DIETARY LIPIDS AND CORONARY HEART DISEASE: OLD EVIDENCE, NEW PERSPECTIVE

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I. INTRODUCTION

It is not in doubt that coronary heart disease (CHD) represents a major, often the major, cause of death and ill-health in developed industrialized countries. In the U.K., CHD accounts for about one third of male and a quarter of female deaths. While the peak age of death from CHD is 70-74 for men and 75-79 for women, particular concern has been expressed that it is principally a cause of premature death especially in middle aged men, with grave social and economic consequences. Thus in 1988, 8% of all male deaths occurred before the age of 65 years due to CHD. While it is likely that as yet ill-defined inherited factors predispose individuals to premature heart disease, a consensus regards environmental factors as more important, although the variety of factors that has been suggested to be involved is bewildering. Of these, diet, smoking habits and physical activity have received most attention as being potentially subject to modification. Given

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ABBREVIATIONS

ADP—Adenosine diphosphate
AHA—American Heart Association
apo—apolipoprotein
CHD—Coronary heart disease
DHA—Docosahexaenoic acid
EPA—Eicosapentaenoic acid
FH—Familial hypercholesterolaemia
HDL—High density lipoprotein
HMG-CoA—Hydroxy methylglutaryl-CoA
IHD—Ischaemic heart disease
LDL—Low density lipoprotein
LRC-CPPT—Lipid Research Clinics Primary Prevention Trial
MI—Myocardial infarction
MRFIT—Multiple Risk Factor Intervention Trial
MUFA—Monounsaturated fatty acids
PAI—Plasminogen activator inhibitor
POSCH—Program on the Surgical Control of the Hyperlipidaemias
P/S ratio—Ratio of polyunsaturated to saturated fatty acids
PUFA—Polyunsaturated fatty acids
SFA—Saturated fatty acids
TC—Total cholesterol
tPA—Tissue plasminogen activator
VLDL—Very low density lipoprotein
WHO—World Health Organization

Shorthand nomenclature for fatty acids: number before colon represents carbon chain length; number after colon represents number of double bonds.
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this background, it is not surprising that individual governments and international organizations have drawn up recommendations for the prevention of CHD. These may be similar to, but should be distinguished from, recommendations for the management of CHD in those already diagnosed as having the disease.

It is probably true to say that smoking and dietary modification aimed at controlling plasma lipid concentrations, have been the major targets of public education campaigns. This review is concerned only with dietary lipids but the reader is entreated to keep in mind three cautions that set dietary lipids in context. First, diet is but one of many environmental factors that might be targeted and there is controversy about its relative importance; second, dietary fat is but one of many dietary factors that might be implicated in the development of CHD; third, plasma lipids, although having been given overwhelming emphasis in the formulation of public policy, are not the only predictors of CHD that might be influenced by dietary lipids.

It is not the purpose here exhaustively to review the role of diet in influencing plasma lipoproteins or the evidence that various plasma lipoproteins confer risk for or protection from CHD. This has been well covered in other reviews. The reviewer holds the view that the roles of diet and of dietary lipids in particular, have been overemphasized; that the relationships between dietary lipids and CHD have been oversimplified; and that, as a result, much of public policy is at best inefficient and at worst misguided. This paper will begin by describing the main tenets of the "lipid hypothesis" (Section II) and reviewing the main evidence for it, relying extensively on excellent reviews by other authors rather than on original material (Section III). In Section IV, the weaknesses of the lipid hypothesis will be reviewed by extensive reference to original literature, and alternative and, perhaps, more productive research areas will be discussed.

This paper is mainly concerned with the science underlying our understanding of how lipids may be involved in the development of CHD rather than with public education designed to control the disease. Any truly comprehensive theory of the development of CHD must take account of certain well established characteristics of the disease and be able to explain them. Thus, CHD is more prevalent in males than females, at least until women reach their menopause, and in countries like the U.K., there are large differences in CHD rates between socially deprived and advantaged groups. Moreover, there are very large regional differences even within a country with a small land area like the U.K., as well as substantial differences between ethnic groups that are independent of geographical location. CHD prevalence is dependent on latitude, altitude and climate. Most importantly, there have been changes in CHD rates, increasing in developed countries in the early and middle part of the twentieth century to a peak and then declining. In other countries, including some in Africa and Eastern Europe, rates have been low until very recently when they started to climb dramatically.

The term "CHD" covers a complex set of contributory conditions. Failure to find a satisfactory all-embracing explanation may in part be due to failure to realize that any environmental factors being considered, for example, dietary fatty acids, influence these different aspects of the disease in different ways. Section II will, therefore, begin with a brief review of the nature of CHD.

II. THE LIPID HYPOTHESIS OF CORONARY HEART DISEASE

A. A Brief Description of Coronary Heart Disease and Contributory Vascular Diseases

1. Coronary Heart Disease

Coronary heart disease (CHD) is a condition in which the main (coronary) arteries supplying the heart are no longer able to supply sufficient blood, and therefore oxygen, to the heart muscle (myocardium), which may then quickly die. A heart attack (myocardial infarction) is caused by a thrombus forming and completely blocking the heart’s blood supply. The condition of oxygen starvation that damages, or kills the myocardium is
known as ischaemia so that coronary heart disease is often referred to as ischaemic heart disease (IHD). The terms CHD and IHD will be regarded as synonymous here.

The main cause of reduced blood flow is the accumulation of plaques mainly in the intima of the arteries, a disease known as atherosclerosis. This begins in early life and progresses to differing degrees in all peoples of the world over a lifespan. Thrombosis, in contrast, occurs rapidly and is most likely to be fatal when the arteries are already narrowed severely by atherosclerosis.

2. Atherosclerosis

Atherosclerosis is a disease of arteries characterized by local thickening of the inner coat or intima, leading to irregular narrowing of the blood vessels (stenosis), which may restrict the flow of blood, and by a reduction in elasticity of the vessels (arteriosclerosis).

The complex layering of elastic and muscle tissues in normal blood vessels enables the vessels to constrict and also to relax in time with the pulse wave as blood is pushed along the arteries by the activity of the left ventricle of the heart or by the right ventricle in the case of the arteries supplying the lungs. The outer layer of the blood vessel consists of connective tissue and adipose tissue layers that contain a network of small blood vessels and lymphatic channels which partially penetrate into the outer elastic-muscle tissue layer.

In a vessel diseased by atherosclerosis, there is marked thickening of the intimal layers (i.e. those closest to the arterial lumen and the blood). The material that causes the thickening of the initial layers is known as atherosclerotic plaque. The plaque is rarely evenly spread over arteries but occurs at focal points. The composition of a plaque is often discussed in terms of two main characteristics: (1) a proliferation of smooth muscle cells and connective tissue and (2) an accumulation of lipid in "foam cells", so-called because under the microscope they have a foamy appearance due to the presence of numerous small lipid droplets. An accumulation of foam cells is generally described as a "fatty streak" and is generally believed to be one of the earliest recognizable facets of atherosclerosis. They are found from childhood onwards but may not always be the precursors of mature established lesions.

Plaque that has developed over a long period of time is, however, more complex than suggested by this description. Because the early stages of atherosclerosis involve a response to some kind of injury to the arterial intima, the components of the plaque are characteristic of damaged tissues. Thus, in addition to the lipid-filled foam cells, connective tissue and smooth muscle cells, there are deposits of calcium, thrombi, ulcerations, cell debris and ceroid, an intractible or basically inert material resulting from lipid peroxidation. The composition of plaque changes with age and as the material expands to narrow the artery.

The cellular tissue (including the foam cells) is more predominant in younger patients but the lesions are dominated by dense fibrous and calcified tissue with increasing age. As the artery becomes narrower, the proportion of cellular fibrous tissue decreases while that of foam cells and dense fibrous tissue increases.

The question of how atherosclerosis is initiated is one of continuing research and a subject of some controversy. Better understanding of this question will be crucial to understanding whether lipids play an initiating or a facilitating role. Although Steinberg and Witztum proclaim that "... there is no longer any doubt about the causative relationship between hypercholesterolaemia and premature atherosclerosis", not all would concur and there is much cause for argument about the interpretation of the word "causal".

In general, there have been two major hypotheses: (1) the "response-to-injury" and (2) the "monoclonal" hypotheses. In the latter, it is proposed that a single cell mutates under the influence of viruses or chemical agents to produce what is virtually a tumour. The response-to-injury hypothesis probably has the greater support and proposes that the initiation of atherosclerosis involves injury to the arterial intima. This could theoretically be caused by chemical factors, for example, the products of smoking which contain high concentrations of free radicals, or by antibodies formed in an immunological
reaction, perhaps to an infection, or normal blood constituents such as lipoproteins. Alternatively the damage could be mechanical. Stehbens et al. argue for the involvement of turbulent blood flow which causes arterial fatigue and argue against a major initiating role for plasma LDL or a modified form of LDL.

Whatever the precise initiating factor, during the early development of atherosclerosis, various events are concurrently taking place which are essentially an attempt to repair the initial damage to the intima. Monocytes from the blood adhere to the sites of injury, migrate into the intima and become activated to form macrophages. These cells have surface receptors called "scavenger receptors" that take up LDL particles that have been modified by peroxidation but do not take up native, unmodified LDL.

As the lesion develops, the macrophages become engorged with lipid to become foam cells. The characteristic lipid of these cells is cholesteryl ester containing predominantly linoleic acid (intracellular lipid). The developing lesions become complicated by the accumulation of extracellular lipid which is also cholesteryl ester, but its characteristic fatty acid is oleic acid. This comes from plasma LDL which becomes immobilized with fibrin in the lesion.

Another event, caused by the release of various growth factors, is the proliferation of smooth muscle cells. Although these contain some lipid, they are not the main precursors of foam cells as was once thought. The proliferating smooth muscle cells secrete mucopolysaccharides and the connective tissue proteins collagen and elastin in the lesion. Necrosis of cells within the lesion, including the foam cells gives rise to the necrotic lipid-rich core of the advanced lesions. Complications leading to thrombosis (see Section II. A.3) may or may not follow.

While there are clearly many steps in the biochemical and pathological progression of the disease, in terms of its clinical outcomes, atherosclerosis may be seen as developing in two stages: (1) a quiescent stage lasting many decades, that is asymptomatic; (2) an active stage at which complications become manifest pathologically and clinically and which involve haemorrhage and tissue ischaemia.

3. Coronary Thrombosis

The endothelial surfaces of blood vessels that are exposed to the blood are subject to continual wear and tear. The body has developed a complex repair system in which various elements of the blood associate together to form a thrombus that seals the wound. Briefly, wound healing is effected by: (1) the aggregation of platelets and (2) by the formation of a clot composed of strands of the protein fibrin. The normal undamaged endothelial surfaces of the blood vessels inhibit the induction of the blood clotting "cascade" that ends in the conversion of fibrinogen to fibrin. As soon as damage occurs to the endothelium, a specific cell membrane protein, "tissue factor", which is normally concealed from the blood, is exposed. This then interacts with factor VII to trigger the cascade that ends in a wound-sealing thrombus, incorporating various proteins. Endothelial damage also triggers the platelet aggregation system. Interaction of collagen with its receptor on the platelet membrane is an early step in the induction of aggregation. Arachidonic acid, esterified in membrane phospholipids is released by phospholipase A2, and converted into thromboxane, that stimulates aggregation. It also constricts the blood vessel to assist clotting, thus assisting in anchoring the developing thrombus.

