoa butter	59	–	266	769	1746	88	29
Coconut	23	–	18	296	1322	319	136
Corn	–	–	2691	702	7722	468	117
Olive	–	–	28	14	1310	29	58
Palm	26	–	358	204	1894	51	25
Peanut	–	–	360	2160	1536	192	72
Rape	–	612	1530	–	3549	122	306
Soybean	–	–	720	720	1908	108	108
Sunflower	–	–	313	313	2352	156	588

Influence on cholesterol metabolism

There is no known direct role for plant sterols in human nutrition. Unlike cholesterol and the plant-derived essential fatty acids, linoleic and α-linolenic acids, they are not found in the membranes of human cells. Apparently, only the cholesterol structure is able to support the structure and function of animal membranes. Any attempt to substitute cholesterol with other sterols has led to non-functioning membranes.

It is generally considered that plant sterols are poorly absorbed from the small intestine but again the subject has been poorly studied. The Finnish workers (63) found that the serum concentration of all the plant sterols was less than 1% of the cholesterol concentration. Since the 1950s it has been known that plant sterols inhibit cholesterol absorption. This does not necessarily influence serum cholesterol since synthesis of cholesterol in the liver is under feedback control from cholesterol entering from the diet but some studies have shown a degree of cholesterol lowering. It was discovered, however, that sitostanol, a 5-α saturated derivative of sitosterol was much more effective in reducing cholesterol absorption and serum cholesterol concentration than sitosterol itself and this fact has sometimes been exploited in therapy for hypercholesterolaemia (65,66).

Recent research

Miettinen and colleagues (67) demonstrated significant blood cholesterol reduction in people with mildly elevated blood cholesterol by the regular use

of a specially formulated margarine that contained sitostanol. Sitosterol was prepared from wood, hydrogenated to sitostanol, and then interesterified with rapeseed oil to produce sitostanol esters. The margarine used in the study was also based on rapeseed oil and was blended with the sitostanol esters so that an 8 g portion of margarine provided 1 g sitostanol.

In their study, 153 participants were instructed to replace 24 g/day of their normal fat intake with a conventional rapeseed oil based margarine for 6 weeks after which they were randomized into two groups, one (51 subjects) continuing to use the conventional margarine, the other using the margarine blended with sitostanol. The latter received 3 g/day sitostanol. After 6 months, people in the sitostanol group were randomly assigned to one of two equal groups, one continuing to receive 3 g/day, the other 2 g/day, sitostanol. Fasting blood samples were taken at appropriate points throughout the study and analysed for total cholesterol, HDL-cholesterol, campesterol and triacylglycerols. LDL were estimated using a formula that predicted their concentration from the concentrations of the other constituents.

No untoward symptoms were reported by the people receiving the sitostanol. After a year, blood cholesterol decreased from the baseline value on average 10.2% in people given the sitostanol margarine and increased on average 0.1% in the control group. This equated to a reduction of 0.63 mM in subjects receiving sitostanol compared with controls (P <0.001). It also compares very favourably with more conventional dietary measures to reduce blood cholesterol, for example increasing the ratio of *n*–6 polyunsaturates to saturates. Most of the cholesterol reduction resulted from a fall in LDL-cholesterol; there were no significant changes in HDL-cholesterol or triacylglycerols and no significant differences between the effects of 2 g/day and 3 g/day of sitostanol.

The study was well-designed, conducted and controlled and the numbers in each group were substantial. One year is much longer than is normally devoted to most nutritional studies of this type and the study involved some painstaking work. The content of the plant sterol in the margarine fat was considerable (20%) and the authors were wise to monitor for possible side-effects of the treatment. None were observed.

Apparently, the subjects could distinguish the taste of the two products, thereby to some extent invalidating the claim that the study was ‘double-blind’. As against this, it was claimed that the subjects could not decide which was the preferred taste, so that the difference did not influence total intake of either of the margarines.

It is fair to ask whether the background diet was well enough controlled: the subjects were effectively consuming their habitual diets with the exception of the 'control' or 'treatment' margarine. Food intake was not monitored until 9 months into the trial and again at 14 months when the subjects had been back to their normal diets again for two months. This provided evidence that there were minimal differences between subgroups in terms of dietary total fat, fatty acids or cholesterol intakes at these times. No data were presented for the consumption of other nutrients, some of which might have had effects on blood cholesterol (animal/vegetable protein, fibre among others). It would have been interesting and perhaps important to have been told how much natural plant sterols were being consumed in the food. However, as against these arguments, the authors could reasonably claim that this was a blinded controlled experiment that had a positive outcome so that criticisms about the composition of the rest of the diet might be academic.

The margarine (both 'control' and 'treatment') contributed 24 g per day of fat to the diet. Supposing the total fat consumption to be 80–100 g/day (we are not told exactly how much in grams was consumed — only that it represented 35% of energy), this is about a quarter of fat intake. Subjects were taught to replace part of the fat in their diet with this product but there was no indication of how they did this or what fat was actually replaced. The 'treatment' groups received a maximum of 3 g/day of the plant-derived sterol — about ten times as much as in normal diets. Since the vegetable foods that supply plant sterols in the normal diet tend to have a low-fat content, it would be quite difficult, even for a vegetarian, to achieve anywhere near the intake of plant sterols from a normal diet as was consumed in this study. Moreover, it is known from previous studies that naturally-occurring plant sterols have less potent cholesterol-lowering ability.