The associated fibrinolytic pathway exists essentially to dissolve the fibrin in a thrombus and is effected by plasmin, which is normally present in the inactive form plasminogen. The inactive zymogen has to be activated by proteinases termed tissue plasminogen activators (tPA). Plasma also contains many proteinase inhibitors including plasminogen activator inhibitors (PAI-1 etc.). Complex interplay between these various factors keeps the fibrinolytic system in check. Thus, in the normal physiological state, once a thrombus has performed its task and the wound has been repaired, there is a mechanism for dissolving the thrombus so that it can cause no partial or complete obstruction. A defect in one or more of these processes can result in a thrombus detaching itself and, if it blocks
a coronary vessel, precipitating a myocardial infarction. Davies and Thomas demonstrated, by carefully conducted autopsy studies, that obstruction of the coronary artery is involved in three-quarters of cases of sudden cardiac death, although there are disagreements about this. The potential importance of coronary thrombosis in coronary heart disease mortality has at times been under-emphasized because standard autopsy techniques do not detect even a majority of thrombi in coronary arteries. However, the inability to detect a thrombus in cases of sudden cardiac death does not necessarily mean that a thrombus did not occur, since it may have been dissolved by the time an autopsy was carried out.

A combination of factors may influence the likelihood of thrombus formation, including: alterations in the character of the blood and of particular blood proteins; disturbances in blood flow (particularly the development of turbulence) and damage to, or alteration in, function of the endothelial cells. The turbulent blood flow itself, and sometimes alterations to the composition of the blood, may cause actual injury to the epithelium. Increased numbers of platelets and increased concentrations of activated aggregation and coagulation factors are found in places where blood flow separates and forms vortices. Fast flowing blood will dislodge platelets from the surface of the thrombus and the aggregatory factors will be diluted so that, unless flow is slowed or arrested in these vessels, the likelihood of the formation of a thrombus big enough to block an artery is reduced. Connective tissue is important in the initiation of thrombi in injured and healthy arteries. Rupture of atherosclerotic plaque frequently leads to formation of an occlusive thrombus in a coronary artery.

Modern concepts of the development of atherosclerosis and thrombosis postulate a degree of interrelationship between the two processes. Blood lipids and tissue lipids are involved in several ways. The influence of dietary lipids on the activation of the blood coagulation and platelet aggregation systems is discussed in Section IV.B.2.

4. The Concept of Risk Factors

The concept that certain physiological characteristics of individuals might have predictive value stemmed from the work of Ancel Keys and others in the late 1940s and early 1950s and has been reviewed by Paul. These predictive variables became rather loosely known as “risk factors” and the term gradually came to be used for not only physiological and biochemical characteristics of individuals but also aspects of lifestyle, such as smoking habits, behaviour patterns, and dietary characteristics. Several very powerful “risk factors” that are highly predictive of later CHD are not susceptible to modification, including increasing age, masculinity and genetic predisposition to premature CHD. Consequently, most attention has been paid to those “risk factors” that are, or should be, readily susceptible to modification and these have been mainly: diet, plasma total cholesterol, blood pressure, and smoking habit. Of course, these risk factors are not entirely independent but have often been treated as if they were. Moreover, although their identification has given powerful impetus to preventive programmes, they are derived from statistical associations and, therefore, cannot clearly establish cause and effect relationships.

5. Summary

CHD is a complex and often ill-defined condition. It may be fatal, generally as a result of a myocardial infarction but a proportion of deaths are unexplained and recorded in publications as “(unexplained) sudden death”. Fatal myocardial infarctions and unexplained sudden deaths contribute to the greater part of statistics for CHD mortality. The disease may not, however, be fatal. It may be recognized in the form of symptoms such as angina pectoris, non-fatal myocardial infarctions, and so-called ventricular fibrillation (irregular electrical activity of the heart). These manifestations are often grouped together and recorded in the literature as “coronary events”. It is also important
to recognize that much vascular and heart disease may be completely asymptomatic and may, therefore, progress over a number of years without anyone having any knowledge of it. This may be particularly true of atherosclerosis and of degeneration of the myocardium.

The importance of these remarks lies in the problem of trying to interpret scientific evidence concerning the relationship between diet and CHD when in reality the disease is so ill-defined. Understanding and interpreting relationships between diet and atherosclerosis or thrombosis are more straightforward since these specific diseases are more easily defined and categorized. However, the literature on human coronary disease is rarely concerned with these specific well-defined facets but rather it relates to symptoms or end-points that may have quite different and certainly ill-defined aetiologies. Moreover, CHD and atherosclerosis are sometimes either treated as synonymous or there is confusion about which condition is being described. Furthermore, the thrombotic phase of the disease has been under-researched. This may have obscured or confused the true role of dietary lipids.

B. Description of 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 in animals given diets rich in cholesterol. Among the observations were that the animals developed high concentrations of plasma lipids. It was not until the early 1950s that wide interest grew in the effects of different dietary fats on plasma cholesterol in experimental animals and man. Much of this early work is reviewed by McGandy and Hegsted but the interested reader is encouraged to go back to some of the original classical papers. In addition to this experimental work, Keys and his colleagues were engaged in the classic epidemiological investigations of the Seven Countries Study that produced cross-cultural evidence for associations between dietary saturated fatty acids (SFA), plasma cholesterol and CHD mortality. Together this early work gave rise to the lipid hypothesis.

For the purposes of this paper, the lipid hypothesis will be reviewed as comprising four tenets:

1. Diets containing a high fat/saturated fatty acid/cholesterol content lead to high concentrations of cholesterol (particularly LDL-cholesterol) in plasma.

2. A high plasma cholesterol (particularly high LDL-cholesterol) presents a high risk for coronary heart disease (CHD) and leads to a high CHD morbidity and mortality.

3. Reducing the amount of fat/saturated fatty acids/cholesterol in the diet will result in a reduced concentration of cholesterol (particularly LDL-cholesterol) in plasma.

4. Reducing the concentration of cholesterol (particularly LDL-cholesterol) in plasma will result in a lower risk of CHD and eventually a lower morbidity and mortality.

Different authors pose the lipid hypothesis in different ways. Some concentrate on the effects of dietary lipids on blood cholesterol; of these, some will emphasize total fat, others saturated fatty acids and still others cholesterol. Others will emphasize the relationship between high blood lipids and coronary disease, irrespective of the aetiology of the high blood lipids. This paper investigates the all-embracing view expressed by the above four tenets.

In describing the hypothesis in this way, it appears that relationships are simple. This is not so. The four tenets set out above give little hint of the complex relationships between blood lipids and total dietary fat, saturated, monounsaturated, trans and polyunsaturated fatty acids and dietary cholesterol. They do not consider whether it is the absolute intake of these dietary components that is important, the ratio between the different fat components, or between fat and other energy-providing constituents of the diet. Neither do they make sufficient distinction between the types of plasma lipoproteins, of which cholesterol is a constituent. Finally, the concepts embodied in the lipid hypothesis date from an era when attention was concentrated almost entirely on the atherosclerotic
component of CHD and the role of lipids in contributing to atherosclerosis. It did not consider whether lipids might play a role in the thrombotic episode.

Section III will review briefly the evidence for the four tenets of the lipid hypothesis. I will concentrate on recent research that has tended to modify or confirm previous views, referring to other reviews for the older, more established literature.

### III. EVIDENCE FOR THE LIPID HYPOTHESIS

#### A. Tenet 1: Dietary Lipids Influence Plasma Cholesterol

1. **Dietary Cholesterol**

   Addition of cholesterol to the diets of many species of experimental animals elicits a rise in plasma cholesterol concentration. Rabbits are particularly sensitive and so are many types of monkeys while rats are relatively insensitive. The relevance of these animal experiments to an understanding of human physiology is highly questionable.

   The influence of dietary cholesterol on plasma concentration in man is in general less pronounced than in other primates. Many carefully supervised experiments with subjects in metabolic wards have demonstrated small but significant rises in plasma cholesterol in response to dietary cholesterol. In contrast, studies with "free-living" subjects seem to have shown little or no effect.

   Pure cholesterol added to diets had no influence on human plasma cholesterol concentration in man. Experimenters have subsequently relied on supplementing the diet with eggs because of their very high content of cholesterol (about 270 mg per egg). While some experiments conducted under well controlled conditions showed modest (10–20%) rises with one or two eggs when compared with a controlled diet of low cholesterol content, others found rises of less than 10%. Yet others found similar plasma cholesterol concentrations in men ingesting as many as 11 or as few as 2 eggs per week.

   Certain individuals respond strongly and others weakly to dietary cholesterol (hyper- and hypo-responders). This phenomenon is seen in a variety of animals and in man and is discussed in greater detail in Section IV.A.2.

   McNamara has analysed the results of 68 clinical trials representing 1490 subjects. A mean increase of 2.3 (SD, 0.2) mg/dl plasma cholesterol (0.059 mM) was observed for every 100 mg per day increase in dietary cholesterol. It should be stressed that such meta-analyses gives mean results for a large number of individuals and that predictive formulae, such as those of Keys et al. and Hegsted et al. hold only for group or population means, not for individuals.

   Some epidemiological observations also suggest that there is a linear association between dietary and plasma cholesterol. However, because foods that are rich in cholesterol also tend to have a high proportion of saturated to unsaturated fatty acids, it is difficult to distinguish clearly between the two influences. In this review, I shall use the word "association" when data are from epidemiological studies in which other confounding factors could be present and reserve the word "relationship" for situations when a clear cause and effect relationship is apparent.

2. **Saturated Fatty Acids**

   Although early research did indicate that not all saturated fatty acids were equivalent in their cholesterol-raising effects, the use of a single all-embracing term for saturates in the Keys equation has tended to obscure this fact and it is only recently that serious attention has been given to these differential effects. Early work indicated that fatty acids with chain lengths up to and including 10 carbon atoms do not influence plasma cholesterol 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". Lauric (12:0), myristic (14:0) and palmitic...
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(16:0) acids have 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. Early work found roughly equal effects of lauric, myristic and palmitic acids or effects in the order myristic > palmitic > lauric. Recently, the differential cholesterolaeemic effects of SFA of different chain length have been re-evaluated as discussed in Section IV.A.2.

Cross-cultural epidemiological studies have generally demonstrated correlations between the average consumption of saturated fatty acids and the mean plasma total cholesterol concentration. Thus, the Seven Countries Study of Keys et al. indicated a high correlation \( r = 0.87 \) between the percentage of total calories from saturated fatty acids and plasma total cholesterol. The finding that weak or absent correlations between individual dietary intakes, plasma lipoprotein concentrations and CHD risk within populations is explained by Blackburn as being mainly due to the weak and variable measures with which we attempt to characterize an individual's diet, and to the vagaries in blood lipoprotein levels and their measurement.

3. Monounsaturated Fatty Acids

Keys et al. found that monounsaturates were "neutral" in their effect on plasma cholesterol and did not include a term for them in their equation. Recently, this question has been re-evaluated. Broadly, two types of experiments have been conducted. In the first type, diets of equal (and relatively high) fat content have been compared, differing only in the fatty acid composition, with either saturates, monounsaturates or n-6 polyunsaturates predominating. In the second type, carbohydrates have been substituted with fat rich in monounsaturates so that a high fat monounsaturate diet has been compared with a low fat diet. While not all studies were well controlled, most found that monounsaturates, when substituted for saturates, lowered plasma total cholesterol concentration as effectively as n-6 polyunsaturates, although the findings were not entirely consistent. The lowering was almost entirely associated with the LDL fraction. When substituted for carbohydrates, they resulted in a similarly low plasma cholesterol (LDL) but did not elicit the rise in VLDL (and, therefore, triacylglycerols) often seen with high carbohydrate diets.

4. Fatty Acids with trans Unsaturation

The digestion, absorption, transport and catabolism of fatty acids containing trans unsaturation differs little from their cis counterparts. However, because of certain similarities in properties to the saturated fatty acids, namely the "straight" shape of the molecules, enabling the lipids to pack together in crystalline array, their higher melting points and their tendency to be esterified in position 1 of membrane phospholipids, it has been suggested that, for purposes of dietary advice, they should be treated like saturated acids. There have been few studies to evaluate the effects of these fatty acids on plasma cholesterol in man and these have been contradictory. The view that trans fatty acids do not have an hypercholesterolaemic effect has been modified somewhat since the publication by Mensink and Katan. In this study, in which fat provided 40% of energy, the inclusion of trans-18:1 as the predominant fatty acid (11% of energy; 14 g/day) and a roughly equal quantity of cis-18:1 resulted in significantly higher total and LDL-cholesterol than a corresponding diet in which all the monounsaturated fatty acids were in the cis-configuration. The elevation of total plasma cholesterol by the "trans diet" was significantly less than promoted by a diet in which saturated fatty acids predominated (20% of energy). From the limited data presented on pre-trial plasma lipid concentrations, it is apparent that while cis-monounsaturates slightly lowered plasma total cholesterol (4.75 mm pre-study to 4.46 mm at the end), the trans-unsaturates had no effect (4.75 mm pre-study to 4.72 mm at the end). On this basis, trans fatty acids have no particularly
striking hypercholesterolaemic properties, although the conclusions must be limited because a control for the subjects' habitual diet was not included in the study. Whereas both saturates and cis-monounsaturates raised plasma HDL-cholesterol, the trans diet caused a lowering of this fraction. More research is needed to confirm this observation and to investigate the effects of chain lengths other than 18 carbons.

5. Polyunsaturated Fatty Acids

Just as the term "saturates" embraces a wide range of structures each with different physiological activities, so the term "polyunsaturates" is equally broad and non-specific. An enormous number of studies has left little doubt that a major effect of consuming n-6 polyunsaturated fatty acids, in substitution for saturated fatty acids, is a lowering of plasma cholesterol, principally the LDL fraction. There is little effect on HDL-cholesterol provided that the contribution of linoleic acid is not much more than 12% of dietary energy or the ratio of polyunsaturated to saturated fatty acids (P/S) is not much more than 1.0. These conditions are unlikely to occur in most self-selected diets in developed countries. There is also little effect of exchanging n-6 PUFA for SFA on VLDL. The experiments that have led to these conclusions have been conducted with diets containing relatively large proportions of energy as fat: between 30 and 40% of energy. The fact that these effects are similar to those obtained by substituting monounsaturated for saturated fatty acids suggests that the effect may be due more to a reduction in saturates intake than an increase in unsaturates.

In contrast, the effect of dietary n-3 fatty acids is to reduce the concentration of VLDL and since the major lipid component of these lipoproteins is triacylglycerol, the chief response is a lowering of plasma triacylglycerol concentrations. Only at very high intakes of fish oils is there a lowering of LDL or total cholesterol. The effect seems mainly confined to the very long-chain n-3 PUFA since linseed oil, which has a high content of 18:3n-3, is ineffective at similar doses.

Just as there are wide differences in individual responses to dietary cholesterol, so there are hyper- and hypo-responders to dietary fatty acids. It is apparent that one can find subjects with consistently high or low responses but that total insensitivity is rare.

6. Proportion of Fat to Carbohydrate

A "high fat" diet is by definition a "low carbohydrate diet". International comparisons tend to show that plasma lipoprotein concentrations, in particular LDL, are lower in populations that on average consume diets with a low fat, high carbohydrate content. Early experimental work was more concerned with the type rather than the amount of fat. The careful studies of Hegsted et al. recorded similar concentrations of LDL on low-fat (about 22% energy as fat) and high PUFA (40% energy as fat) diets. However, during the substitution other variables were changed: for example, on the lower fat diet, the intake of dietary cholesterol was also reduced and although no details were supplied, the content of dietary fibre was probably increased. The presence of these confounding factors reduces the certainty that effects observed were specifically due to changes in total dietary fat level. Moreover, no careful dose–response experiments seem to have been done. The concentration of plasma LDL is similar when fat which is predominantly mono- or polyunsaturated is replaced by carbohydrate but lower when the substitution is with fat in which saturated fatty acids predominate. In at least one study in which carbohydrate was substituted for mainly saturated fatty acids, there was a greater lowering of LDL cholesterol than of apoB suggesting a reduction in the size but not the number of LDL particles.

Diets high in carbohydrate and low in fat tend to raise the concentrations of VLDL and, therefore, triacylglycerols in plasma. Once again, there are wide differences in the response of individuals, some people being much more sensitive to carbohydrate than
others. Plasma HDL-cholesterol and also apoA concentrations are lowered on a high carbohydrate diet suggesting a reduction in the total number of HDL particles.\textsuperscript{38}

7. 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.\textsuperscript{92} The way in which fatty acids are distributed may influence plasma cholesterol irrespective of the overall composition of the fatty acids.\textsuperscript{91} Thus linoleic acid is more hypocholesterolaemic\textsuperscript{235} and saturates more hypercholesterolaemic\textsuperscript{146} 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.\textsuperscript{38}

8. Mechanisms

Differences between individuals in their responses to dietary cholesterol might be accounted for by differences in: absorption of dietary cholesterol; cholesterol biosynthesis; output of LDL by the liver or in the receptor-mediated clearance of LDL from plasma; sterol and bile acid excretion from the body or the accumulation of cholesterol in body tissues.\textsuperscript{24,38} Differences between individuals in cholesterol absorption and in the capacity to regulate cholesterol biosynthesis to compensate for dietary intake clearly exist.\textsuperscript{152} 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\textsuperscript{38} 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.

Likewise, 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.\textsuperscript{24,38,204} Dietary fatty acid composition may influence: (1) the excretion of bile acids that occurs at each passage of the entero-hepatic circulation; (2) the production of cholesterol and of apoB-containing lipoproteins; (3) the catabolism of LDL; (4) the cholesteryl ester content of each LDL particle in the plasma.

The "hypercholesterolaemic" SFA appear to suppress the receptor-mediated clearance of LDL from plasma.\textsuperscript{38} 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\textsuperscript{41,105,245} and a slight rise in fractional catabolic rate.\textsuperscript{105}
B. Tenet 2: High Plasma Cholesterol Increases the Risk of CHD

1. Evidence from Experimental Animals

Animal experiments provided some of the first indications that fatty substances in the blood, the concentrations of which could be elevated by diets rich in fat and cholesterol, were associated with the development of atherosclerosis. As early as the mid-nineteenth century, Virchow\textsuperscript{253} expounded a theory that fatty substances in the blood were imbibed by the arterial wall leading to degenerative changes. Anitschkow\textsuperscript{5} described numerous experiments in which arterial lesions were produced in rabbits by giving them diets in which cholesterol was dissolved in vegetable oil. He wrote that "from the morphological point of view, arterial cholesterol atherosclerosis in rabbits is in many respects analogous to human atherosclerosis". Blackburn\textsuperscript{25} states that "lesions resembling the atherosclerotic plaque can only be produced by dietary manipulations of blood lipoproteins". A notable finding is the very wide variation between species in susceptibility to atherosclerosis induced by diet and the considerable variability in individuals within species.\textsuperscript{8,122} Nearly all the research is concerned with atherosclerosis, since it is unusual, although not unknown\textsuperscript{226} to find coronary heart disease and coronary thrombosis in animals. The considerable work on diet, blood lipids and atherosclerosis in animals has been extensively reviewed.\textsuperscript{8,108,122,195}

2. Evidence from Familial Dyslipoproteinaemias

Familial dyslipoproteinaemias are inherited disorders resulting in abnormally elevated or reduced concentrations of one or more plasma lipoprotein fractions. The commonest is familial hypercholesterolaemia (FH), an autosomal dominant disorder of lipoprotein metabolism characterized by mutations of the low density lipoprotein receptor resulting in an accumulation of low density lipoprotein cholesterol in the plasma.\textsuperscript{75} Heterozygous familial hypercholesterolaemia has been estimated to affect 1 in 500 of the British population.\textsuperscript{75} Most affected subjects remain undiagnosed.

Patients with FH are at greater risk of CHD than those with polygenic hypercholesterolaemia and one report has suggested that there is a 52% chance of fatal or non-fatal CHD by the age of 50 in men and 12%, in women.\textsuperscript{215} This and most other studies have been retrospective analyses but a recent prospective study found that FH was associated with substantial excess mortality in young adults but not in older patients.\textsuperscript{207} Many authors have described the extensive atherosclerotic lesions in persons with FH, whose LDL concentrations may be of the order of 10–15 mM in heterozygotes and up to 30 mM in homozygotes compared with 5–6 mM for the general population.\textsuperscript{75} These findings are said to provide the most persuasive evidence for a direct link between plasma LDL and CHD.

3. Evidence from Epidemiology

Epidemiological studies fall mainly into three categories: cross-cultural, within-country cohort, and migration studies. The most influential of the cross-cultural studies has been the Seven Countries Study of Keys \textit{et al.}\textsuperscript{113,115} which indicated a strong independent correlation between the consumption of SFA as a percentage of dietary energy and fatal CHD incidence.

Several cohort studies have shown that individuals with a high plasma cholesterol concentration at baseline are more likely to develop CHD over the next 10–20 years.\textsuperscript{15,81,212,246} Twenty or so prospective studies in different countries have now shown a strong graded relationship between plasma total cholesterol and CHD, occurring in both sexes and being independent of all other measured risk factors.\textsuperscript{137} Other studies of populations that have migrated from areas of low CHD incidence to areas where incidence is high have also supported a strong influence of plasma cholesterol on CHD.\textsuperscript{268}
C. Tenet 3: Reducing the Intake of Dietary Saturated Fatty Acids and Cholesterol will Reduce the Concentration of Plasma Cholesterol

1. Evidence from Experimental Animals

Numerous experiments with experimental animals indicate that replacing SFA by UFA or carbohydrates generally results in lower concentrations of plasma total cholesterol. Wide differences in responsiveness have been revealed, rabbits tending to exhibit an exaggerated response to SFA and cholesterol; dogs and rats a limited response. Even among non-human primates, there are large differences, some eliciting a large rise in plasma LDL in response to SFA and cholesterol, others being very resistant.

2. Evidence from Intervention Trials

Many individually controlled dietary studies have been conducted to investigate the effects of dietary fat modification on plasma lipid and lipoproteins in human subjects. Some of these were discussed in Section III.a and there have been numerous reviews. Briefly, replacement of SFA with cis-MUFA or n-6 PUFA reduces plasma total and LDL-cholesterol. For every 1% of energy of SFA replaced by linoleic acid, there is, on average, a reduction in plasma total cholesterol of 0.13 mmol (5 mg/dl). Intakes of up to 12% of energy as linoleic acid do not affect HDL-cholesterol but amounts of linoleic (but not oleic) acid in excess of this cause a reduction in HDL. Plasma total cholesterol can also be lowered by increases in linoleic acid up to 4% of energy without concomitant reduction of SFA but increases between 4 and 10% have little additional benefit.