The authors did not measure cholesterol absorption directly but they did measure the plasma concentration of campesterol, which is known to be directly related to cholesterol absorption. The decrease in plasma campesterol seen in subjects consuming the sterol ester margarine suggests that these subjects also absorbed less cholesterol than controls. In view of what is known about cholesterol homeostasis (see influence on cholesterol metabolism above), it is surprising that, if the only effect of the plant derived sterol was to inhibit cholesterol absorption, this in-built compensation mechanism did not operate to maintain blood cholesterol constant. Instead, plasma cholesterol fell. The subjects in this study may not have been good 'compensators' (i.e. the synthesis of cholesterol in the liver was insensitive to dietary cholesterol). As they were 'moderately hypercholesterolaemic', this may indeed have been the explanation. Alternatively, the plant derived sterol may have been exerting

its influence by an independent and as yet unknown mechanism and this deserves further research.

It is clear that we have neglected the plant sterols as potentially important dietary components and that much more needs to be known about their physiological effects. It is well known that whereas blood cholesterol responds to simple dietary changes (e.g. the ratio of *n*–6 PUFA to saturated fatty acids) under well-controlled conditions in metabolic experiments, it does not appear be influenced very much by dietary changes in 'real life'. It may be that normal mixed diets contain many components (some of which, like the plant sterols, are not nutrients) that influence blood cholesterol in either direction, thereby damping down or obscuring the effects of changing dietary fatty acid composition.

When I first wrote the article in *Lipid Technology* on which this section is based, I expressed the opinion that the results presented here, though important and interesting, may have more pharmaceutical than nutritional relevance. Since then, however, a margarine called Benecol based on the experimental one described above has been launched by Raisio in Finland and has become enormously successful. Unilever is about to launch a margarine called Take Control containing soybean sterol ester. Both products have passed review by the US FDA. No doubt other products will follow. There may be interest by food manufacturers in fortifying or supplementing other foods with plant derived sterols, thereby extending the concept of functional foods and the range of those foods available.

References

1. Ministry of Agriculture, Fisheries and Food (1994) Annual Report of the National Food Survey Committee. Her Majesty's Stationery Office, London.
2. Rhee, K.S. (1992) In: Fatty Acids in Foods and their Health Implications (C.K. Chow, ed), Marcel Dekker, New York, pp.65–93.
3. Watts, G.F. *et al*. (1988) *British Medical Journal*, 296, 235–237.
4. Wood, J.D. (1984) In: Fats in Animal Nutrition (J. Wiseman, ed), Butterworths, London, 407–435.
5. McDonald, I.W. and Scott T.W. (1977) *World Review of Nutrition and Dietetics*, 26, 144–207.
6. Sieber, R. (1993) *Lebensmittel Wissenschaft und Technologie*, 26, 375–387.
7. McNamara, D.J. (1990) *Advances in Meat Research*, 6, 63–87.
8. Pariza, M. *et al*. (1996) *FASEB Journal*, 10, 3227.
9. Freckleton A M *et al.* (1989) In: The Human Food Chain (C.R.W. Spedding, ed.) Elsevier Applied Science, London, pp.17–57.