The effects of n-3 PUFA are not comparable to those of the n-6 family. Long chain n-3 PUFA, such as the eicosapentaenoic and docosahexanenoic acids of certain fish oils, lower plasma triacylglycerols and VLDL concentrations but not LDL-cholesterol, while the precursor EFA, α-linolenic acid, does not have this effect at comparable doses. Many intervention trials that have been conducted to test the hypothesis that modifying dietary fat will reduce the incidence of CHD, have, of course, yielded information on the extent of plasma cholesterol and plasma lipoprotein modification. These have been influential in supporting arguments for the benefits of extensive changes in dietary fat in Western populations and have been reviewed in detail. While some trials were disappointing in the extent of cholesterol lowering achieved, in many others, reductions of between 13 and 16% were achieved over periods of one to five years. Adherence to diet is an important factor and is likely to be poor if the choice of desirable foods is reduced because some are regarded as “atherogenic”. In this regard, it has been demonstrated that meat can form a perfectly acceptable part of a cholesterol-lowering diet as long as the fat is carefully trimmed. Plasma cholesterol reductions of 9–18% were achieved by this means.

3. Evidence from Migration Studies

The most widely quoted study is the Ni-Hon-San study of Japanese who migrated to Honolulu and San Francisco. Those who migrated to California became taller, heavier, more obese and more sedentary. They ate more saturated fatty acids and cholesterol and consumed less alcohol and carbohydrates than their counterparts in Japan. In general, the Hawaiian Japanese had risk factor values and degree of risk intermediate between those in Japan and California. Age-adjusted death rates from CHD are consistent with an association between consumption of saturated fatty acids and cardiovascular events.

4. Trends in Food and Nutrient Consumption

Whereas longitudinal data are available in many countries to illustrate trends in the consumption of dietary fatty acids, corresponding data on time-related trends in risk
factors, particularly plasma cholesterol are scarce.\textsuperscript{17,25} The limited knowledge of trends in nutrient consumption, risk factors and CHD mortality will be discussed together in Section III.D.4.

D. Tenet 4: Reducing the Concentration of Plasma Cholesterol will Reduce the Risk of CHD

1. Evidence from Intervention Trials: Drugs

Most of the evidence relating modification of plasma lipids to changes in CHD incidence has come from intervention trials set up specifically to test the lipid hypothesis. Although this review is concerned principally with the effects of changing dietary lipids, it is necessary to consider the results of trials in which drugs were the lipid-lowering agents, since many claims for the validity of the lipid hypothesis rely to a great extent on the results of drug trials. 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 of subjects to the treatment is likely to be stricter. Results have been extensively reviewed.\textsuperscript{102,247} Briefly, it is claimed that these results have indicated that when the initial plasma cholesterol is in the upper range of the distribution, for every 1\% reduction in an individual's plasma total cholesterol, a 2\% reduction in CHD risk can be expected. The substantial reductions in plasma cholesterol and in coronary events, especially from the LRC-CPPT\textsuperscript{132,133,134} and Helsinki\textsuperscript{67} trials have been cited as powerful evidence for the benefits of aggressive policies to reduce plasma cholesterol in the general population.\textsuperscript{3,246} It has also been assumed that these results can be extrapolated to reduction in plasma total cholesterol achieved through dietary changes in lower risk men, women and the elderly.\textsuperscript{60,134}

2. Evidence from Intervention Trials: Surgery

This evidence is included here since, like the evidence from drug trials, it has been cited as providing powerful support for the idea that reduction of plasma cholesterol reduces the risk of coronary events significantly. The most influential study has been the Program on the Surgical Control of the Hyperlipidaemias (POSCH).\textsuperscript{33} Partial ileal by-pass surgery reduced plasma total cholesterol by 23\% ($P < 0.00001$) and LDL-cholesterol by 38\% ($P < 0.0001$), while HDL-C was 4\% higher ($P < 0.02$) compared with the control group. Coronary events (CHD deaths and non-fatal myocardial infarctions) were 35\% lower ($P < 0.001$) than in the control group. Disease progression as measured by coronary arteriography was reported to be significantly less in the surgical group.

Other evidence linking dietary fat, plasma lipids and atherosclerosis, comes from post-mortem examinations, which, in cross-cultural studies, reveal strong correlations between habitual dietary fat consumption and the frequency of advanced atherosclerotic lesions.\textsuperscript{25,148} Finally, lipid lowering, mainly by drugs, results in significant reductions in atherosclerosis as demonstrated by angiographic techniques.\textsuperscript{27}

3. Evidence from Intervention Trials: Diet

The results of the major primary\textsuperscript{43,48,62,66,101,168,185,246,257,265} and secondary\textsuperscript{35,129,191,192,197} trials have been extensively reviewed.\textsuperscript{102,189,247} In many, there were significant reductions in CHD deaths and especially non-fatal coronary events.

A distinction can be made between trials that employed dietary modification as the only treatment, of which there are few, and those in which diet was combined with modification of other risk factors including smoking,\textsuperscript{101,168,257,265} control of blood pressure\textsuperscript{168,257,265} and control of energy expenditure and or weight loss.\textsuperscript{265} Thus the most encouraging results in terms of the reduction in plasma total cholesterol and in coronary events came from the Oslo Study\textsuperscript{101} which also incorporated advice on smoking reduction so that the influence of diet alone remains uncertain.
Studies that employed diet alone have tended to achieve reductions in total plasma cholesterol mainly by exchanging n-6 PUFA for SFA to achieve P/S ratios that were approximately six-fold higher than in the average diet at the time of the studies. While total mortality was unaffected, coronary deaths plus non-fatal coronary events were significantly reduced. The Finnish hospitals study is especially noteworthy as being one of the few trials that has included women.

4. Trends in Food and Nutrient Consumption and in Risk Factor Levels

In several developed countries, CHD mortality increased to a peak in the middle of this century and then started to decline. The time at which the peak occurred and the rate of decline after the peak have differed considerably between countries. Thus, after an abrupt rise in CHD mortality in the U.S.A. after world war 2, there was a peak in 1968. Thereafter, there was a sharp decline. Similar events occurred in Australia, New Zealand, Canada, Israel and Finland, while in others, such as the U.K., the decline started later and was less steep. In some Eastern European and several developing countries the incidence, having been previously rather low, has now started to increase. Various authors have cited parallel changes in fat, particularly animal fat, consumption and changes in the P/S ratio. Thus Beaglehole and colleagues find that in New Zealand, where the decline in CHD has been particularly striking, “favourable national trends in food and tobacco consumption were estimated to account for up to 50% of the decline in mortality between 1968 and 1981". In the United States, Blackburn states that “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 United States, largely explainable by known changes in the composition of the diet during this period”. Such changes include a reduction in the consumption of animal fat and a steady increase in the P/S ratio.

IV. WEAKNESSES OF THE LIPID HYPOTHESIS

Those who claim a dominant role for dietary lipids in the genesis of CHD and who, on those grounds, advocate radical changes in consumption of dietary fats in countries where CHD is prevalent or showing signs of increasing, argue that these views are based on a consistency of evidence from (a) animal experiments; (b) epidemiological data; (c) patients with familial dyslipoproteinaemias and (d) intervention trials. These arguments have been summarized in the preceding section. This section will examine the evidence in each of these categories to test its strength. In general, the main weaknesses are concerned with: (a) insufficient correspondence between animal and human forms of the disease; (b) confounding factors in the epidemiological evidence as well as over-extrapolation of unrepresentative data; (c) lack of correspondence between the disease seen in cases of familial dyslipoproteinaemias and the generality of human atherosclerosis; (d) poor design or poor control of intervention trials as well as over-extrapolation of unrepresentative data and finally (e) deliberate disregard of evidence that does not fit with preconceived ideas.

The evidence in relation to tenets 1 and 2 will be discussed together since many studies consider these two aspects without making any clear separation, while other studies consider the direct link between dietary lipids and arterial disease without discussing the intermediate effects on plasma lipids.

A. Tenets 1 and 2: Dietary Lipids Influence Plasma Cholesterol, which in turn, is a Powerful Predictor of CHD

1. Evidence from Experimental Animals

Animal models are vital for establishing basic biological principles and mechanisms. Many problems and disadvantages of human experimentation are avoided because animals
can be studied under much more rigorously controlled conditions. Many strict impositions are ethically impossible or impractical with human subjects. It is simpler, with animals, to change one factor at a time and from a strictly scientific point of view, results should be much easier to interpret. By the same token, such rigorous control cannot possibly mimic the normal conditions of human life in which there may be important interactions between diet and other lifestyle factors. In particular, physical exercise is rarely controlled and there is increasing evidence that this may have profound effects on plasma lipoprotein profiles (see Section IV.A.2).

(a) Dietary lipids and plasma lipoproteins. In terms of dietary influences on plasma lipoproteins, there are wide differences between animals in respect of lipoprotein profiles and lipoprotein metabolism.\textsuperscript{17} It could be argued that much of the work with rats, which comprises a large proportion of the literature, is irrelevant because of the relative lack of response of plasma LDL in these animals to dietary SFA and cholesterol. Thus, to measure an effect in rats it has usually been necessary to give quantities of cholesterol that bear no resemblance to those consumed by man and to add cholic acid to the diet.\textsuperscript{24} In man and in many other animals, the apoB/E receptor plays a major role in regulating the rate of removal of LDL from plasma as well as its rate of synthesis.\textsuperscript{76,77,96} However, there may be major differences between man, in which LDL receptor activity is generally low and in whom, therefore, LDL cholesterol concentrations are primarily determined by rates of synthesis, and many animals in which rates of removal may be paramount.

The main lesson from more recent studies on non-human primates has been the very wide range of response in plasma lipoproteins to changes in dietary fatty acid and cholesterol composition and this provides a useful model for the wide variability in human responses.\textsuperscript{24,98,108}

In summary, although use of experimental animals has been crucial to our knowledge of basic biochemical pathways, differences in some aspects of lipoprotein metabolism have largely been ignored by those who use animal experiments as supporting evidence for the lipid hypothesis. Major differences in living conditions that may interact with diet to influence plasma lipoprotein profiles have also largely been ignored.

(b) Dietary lipids, blood lipids and atherosclerosis. Atherosclerosis is not exclusively a human disease; it affects other animals as well. It may occur spontaneously in reptiles, rats, rabbits, cats, pigs, dogs, sheep, bears, cattle, horses, elephants, primates and wild as well as domestic birds, despite the fact that many of these animals are predominantly vegetarian.\textsuperscript{122}

A major difficulty arises in terms of assessing the relevance of animal experiments to man\textsuperscript{108,122,195,236} and the appropriate “animal model” has to be chosen with care. Rabbits given cholesterol in the diet have a high plasma LDL and exhibit severe arterial lesions.\textsuperscript{122} However, rabbits do not normally consume cholesterol and the amounts given have usually been many times greater than those ever consumed by man. Moreover, some pathologists have argued that the lesions that develop under such conditions differ from the complex lesions of human atherosclerosis.\textsuperscript{122,221} The lipid-containing lesions in these experimental animal models are xanthomatous and differ topographically, macroscopically, microscopically and electron microscopically from the spontaneous atherosclerosis in man and other animals\textsuperscript{221} and compare closely with the type of pathology seen in human beings with familial hypercholesterolaemia (Section IV.A.3).

Thickening of the intima occurs at sites of particular turbulence. This is later followed by lipid accumulation. The reason why arteries are more prone to thickening than veins is because of the greater turbulence in these vessels. Yet in experimental models, veins can develop atherosclerosis if subjected to greater haemodynamic stress.\textsuperscript{226} Thus, lesions can be induced in the vessels of animals, such as ruminants, with very low plasma cholesterol concentrations, so that the presence of an excessive lipoprotein concentration is not a prerequisite for lesion formation, in direct contradiction to the statement of Blackburn\textsuperscript{25} (Section III.B.1).
2. Experimental Evidence in Human Beings

(a) **Dietary lipids and plasma lipoproteins.** The lipid hypothesis as defined in this review and as generally stated (in whatever form) considers only the effect of dietary lipids on plasma total cholesterol and of plasma total cholesterol on CHD risk. At the time when the lipid hypothesis first began to be formulated, the methodology for lipoprotein separations was cumbersome and required large sample sizes. There was little appreciation of the pathways of metabolism of the different lipoproteins, the relationships between them, or their relative influences on arterial disease processes. Knowledge of the crucial roles of the apolipoproteins was rudimentary. Even now, with vastly increased knowledge of the detailed interrelationships between the lipoproteins, their removal from plasma by receptor-mediated processes and their differential influences on arterial disease progression and CHD risk, policy statements referring to management of plasma lipoprotein profiles, almost always consider total cholesterol, and hardly ever, LDL, HDL and VLDL.

Even now, many still use the Keys/Hegsted equations, formulated in the 1960s to predict the response in plasma cholesterol to a change in dietary fat intake from the known intakes of total saturated and polyunsaturated fatty acids (SFA, PUFA) and cholesterol. This is despite their limitations, namely: (1) SFA and PUFA are broad terms covering a wide variety of chemical structures. Different types of SFA and PUFA have widely different effects on plasma cholesterol; (2) the equations did not include a term for MUFA, even though oleic acid is quantitatively the most important fatty acid in the diet and in the body; (3) the recognition that changes in plasma total cholesterol are of limited significance and that changes in individual lipoproteins are more closely related to vascular health, in particular the ratio of HDL to LDL; (4) they do not take into account interactions between fatty acids at different levels of intake.

Briefly, from publications reviewed in Section III: (1) Increasing the dietary intake of cholesterol increases plasma cholesterol by only a small extent (about 2 mg/dl for every 100 mg cholesterol consumed daily) and this is mainly attributable to increases in the LDL fraction. (2) Substitution of saturated by monounsaturated or n-6 polyunsaturated fatty acids leads to a reduction of the concentration of LDL-cholesterol. HDL-cholesterol is not reduced unless linoleic acid represents more than about 12% of dietary energy. (3) Addition of long chain, n-3 PUFA (mainly 22:5 and 22:6) to diets leads to a reduction in the concentration of VLDL but not LDL or HDL. (4) Replacement of dietary SFA with carbohydrates leads to decreased concentrations of LDL and HDL and, in some people, increased concentrations of VLDL. (5) Replacement of dietary n-6 PUFA with carbohydrates leads to increased LDL-cholesterol concentration with little change in HDL and in some people, increased concentrations of VLDL.

These are the average changes that can be observed in subjects under carefully controlled experimental conditions, given defined diets in which only the specified nutrient changes are made and in which other variables that may influence plasma lipoproteins are kept reasonably constant.

(b) **Dietary cholesterol.** Advice to the general public to reduce dietary intake of cholesterol has ignored (i) the weak influence of dietary cholesterol on plasma cholesterol; (ii) the impossibility of predicting any individual's response, due to inter- and intra-individual variability and (iii) the complex interactions between fat and cholesterol and the differential effects on lipoprotein fractions.

The influence of dietary cholesterol has been grossly exaggerated and, indeed, neither scientists, public agencies nor the media have been too scrupulous about distinguishing between dietary and plasma cholesterol. In the U.S.A., a principal vehicle for public education about lipids and disease risk is the National Cholesterol Education Program. Although the term cholesterol in its title presumably refers to plasma cholesterol, many media activities, purporting to inform the public, portray the problem as one of dietary cholesterol. In reality, the advice to reduce cholesterol intakes from 450 to less than
300 mg/day would result in a decrease in average plasma total cholesterol concentrations of 3–4 mg/dl or slightly less than 2%. Somewhat larger reductions could be achieved by relatively modest reductions in body fat.\textsuperscript{31}

Human beings exhibit wide differences in response of plasma cholesterol to dietary cholesterol.\textsuperscript{24} In one study\textsuperscript{12} of a group of 75 experimental subjects, 52 (69\%) were able to compensate for intakes of cholesterol between 200 and 900 mg/day by maintaining a constant plasma cholesterol, whereas 23 subjects did not compensate and exhibited rises in plasma cholesterol greater than 5\% (mean 12\%). Further investigation demonstrated that those subjects who compensated for increased cholesterol intake by maintaining constant plasma cholesterol concentration, were able to reduce their rate of cholesterol biosynthesis significantly ($P < 0.001$). The non-compensators exhibited no significant reduction in cholesterol biosynthesis. This experiment also showed that there were very large differences in the proportion of dietary cholesterol absorbed (from about 1 to 9 mg/kg body weight/day) and that the degree of suppression of cholesterol synthesis was a linear function of the amount of cholesterol actually absorbed by the subjects. This research is important in that it is one of the few studies to utilize this powerful combination of techniques to relate plasma cholesterol to the processes of absorption and endogenous synthesis in human beings. Some individual people may appear to be hyper-responsive in one experiment and hypo-responsive in another. This is because the plasma cholesterol of individuals is subject to a periodicity. The question of plasma cholesterol variation in animals and man has been reviewed by Kritchevsky\textsuperscript{123} and Durrington.\textsuperscript{53} The within-person variability is independent of diet and calculated to be responsible for about 25\% of the apparent variance in response between subjects, even if as many as 12 independent blood samples are used to determine each person’s plasma cholesterol.\textsuperscript{24} An interesting demonstration of cholesterol fluctuation was described by Groover \textit{et al.}\textsuperscript{85} Over a period of 5 years, Groover examined military personnel at The Pentagon in Washington, U.S.A. In a majority of subjects, plasma cholesterol concentration fluctuated between 20 and 40\%. In 37 out of 177 subjects, the variation was over 50\%. The cause of the fluctuations has not been defined, although “stress” has been implicated. Other factors affecting the plasma cholesterol concentration at any one time are the position of the subject when the sample is taken, time of day and season of the year. In one study conducted by WHO in the northern hemisphere (cited by Durrington\textsuperscript{53}) there were maxima in April and November and minima in February and June of the same year amongst subjects taking part. The difference in concentration between the highest and lowest points was 16 mg/dl (0.41 mM). For these reasons, reliance should never be placed on a single plasma cholesterol measurement and yet it almost invariably has been.

Eddington \textit{et al.}\textsuperscript{55,56} confirmed the complexity of interrelationships that determine the effects on individuals of particular combinations of cholesterol and fatty acid intakes. When the diet was relatively low in total fat content, plasma cholesterol concentrations in most people were remarkably independent of the amount of cholesterol present in the food. In these studies, consumption of eggs, used as the major source of cholesterol, varied between two and nine per week. Most importantly, the higher levels of intake did not seem to raise plasma cholesterol concentration significantly in people who had been categorized as “hyper-responders” to dietary cholesterol in a sub-set of experiments. The authors considered that although the level of dietary fat was relatively low, liver cholesterol synthesis would be inhibited because the level of saturated fatty acids was held below a level that would normally stimulate cholesterol production.

Dietary cholesterol may not influence all lipoprotein classes equally. Thus, Appelbaum-Bowden \textit{et al.}\textsuperscript{7} and Sacks \textit{et al.}\textsuperscript{201} found that supplementation of diets with eggs significantly increased plasma LDL but not total cholesterol concentration. This implies a fall in HDL. Beynen and Katan,\textsuperscript{23} however, observed an increase in HDL as well as LDL after cholesterol feeding.

\textbf{(c) Dietary fatty acids.} There are at least two difficulties in the interpretation of much experimental work, even that which appears to have been well designed and conducted:
first the difficulty of changing only one variable and second, of assessing dietary interactions that may arise in practical mixed diets as distinct from experimental diets incorporating “pure” fats and oils.

All natural fats contain mixtures of fatty acids, never a single type. Therefore, when authors report the cholesterol-aemic effects of “saturates”, “monounsaturates” or “polyunsaturates”, it must be remembered that what the experiments really show is an effect due to the balance of the fatty acids in the dietary fat(s) used. It would be advantageous if nutritionists would agree to abandon the terms “saturated fat” and “polyunsaturated fat” and refer only to saturated or polyunsaturated fatty acids.

Natural oils contain minor lipid fractions in addition to the triacylglycerols that are usually the major interest of the research reported. The mix of minor components is different in each oil and may influence lipoprotein metabolism in ways that are as yet ill-defined. Thus, maize oil, which has often been used as the archetypal “polyunsaturated fat”, contains a particularly large proportion of tocopherols, especially γ-tocopherols. Maize and palm oils also contain tocotrienols, that inhibit HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. A common fault, therefore, is to assume that the treatments being compared differ only in their fatty acid compositions, whereas if one treatment uses entirely animal fats while the other employs only vegetable oils, the diets will differ markedly not only in their cholesterol contents but also in many other “minor” components.

Palm oil does not result in the high plasma cholesterol concentrations that might be expected on the basis of its fatty acid composition (50% saturates). This may be due to its high tocotrienol content, its MUFA content, or the stereospecific distribution of fatty acids in its triacylglycerols, but the precise mechanism is unknown. Milk does not have the cholesterol-raising properties that might be expected from its fat composition, raising the possibility that a factor in the non-fat fraction may oppose the hypercholesterolaemic tendency of the fatty acid fraction.

Ever since the classic experiments of Hegsted, Keys and colleagues, in the 1960s, there has been controversy about the relative hypercholesterolaemic effects of different SFA. It has been clear that chain length is important. Thus, there is essentially no influence on plasma cholesterol of SFA with chain lengths up to 10:0, nor from 18:0 (stearic acid). In carefully controlled experiments with different species of monkeys chosen to simulate human “high”, “medium” and “low” responders, Hayes and colleagues found that 12:0 and 14:0 were considerably more potent than 16:0 in raising plasma TC and LDL-C. Similar results were obtained by Ng et al. in human subjects, although the relative potencies of 12:0 and 14:0 remain in question; published results suggest that 14:0 is more effective than 12:0. Hayes and colleagues 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 two distinct levels: (i) at 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 the level of 18:2 in the diet; (b) the level of dietary cholesterol and (c) on the initial concentration of LDL-C 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, which will no doubt be refined as more experimental data becomes available in man and dose–response effects are established, 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 TC and LDL-C concentrations. Thus, discrepancies between the original studies of Hegsted and Keys99,116 which observed no cholesterolaemic effect of 18:1 and more recent studies140,156 which observed an hypocholesterolaemic effect, can be explained. Hegsted and Keys99,116 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 studies140,156 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 an hydrophobic amino acid sequence in the receptor protein that needs a 14C 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 and these new concepts emphasize the need to focus on individual dietary fatty acid interactions rather than “fats” in general or complex mixtures of SFA and UFA. 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. The findings also underline the need for caution in extrapolating the results of experiments designed to investigate the lipidaemic effects of exchanging one dietary fat for another to whole foods where there may be complex nutrient interactions occurring. It also cautions against the ready assumption that effects seen are necessarily a result of fatty acids.

(d) Energy balance and blood lipids. Several epidemiological studies have suggested that those with higher energy expenditure are at lower risk of CHD79,153,163 (see Section IV.A.5). Whether the mechanism involves an effect of exercise upon plasma lipoprotein profiles or upon regulation of the adipose tissue mass is unknown. Obesity, particularly when characterized by a central or “android” distribution of adipose tissue is now recognized as being an independent risk factor for CHD112 but also influences plasma lipoprotein profiles. Kaplan112 proposed that glucose intolerance, android obesity, hypertriglyceridaemia and hypertension all work through a common factor, namely hyperinsulinaemia.