10. Department of Health and Social Security (1984) Report on Health and Social Subjects, 28. Her Majesty's Stationery Office, London.
11. Gurr, M.I. *et al.* (1989) *Nutrition Research Reviews*, 2, 63–86.
12. Ferro-Luzzi, A. *et al.* (1984) *American Journal of Clinical Nutrition*, 40, 1027.
13. Gurr, M.I. (1989) In: Fermented Milks and Health, NIZO, Ede, The Netherlands, pp.77–87
14. Agerbaek, M. *et al.* (1995) *European Journal of Clinical Nutrition*, 49, 346–352.
15. Richellsen, B. *et al.* (1996) *European Journal of Clinical Nutrition*, 50, 811–815.
16. Gilliland, S.E. (1989) *Journal of Dairy Science*, 72, 2483.
17. Gibney, M.J. and Burstyn, P.G. (1980) *Atherosclerosis*, 35, 339.
18. WHO Study Group (1990) Diet, Nutrition and the Prevention of Chronic Disease. World Health Organization Technical Report Series 678, World Health Organization, Geneva.
19. Mela, D.J and Raats, M. (1995) In: Advanced Dairy Chemistry, Volume 2: Lipids, 2nd edition, (P.F. Fox ed.) Chapman and Hall London pp.403–432.
20. Gurr, M.I. and Harwood, J.L. (1991) Lipid Biochemistry: An Introduction, 4th edition, Chapman and Hall, London.
21. Parodi, P.W. (1996) *Australian Journal of Dairy Technology*, 51, 24–32.
22. Parodi, P.W. (1994) *Australian Journal of Dairy Technology*, 49, 93–97.
23. Kivinen, A. *et al*. (1992) Milchwissenschaft, 47, 694–696.
24. Heiferman, A. *et al*. (1981) *Israel Journal of Dental Medicine*, 29, 61–63.
25. Williams, R.J. *et al*. (1973) *Proceedings of the National Academy of Sciences (USA)*, 70, 710–713.
26. Kepler, C.R. *et al*. (1966) *Journal of Biological Chemistry*, 241, 1350–1354
27. Fogerty, A.C. (1988) *Nutrition Reports International*, 38, 937–944.
28. Chin, S.F. *et al*. (1992) *Journal of Food Composition and Analysis*, 5, 185–197.
29. Ip, C. *et al*. (1994) *Cancer Research*, 54, 1212–1215.
30. Pariza, M.W. and Hargraves, W.A. (1985) *Carcinogenesis*, 6, 591–593.
31. Ha, Y.L. *et al*. (1987) *Carcinogenesis*, 8, 1881–1887.
32. Huang, Y-C. *et al*. (1994) *Nutrition Research*, 14, 373–386.
33. Lee, K.N *et al*. (1994) *Atherosclerosis*, 109, 19–25.
34. Van Aerde, J.E. and Clandinin, M.T. (1993) *Canadian Journal of Physiology and Pharmacology*, 71, 707–712.
35. Forsyth, J.S. (1998) *Nutrition Research Reviews*, 11, 255–278.
36. Goedhart, A.C. and Bindels, J.G. (1994) *Nutrition Research Reviews*, 7, 1–23.
37. Jensen, R.G. (1996) *Progress in Lipid Research*, 35, 53–92.
38. British Nutrition Foundation (1992). Report of the Task Force on Unsaturated Fatty Acids. Chapman and Hall, London.
39. Cruz, M.L.A. *et al*. (1994) *Pediatric Research*, 35, 135–140.
40. Filer, L.J. *et al*. (1969) *Journal of Nutrition*, 99, 293–298.

41. Quinlan, P (1996) *Oils–Fats–Lipids 1995: Proc. 21st World Congress of the Int. Soc. for Fat Research*, The Hague, 1995 (W.A.M.Castenmiller, ed.). P.J.Barnes and Associates, Bridgwater, pp.587–592.
42. Widdowson, E.M. *et al*. (1975) *British Medical Journal*, 1, 653–655.
43. Carlson, S.E. *et al*. (1992) *American Journal of Clinical Nutrition*, 58, 35–42.
44. Department of Health (1991). Dietary Reference Values for Food Energy and Nutrients for the United Kingdom. Report on Health and Social Subjects, 41, HMSO, London.
45. Christie, W.W. (1978) *Progress in Lipid Research*, 17, 111–205.
46. Gunstone, F.D. *et al*. (eds) (1986) The Lipid Handbook, 1st edition, Chapman and Hall, London.
47. British Nutrition Foundation (1990) Complex Carbohydrates in Foods, Chapman and Hall, London.
48. Van Itallie T B and Kachadurian A K (1969) *Science*, 165, 811.
49. Grundy, S.M. and Denke, M.A. (1990) *Journal of Lipid Research*, 31, 1149–1172.
50. Sickinger, K. (1975) In: The Role of Fat in Human Nutrition (A.J. Vergroesen, ed.) Pp.115–209 Academic Press, London.
51. Babayan V K (1974) *Journal of the American Oil Chemists' Society*, 51, 260.
52. Sundram, K. *et al*. (1992) *British Journal of Nutrition*, 68, 677–692.
53. Wood, R. *et al.* (1993) *Journal of Nutritional Biochemistry*, 4, 286–297.
54. Truswell, A.S. *et al.* (1992) *Nutrition Research*, 12, S43–S52.
55. Ng, T.K.W. *et al.* (1992) *Journal of the American College of Nutrition*, 11, 383–390.
56. Hornstra, G. *et al*. (1991) *Atherosclerosis*, 90, 91–93.
57. Qureshi, A.A. *et al.* (1986) *Journal of Biological Chemistry*, 261, 10544.
58. Vorster, H.H. *et al.* (1997) *Nutrition Research Reviews*, 10, 115–135.
59. Hornstra, G. (1988) *Oleagineux*, 43, 75.
60. Sylvester P W *et al.* (1986) *Cancer Research*, 46, 757.
61. Sundram, K. *et al.* (1989) *Cancer Research*, 49, 1447–1451.
62. Gurr, M.I. (1998) *Lipid Technology*, 10, 63–65.
63. Miettinen, T.A. *et al*. (1990) *American Journal of Epidemiology*, 131, 20–31.
64. Gregory, J. *et al*. (1990) The Dietary Survey of British Adults. Her Majesty's Stationery Office, London.
65. Miettinen, T.A. *et al*. (1994) *Atherosclerosis*, 105, 217–226.
66. Gylling, H. *et al*. (1994) *Diabetologia*, 37, 773–780.
67. Miettinen, T.A. *et al*. (1995) *New England Journal of Medicine*, 333, 1308–1312.