Exercise increases the demands of muscles for fuel and draws on plasma lipoproteins rich in triacylglycerols as its source. The triacylglycerol fatty acids in transport as plasma lipoproteins are derived either directly from the diet, or from adipose tissue stores. Physical activity may, therefore, be expected to influence circulating concentrations of lipid fuels and a number of studies confirm this assumption. In general the effects of exercise are to reduce postprandial chylomicron triacylglycerols256 and fasting VLDL,256 and to increase HDL119,258,259 and the ratio of HDL/LDL.258,259 In some studies exercise was accompanied by weight loss258,259 but in others weight was maintained119,256 suggesting that exercise can influence plasma lipoproteins independently of changes in adipose tissue stores.

A common finding is that exercise causes an increase in the activity of muscle lipoprotein lipase, consistent with increased utilization of VLDL119,256 and this might be postulated as a key step in the regulation of plasma lipoprotein metabolism. In general, the focus has been upon apoB/E receptors as the major mechanism for the regulation of LDL concentration. But LDL arises from the catabolism of VLDL via IDL and the initial step in VLDL catabolism is lipoprotein lipase. According to Eisenberg,56 the principal function of HDL is to receive surface remnants of the catabolism of triacylglycerol-rich lipo-
proteins. Thus, exercise drives up the concentration of HDL via increased activity of lipoprotein lipase generating increased catabolism of VLDL. Such catabolism also generates LDL. However, as a result of exercise, LDL concentrations either remain the same or fall, indicating that there is also an influence on apoB/E receptor activity.

Training of muscles by frequent exercise leads to increased numbers of mitochondria, blood capillaries and a higher lipoprotein lipase activity to service higher demands for oxygen and fatty acid fuels. The lipoprotein balance is shifted away from triacylglycerol-rich lipoproteins and LDL and towards HDL. The reverse occurs in the muscles of sedentary individuals. Exercise can, therefore lead to profound changes in energy metabolism and its role in mediating changes in lipoprotein patterns has been grossly underemphasized compared with the role of the diet.

Finally, there is also a wealth of evidence for more direct links between physical activity and CHD, whether or not mediated by changes in plasma lipid profiles.

(e) Diet, blood lipids and atherosclerosis. It is of course difficult to design experiments in man directly to demonstrate effects of diet and/or blood lipids on the progression or regression of atherosclerosis. Most reported studies have assessed the progression (or regression) of atherosclerosis by repeated angiography over periods of up to 10 or more years in the absence or presence of surgical or other invasive interventions. A problem in interpreting such studies is the lack of consistency in the methodology or agreement about the degree of stenosis to be regarded as representing significant disease. Thus in one study 40% stenosis was regarded as significant disease whereas in many others greater than 70% stenosis is regarded as significant.

Many angiographic studies have demonstrated that the rate of progression or regression is unrelated to concentrations of blood lipids or lipoproteins, family history, smoking habit, obesity or blood pressure: all “risk factors” regarded as important enough to feature in strategies for disease prevention.

In those trials that do appear to have found a correlation between dietary lipids and/or blood lipids and disease progression or regression, the dietary data were poor and the simultaneous changes in other lifestyle factors made it difficult to distinguish the specific roles of diet and blood lipids. Thus in one study, the energy intakes were extremely low and difficult to reconcile with the body weights quoted. There was a weight loss of 10 kg in the experimental group but none in the control group: this was unremarked in the discussion. Moreover, there were no data on smoking habits, family history or duration of the disease. In another study, the dietary intakes were assessed by three 24 hr recalls at 0, 1 and 2 years of the study and did not seem to be representative of the normal population. It was claimed that risk of developing new lesions was best correlated with total intakes of lauric, oleic and linoleic acids. The biological significance of these findings is obscure since: (i) the difference in plasma total cholesterol between “no lesion” and “new lesion” groups was extremely small and only just statistically significant; (ii) the intake of lauric acid above which increased risk was observed was only 0.22% of dietary energy, making the association very implausible; (iii) oleic acid is the most abundant fatty acid in the diet and in the body and is, moreover, synthesized in the body; the increased risk above a consumption of 11% of dietary energy is again implausible and (iv) linoleic acid is the principal fatty acid associated with a “cholesterol-lowering effect”: the level of intake associated with increased risk in this study (9.7% of energy) is below levels often recommended for cholesterol lowering in patients at high risk of CHD.

The fact that in some patients there may be observed progression of lesions in one location and regression in others, suggest strongly that local factors are far more important than these “global” risk factors.

(f) Dietary lipids and haemostasis. In the scientific discussion that led to the formulation of dietary advice to modify fat consumption, the issue of thrombosis has been almost entirely ignored in the preoccupation with atherosclerosis. Carefully conducted autopsy studies demonstrated that obstruction of the coronary arteries is involved in three-quarters
of cases of sudden cardiac death. The potential importance of coronary thrombosis in CHD mortality has probably been underemphasized because standard autopsy techniques do not detect even a minority of thrombi in coronary arteries. Moreover, the inability to detect a thrombus in cases of sudden cardiac death does not mean that a thrombus did not occur since thrombolysis may have been complete by the time autopsy was carried out.

In human studies, reliance has to be placed on measurements in samples of blood, either whole or fractionated to produce “platelet-rich plasma”. With the latter, it may be difficult to relate observations to events in vivo because the presence of other blood cells in vivo may influence platelet aggregation tendency. Comparison between studies is often difficult because of the use by different authors of different aggregation agonists at different concentrations. Dose-response studies are relatively rare. Another problem of interpretation is the absence of a linear relationship between the dose of agonist, the formation of thromboxanes and the final aggregation of platelets. These problems create large errors in the methods and a high risk of drawing false conclusions from dietary treatments that produce effects, which though small, may be biologically significant. The use of subjects as “their own control” helps to reduce some of the error but does not eliminate it.

The use of bleeding time has many shortcomings and variation within individuals can be very large. However, effects can be so large that they may be detected despite the intrinsic low sensitivity and specificity of the method.

Measurement of factors in the coagulation cascade is subject to a number of limitations. Firstly, they 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. Secondly, immunoassays, which are frequently used, measure all related species of a factor, both active and inactive, and may not, therefore, be particularly useful indicators of coagulant activity in vivo. Thirdly, the activation occurs on vessel surfaces rather than in liquid blood. Fourthly, once the enzymes are removed from their surfaces, they quickly bind to inhibitors so that their activity is neutralized. In recent 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.

This brief account of methodology serves to indicate why the interpretation of experimental results may be difficult and why the results that are available to date seem so inconclusive.

(g) Dietary lipids and platelet aggregation. Studies can be grouped according to whether they involved conducting platelet or bleeding time tests in groups of people (1) who were naturally consuming different diets or (2) 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 Dyerberg and Bang 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 ADP 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”.) 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.
A 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 consumption 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 aggregatory properties as happened in the Zutphen Study. In contrast, ADP-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 that increased dietary linoleic acid is of no consequence for platelet responsiveness to collagen.

There are few data on the effects of the n-9 fatty acids. Diets rich in oleic acid may result in increased proportion of oleic acid in membrane phospholipids at the expense of arachidonic acid but there appears to be little effect on platelet aggregation. In one study that measured bleeding time, giving a diet enriched with a high oleic rapeseed oil resulted in prolonged bleeding time.

(h) 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 factors VII, X, and fibrinogen and most have used fish oil supplements.

Reduction of fat intake as a proportion of dietary energy reduced the concentration in plasma of factor VII 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.

Another important aspect of the haemostatic system that might be affected by dietary fats is the so-called fibrinolytic pathway. This exists essentially to dissolve the fibrin in a clot or thrombus. This is effected by the enzyme plasmin, which is normally present in the inactive form, plasminogen. This zymogen has to be activated by proteases called plasminogen activators including tissue (tPA) and urinary (uPA) forms. Plasma also contains many proteinase inhibitors including plasminogen activator inhibitors (PAI-1 etc.). Complex interplay between these various factors keeps the fibrinolytic system in check.

Study of the effects of diet on these 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 PAI-1 activity after giving dietary fish oil while others have reported no change or a reduction. Likewise, most studies report no change in tPA concentrations in response to fish oils while some report an increase and one an reduction. Fewer studies have examined the effects of the n-6 fatty acids. Again, there have been reports of no effects on tPA or PAI-1 after giving 10 g linoleic acid/day from wheat germ oil or significant reductions in plasminogen, tPA and tPA inhibitor with low fat, high P/S diets. These conflicting results are almost certainly due to technical difficulties in measuring appropriate activity parameters in such a complex system.

The purpose of this sub-section has been to highlight the narrow view of lipids and CHD that has occupied much of the debate so far. Nevertheless, the paucity of information on dietary lipids and haemostasis, and the many contradictions, do not yet allow us to draw any firm conclusions about “prudent diets” in this regard.
3. Evidence from Familial Dyslipoproteinaemias

Although the massive hypercholesterolaemia present in patients with inherited diseases such as FH, in association with a high prevalence of premature deaths due to CHD, has for many authorities provided overwhelming support for the lipid hypothesis, others argue that the pathology of the lesions seen in this disease in no way resembles that of atherosclerosis on the general population.

Stehbens holds that the lesions seen in these patients should more properly be classified as a cholesterol storage disease and do not resemble the complex fibrous lesions of mature atherosclerosis.

Lipid infiltration into the lesion is a relatively late event in atherosclerosis, being preceded by thickening of the arterial intima at specific sites such as forks and bends. Simultaneously, there is thinning of the outer layers of the vessel wall, some progressive dilatation and at times serious encroachment on the lumen by intimal thickening. By contrast, in FH, the early lesion appears to be essentially an accumulation of lipid-laden scavenger (xanthoma) cells in the arterial wall, sometimes sufficient to cause stenosis of even large arteries like the ascending aorta. Eventually, fibrosis occurs, but the usual complications of atherosclerosis are noticeably absent. Thus, aortic aneurysm is never seen, although it would be expected to be frequent if the vascular pathology is really accelerated prematurely severe atherosclerosis. As long ago as 1950, Thannhauser concluded that the vascular lesions of FH are not atherosclerotic on the grounds, inter alia, that cerebral and lower limb vessels were rarely involved in FH. The extensive occurrence of aortic and mitral valvular stenosis as well as extravascular xanthomatous infiltrations or tumor-like nodules are very unlike conventional atherosclerosis.

4. Evidence from Surgical Procedures

The Program on the Surgical Control of the hyperlipidemias (POSCH) was designed to lower plasma LDL substantially and thereby demonstrate marked improvement in atherosclerosis as measured by angiography. The partial ileal by-pass procedure was effective in reducing total cholesterol by 23% and LDL-cholesterol by 38% (P < 0.0001) compared with the control group. HDL-cholesterol was 4% higher (P = 0.02) in the surgical group. These changes were achieved within 3 months and values did not alter, thereafter, for the 10 years of the study.

The stated aim of the trial was “to assess whether lowering the plasma cholesterol level would lead to a reduction in the progression of atherosclerosis”. It was not designed to examine mortality, yet the authors make the statement that “overall mortality and mortality due to CHD were reduced but not significantly so”. This meaningless statement is an example of a commonly used technique designed to mislead the reader into the belief that there has been some positive outcome. If the difference in the numbers did not reach a degree of statistical significance agreed and accepted as the criterion for success at the design stage, then mortality cannot be said to have been “reduced”.

The method for assessing atherosclerosis progression was crude and unscientific, scored by two-member teams, using a global consensus evaluation of disease severity on an 8-point scale scored as from “much worse” to “much better”. Inspection of the data referring to disease progression over the 10 years of the study indicates that although there was a highly significant improvement in the surgical group compared with the control group at all stages of the trial, this must 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 mmol/L, LDL at 2.7 mmol/L and HDL at 1.1 mmol/L. This seems to indicate that there are more important factors resulting in the progression of atherosclerosis than the lipoprotein profile and runs contrary to the
idea that such radical decreases in plasma cholesterol result in significant regression of atherosclerosis.

Both control and surgical intervention groups were allocated to the American Heart Association "step 2 diet", which is a fairly rigorous one (fat < 30% of energy; P/S = 1.4; cholesterol < 200 mg/day). Despite this, there was no change in baseline cholesterol (control group) throughout the 10 years of the experiment. This was not due to opposing changes in lipoprotein fraction that together resulted in a steady total cholesterol value but may have been because: (i) the diet was ineffective in reducing plasma cholesterol; (ii) some other environmental factor tended to cause a rise in total cholesterol or (iii) there was poor adherence to the diet. Other trials in which a step 2 diet has been used have resulted in modest falls in total cholesterol (Section IV.C.3), so it is unlikely that the diet would have absolutely no effect. Adherence to the diet is not commented upon in the paper, illustrating a common weakness of clinical trials that the dietary input, where included, is often very weak. A further question is whether dietary control in the two groups was significantly different. One might intuitively expect that patients hospitalized for surgery may adhere to a specified diet, even after discharge, more rigorously than control subjects who had less direct contact with the clinicians. In many clinical trials there is direct or implied evidence that the control group is treated less rigorously than the treatment group (Section VI.C), a factor that may influence the whole interpretation of the trial’s results.

The patients in this trial were described as "hypercholesterolaemic" although the baseline value was 6.49 (SD, 0.93) mm, so they were not grossly so. The reduction in total cholesterol effected by surgery was 23%: far greater than achieved by the most rigorous diet in the most successful trials (Section IV.C). An improvement from " + " to " + + + " had occurred in 6.4% of surgically treated patients compared with 3.8% of controls. Is drastic by-pass surgery justified for such a meagre improvement? A rigorous diet in this trial produced no benefit at all. The authors' last sentence states: "POSCH results provide new, strong evidence supporting the beneficial effects of lipid modification in the reduction of atherosclerosis progression". Even if the authors just managed to refrain from doing so, many people will unjustifiably assume that dietary modification will achieve similar results and this is not borne out by the facts.

5. Epidemiological Evidence

Atherosclerosis occurs in all human beings of whatever race, geographical location or diet. No population, not even in rural Africa is entirely without it and it is, therefore, not true to say that it is a disease of only "developed" societies.

Differences in clinical outcomes between human beings, therefore, may be due to variations in severity of atherosclerosis, which may determine whether or not and at what stage complications will occur. However, it is known that complications may occur in the absence of marker morphological change. While spasm of smooth muscles in arteries seems to occur when there are plaques present, there are well documented instances when this is not so. Thus, factors within the vessel wall, independent of the atherosclerosis, may have relevance to clinical outcomes. Similarly, it is known that thrombi form and disperse in endothelium which may appear to be normal, i.e. in the absence of atherosclerotic plaque formation.

Epidemiological associations in general relate, not to the disease process of atherosclerosis, but rather to the end-points of coronary heart disease, stroke and sudden death. Since these end-points have a multifactorial basis, one to one relationships between epidemiologically identified factors cannot be expected to be a universal finding.

It is quite clear from Section IV.A. that exchanging one type of fat for another as well as changing the proportion of energy from fat, under well-controlled experimental conditions, results in changes in plasma lipoprotein fractions. The evidence that the fat content of "free-living" people eating self-selected diets has an important influence on plasma lipids is much less convincing.
(a) **International comparisons.** The most cited study in favour of a link between dietary saturated fatty acids and CHD incidence was the famous “Seven Countries Study” of Ancel Keys and colleagues.\(^{113,115}\) Indeed, much of the foundation of the lipid hypothesis can be said to have been laid by this study and one can also conclude that it has had a disproportionate influence on thinking. Graphical representations of the results show a more or less straight line relationship between (a) intake of saturated fatty acids expressed as a percentage of energy intake and 5- or 10-year incidence of CHD and (b) plasma cholesterol concentration and incidence of CHD, although closer examination of the figures shows a more stepped relationship, with incidence rising more sharply when saturated fatty acids represent more than about 15% of energy. There was no such correlation with total mortality. The whole study involved about 12,000 40–59 year old men initially free of CHD. They were from 18 areas in the 7 countries with as few as 509 individuals sampled in Tanushimaru, Japan, versus 2571 US railroad workers.

The emphasis on the association with saturated fatty acids is hardly justified since other aspects of diet and lifestyle in these widely differing communities were also very different. In the case of Japan, the low saturates intake was accompanied by a larger intake of fish oil which we now know may have a degree of protective effect against CHD that is not mediated through plasma cholesterol. Diets low in saturated fatty acids may also be rich in other components, e.g. some kinds of dietary fibre, that have an hypocholesterolaemic effect.

The interpretation that has been placed on the Keys’ Seven Countries Study suffers from an important statistical and logical defect. The seven countries were among 21 member states of the Organization of Economic Co-operation and Development for which statistics were available on average annual consumption of different types of fat and on CHD mortality rates. Japan and Finland were included by Keys “because they were the extremes in vital statistics”. Keys obtained a correlation coefficient for the association between percentage of energy intake as saturated fatty acids and CHD mortality of +0.84. Wood\(^266\) pointed out that there were 116,280 possible ways of obtaining a sample of seven from 21 and that fewer than 10% of these samples of seven would give a correlation coefficient equal to or greater than that obtained from Keys’ selection. The 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 can be drawn from it concerning the relationship between the consumption of saturated fatty acids and CHD. In every selection that included both Japan and Finland, the correlation was significantly greater than zero. Wood’s paper\(^266\) is rarely, if ever, quoted and provides an example of the way in which 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.

Many international studies tend to show that those countries that have on average low intakes of dietary fats also tend to have populations with low average plasma lipid concentrations and low rates of coronary heart disease.\(^{25,266}\) Many of these have been cross-sectional in design and their usefulness is limited in this respect. Other aspects of lifestyle, especially aspects of energy balance, are also quite different and it is virtually impossible to isolate the specific effects of diet.

Too much confidence has been placed in migration studies that often show that those who migrate from a country or region with a low CHD mortality adopt the disease pattern of their adopted country.\(^{139,268}\) Confidence in this assumption must, however, be limited because other lifestyle characteristics (stress, exercise patterns, smoking habit, alcohol consumption etc.) also change. The changes in plasma lipoproteins and disease patterns cannot be attributed to diet with any confidence.

(b) **Within-country comparisons.** Comparisons of individuals or groups of people within countries have not demonstrated significant associations between dietary fat level or composition and blood lipids using a number of study techniques. Thus, in the Western Electric Study,\(^212\) the correlation coefficient between the polyunsaturated to saturated fatty
acid ratio and plasma lipid concentration was small and the Tecumseh Study could not
demonstrate a significant relationship between fat, cholesterol and other macronutrients
in the diet and plasma lipids.

The Framingham Study, began in the late 1940s. Examination of data collected
over the first 20 years on 431 men and 442 women showed that when these subjects were
divided into groups representing tertiles of the cholesterol distribution (below 4.65 mM;
4.65–7.73 mM; over 7.73 mM) there were no differences in their intakes of energy, proteins,
carbohydrates, fat or cholesterol. The same authors found a similar lack of association
between the nature or type of dietary fat eaten and the occurrence of CHD events in
communities as widely different as Puerto Rico and Honolulu. In the Framingham
population, the curve for coronary mortality in men younger than 65 plotted against
plasma total cholesterol concentration is relatively shallow within the range 4.0–6.2 mM
(150–250 mg/dl), rising more steeply after a value of about 6.6 mM (250 mg/dl). With
increasing age, the increased CHD risk due to high cholesterol is no longer seen. In regard
to total mortality, moreover, there is a dramatic decrease in risk in the 65–74 year old group
as plasma cholesterol concentration increases. Similar trends are seen in women, although
less dramatic and with significant deaths only in the older age groups.

The sheer size of the study, its long duration and the numbers of different reports over
time have in a sense, contributed some confusion to the findings, for different aspects of
the study have been reported at different times and different interpretations or emphases
appear to have been given by the authors in different publications. What most people with
an interest in diet, blood cholesterol and CHD tend to recall about the Framingham Study
is the concept that CHD risk rises steeply with increasing blood cholesterol. The numerous
different publications need to be read carefully in order to appreciate that the true
relationships are by no means so simple. Thus, few people are aware that the study has
been conspicuously unable to provide evidence for a relationship between any nutrients
and plasma cholesterol concentrations or CHD risk. Summaries of only part of the data
can give a misleading impression. Thus, Levy and Kannel stated: "The analysis showed
that among young healthy men, ages 31 to 39 years, those with baseline (original) serum
cholesterol levels under 180 mg/dl (4.6 mM) have a slow rate of mortality. Those with levels
ranging from 180 to 220 mg/dl (4.6–5.6 mM) died at slightly faster rate and the rate among
those with levels of 220 to 260 mg/dl (5.6–6.7 mM) was faster still. The most alarmingly
rapid mortality rate was seen in the group exhibiting baseline cholesterol levels in excess
of 260 mg (6.7 mM)." The publication to which they were referring also presented data for
men in three other age groups and for women in the same four age groups. No consistent
association was apparent between serum cholesterol and CHD mortality in any of these
other 7 groups. Someone reading the commentary of Levy and Kannel would, therefore,
get a distorted picture of the overall relationship, because the authors had selectively
reported the one-eighth of the data that supported the consensus view about plasma
cholesterol as a risk factor for CHD, and had selectively omitted the seven-eighths that
did not. Furthermore, the publication of Anderson et al. on the 30-year follow-up of the
Framingham Study concluded that people whose plasma cholesterol fell over a 14-year
period had a higher CHD mortality over the next 18 years.

In the United Kingdom, the Caerphilly and Speedwell Study is a large prospective
cohort study of approximately 2500 men aged 45–59 at entry in 1979–83 in a part of the
country (South Wales) with a particularly high prevalence of heart disease coupled with
a parallel study, involving a similar number of men in Speedwell, a nearby area of England
where the CHD incidence is lower. Total plasma cholesterol and total plasma triacyl-
glycerols were significantly higher in men who had a CHD event compared with those that
did not, whereas HDL-cholesterol concentrations were significantly inversely related.

When the data were analyzed for nutrients, only energy was significantly associated with
incident CHD and in a negative sense. In other words, those men with the highest energy
intakes experienced significantly fewer CHD events. These results are similar to those in
the London Busmen's and Bankers' Study and the Framingham, Puerto Rico and
Honolulu studies. The implications of this apparently paradoxical result are that, if the
Table 1. Dietary Fat Intakes in Major Prospective Studies

<table>
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<tr>
<th>Study</th>
<th>Men who experienced no CHD event</th>
<th>Men who experienced an incident CHD event</th>
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<td>7982</td>
<td>95</td>
<td>35</td>
</tr>
<tr>
<td>Honolulu '47</td>
<td>6632</td>
<td>86</td>
<td>33</td>
</tr>
<tr>
<td>London</td>
<td>287</td>
<td>129</td>
<td>41</td>
</tr>
<tr>
<td>Zutphen</td>
<td>827</td>
<td>142</td>
<td>42</td>
</tr>
<tr>
<td>Boston</td>
<td>891</td>
<td>144</td>
<td>39</td>
</tr>
<tr>
<td>Caerphilly</td>
<td>2197</td>
<td>103</td>
<td>40</td>
</tr>
</tbody>
</table>

subjects are not gaining weight, then those that are consuming more energy are also expending more, possibly by virtue of greater exercise. It is, therefore, possible that increased energy expenditure is protective against CHD.

The Caerphilly Study found no correlations between intakes of dietary fats (or any other major nutrient) and the incidence of CHD. Although there was a positive association between the intake of saturated fatty acids and the concentration of plasma LDL, diet as a whole accounted for only 1–7% of the variance in plasma lipoprotein concentrations and only 1.9% of the variance in plasma total cholesterol. While total plasma cholesterol and HDL-cholesterol were predictive of incident CHD, various haemostatic factors were more strongly predictive. These included fibrinogen concentration, plasma viscosity and white cell count.

The studies reviewed so far used conventional methods for assessment of dietary intakes which have many drawbacks. Berry et al. used adipose tissue fatty acid composition as a more reliable indicator of the fatty acid composition of the habitual diet and concluded that the type of fatty acids consumed explained only a small percentage of the variance in plasma lipids in subjects with plasma lipid concentrations in the normal range.

Keys presented additional information on the Seven Countries Study. In six of the seven countries, pairs of communities were sampled as nearly as possible by language classification. Within countries, there was no overall association between CHD and consumption of saturated fatty acids (r = -0.07).

When the fat intakes of men who experienced no CHD event are compared with those who had an incident CHD event in several major prospective studies, there are insignificant differences between them when expressed either as grams per day or as a proportion of dietary intake (Table 1). 79,124,126,147,153,163

To explain the differences in results between international and national studies, it has been argued that within an affluent population, dietary differences would be small and, therefore, dietary fatty acid and cholesterol intakes would correlate poorly with blood lipids. However, the range of polyunsaturated to saturated fatty acids ratios measured by Berry et al. in their New York City population was very wide, suggesting quite large differences in dietary habits within the population of one city. The Dietary and Nutritional Survey of British Adults also showed significant differences in intakes of total fat and of saturated and polyunsaturated fatty acids between different regions of the U.K. and between different social groups. In the whole sample of men, although the mean fat intake was 104 g/day, the intakes in the lowest and highest 2.5 percentiles were 44 and 171 g/day respectively. While the mean P:S was 0.40 in the complete sample of women, the values in the lowest and highest 2.5 percentiles were 0.17 and 0.91 respectively. Therefore, it seems difficult to sustain the argument that differences in fat intakes within a fairly homogeneous population are not great enough to detect an influence on blood lipids if there were one. Other problems in interpreting population data are that, with few exceptions, the measurement of fat intake has been very poor and the data obtained have been generally unreliable; this is especially true of international data.

This discussion has deliberately concentrated on studies that presented dietary data. There are, however, a number of important studies that examined the association between
plasma lipids (and other “risk factors”) and CHD incidence and mortality but did not consider diet. The Israeli Heart Study\(^7\) is important because it represents a very large number of man-years of observations. Although CHD mortality rose with increasing plasma total cholesterol above 5.6 mm (217 mg/dl) and was inversely proportional to HDL-cholesterol concentration, plasma lipoproteins were less effective in predicting CHD than smoking and hypertension. The study has a number of limitations, one of which is, paradoxically, a result of its length. The prognosis of the influence of plasma cholesterol concentration is based on a single measurement of cholesterol in each man in the trial in 1963. It is well established that several measurements of cholesterol are necessary to establish into which decile of plasma cholesterol concentration the subject should be placed. Over the 23 year period, changes in an individual's cholesterol may have occurred either spontaneously (perhaps as the result of an underlying disease process) or due to therapy. It may also be that the mortality pattern of this population (and also those of Framingham, Caerphilly etc.) may not be relevant to other populations.

As in several other studies of this type, there was a curvilinear relationship between total cholesterol and CHD mortality across the whole distribution (i.e. the risk increased more steeply as total cholesterol increased) but little or no relationship with total mortality. Those who advocate that total cholesterol is the single most important risk factor for CHD use this as an argument that no segment of the population, even those regarded as having relatively low cholesterol, are immune from the risk due to cholesterol. However, the authors of this paper concluded that in the lower three quintiles of cholesterol, the apparently increasing risk is due to the operation of risk factors other than cholesterol.

Another study\(^1\) throws interesting light on the way in which the manner of presentation of results can influence the reader's view of a study. About 76% of men in this prospective study had no symptoms of CHD on entry while some 24% had symptoms. There was no gradient of either CHD or all-cause mortality risk from the lowest plasma cholesterol concentrations to 6.2 mm in the majority without symptoms. Above this concentration risk increased 4.5-fold. The number of men with cholesterol > 6.2 mm was not given but it can be deduced from the distribution curve that the number must have been quite small. It can also be deduced, but was not pointed out in the paper,\(^2\) that most symptomless men (with plasma total cholesterol < 6.2 mm) had no increased risk, whatever their cholesterol within this range and would not, therefore, benefit from the cholesterol-reducing measures proposed in many population-based strategies.\(^3\) The minority with symptoms, however, were different. For them, total and CHD risk increased with increasing plasma cholesterol concentration.

Taken together, these prospective epidemiological studies seem to show that:

1. There is a positive association between plasma total or LDL cholesterol and risk of CHD especially in younger men. The curve is fairly flat in the lower part of the cholesterol distribution but rises more steeply after about 6.5 mm.
2. For the population in general, there is no similar association between plasma TC and total mortality.
3. There is an inverse association between HDL-cholesterol and CHD risk.
4. There is no relationship between dietary lipids and either plasma lipids or CHD mortality.
5. Plasma cholesterol may not be the predominant known risk factor when compared with smoking, hypertension and aspects of energy balance.

**B. Tenet 3: Reducing the Dietary Intake of Saturated Fatty Acids and Cholesterol will Reduce the Concentration of Plasma Cholesterol**

1. "Natural Experiments": Migration Studies

This topic was addressed in Section III.3.C. No epidemiological studies have demonstrated unequivocally that changes in plasma lipids when people move from an environment in which average population concentrations of plasma lipids are high to one in which
they are low are solely or even mainly a result of changes in dietary fats. Such a conclusion would require longitudinal studies of migrating individuals, with careful measurements of dietary intakes and serial measurements of lipoprotein fractions over considerable periods of time, controlling carefully for confounding factors. Such careful observations have not been made.

2. Evidence from Trends in Consumption

It is frequently claimed that changes in dietary fat consumption have had a major impact on downward trends in CHD mortality in several countries in the past two decades (see Section III.D.4). Such claims are as flawed as the claims about migration since so many other lifestyle factors have changed in the same periods of time. A common fault is to equate CHD mortality with disease incidence or prevalence. Thus although CHD mortality has indeed been declining in the USA, its prevalence is still increasing. If the intakes of total vegetable and animal fats are examined in that country over the period 1910 to 1970, during which time CHD mortality is declared to have risen sharply and then declined, there is no relationship with either total fat consumption (assessed by using so-called disappearance figures and other proxies for consumption), which remained more or less constant during that time, or type of fat, which shifted in the direction of an increased P/S ratio continually over the whole period. A member of staff of the National Heart Lung and Blood Institute, which has been at the forefront of encouraging blood cholesterol reduction through dietary fat modification wrote in 1970: “The increased risk of CHD reported to have occurred over this period is not related to dietary fat changes to a very important degree”.

The above conclusions are based mainly on figures for food supply provided by the US Department of Agriculture and have been criticized because they represent available food rather than food actually eaten. They do not take into account wastage or food raw materials used for purposes other than human food. A recent study has recalculated nutrient trends based on an analysis of 171 studies of food intakes of individuals over the period 1920–1985 and the authors suggested that fat intake began to decline at about the same time, or just before, the decline in CHD mortality. However, the data presented do not provide any convincing evidence for a change in total fat consumption (Fig. 2 in Stephen and Wald) and, because of the scatter in the results (Fig. 3), the start of the downward trend in SFA does not convincingly predate the decline in CHD mortality. Moreover, as pointed out earlier, there has been no downward trend in morbidity.

Similarly, figures from the British National Food Survey show that P/S has risen steadily at a time when CHD mortality first increased and then declined. CHD was an unimportant disease in the U.K. in the early part of the 20th century (in contrast to the importance of infectious diseases) yet the total fat consumption then differed little from today.

3. Evidence from Intervention Trials

Table 2 summarizes the effects of diet on plasma cholesterol in the major intervention studies. A problem in comparing such studies is that each trial was different in many respects including: the number of subjects, and, therefore, the power of the study to detect a statistically significant change, the type of diet, the setting in which the study was conducted, the duration and the initial value of the plasma cholesterol. Moreover, any change in a dietary variable will automatically affect a number of other nutrients unless the studies are very well controlled with respect to diet, which was rarely the case in these trials.

The studies can be divided into two groups according to the stringency of the diet. Group 1 includes those using a diet roughly equivalent to the American Heart Association's (AHA) “step 1” diet (total fat < 30% of energy; P/S = 1.0; cholesterol < 300 mg daily; energy intake to maintain desirable weight). Group 2 includes studies in which the diet was
TABLE 2. Dietary Impact on Plasma Cholesterol in Several Intervention Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Duration</th>
<th>Baseline</th>
<th>Change in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(years)</td>
<td>plasma total cholesterol (mm)</td>
<td>cholesterol (%)</td>
</tr>
<tr>
<td>Group 1 (&quot;Step 1 diet&quot;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO European trial (except U.K.)</td>
<td>a</td>
<td>1898</td>
<td>4</td>
<td>6.7</td>
</tr>
<tr>
<td>WHO European trial</td>
<td>b</td>
<td>824</td>
<td>4</td>
<td>5.6</td>
</tr>
<tr>
<td>MRFIT</td>
<td>c</td>
<td>6428</td>
<td>6</td>
<td>6.2</td>
</tr>
<tr>
<td>Gothenburg</td>
<td>d</td>
<td>1473</td>
<td>10</td>
<td>6.5</td>
</tr>
<tr>
<td>North Karelia</td>
<td>e</td>
<td>2535</td>
<td>10</td>
<td>7.1</td>
</tr>
<tr>
<td>Stanford</td>
<td>f</td>
<td>982</td>
<td>2</td>
<td>6.5</td>
</tr>
<tr>
<td>Curcio et al.</td>
<td>g</td>
<td>61</td>
<td>0.5</td>
<td>7.1</td>
</tr>
<tr>
<td>Group 2 (&quot;Step 2 diet&quot; or more stringent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oslo, primary</td>
<td>h</td>
<td>604</td>
<td>5</td>
<td>8.3</td>
</tr>
<tr>
<td>Oslo, secondary</td>
<td>i</td>
<td>206</td>
<td>5</td>
<td>7.7</td>
</tr>
<tr>
<td>MRC Committee</td>
<td>j</td>
<td>169</td>
<td>2</td>
<td>7.1</td>
</tr>
<tr>
<td>Research Committee</td>
<td>k</td>
<td>81</td>
<td>2</td>
<td>6.8</td>
</tr>
<tr>
<td>Rose et al.</td>
<td>l</td>
<td>13</td>
<td>2</td>
<td>6.8</td>
</tr>
<tr>
<td>Minnesota</td>
<td>m</td>
<td>4541</td>
<td>1</td>
<td>5.4</td>
</tr>
<tr>
<td>Finnish Mental Hospitals</td>
<td>n</td>
<td>300</td>
<td>4.5</td>
<td>7.0</td>
</tr>
<tr>
<td>Los Angeles Veterans</td>
<td>o</td>
<td>163</td>
<td>2</td>
<td>6.1</td>
</tr>
</tbody>
</table>

a, Factory population, high risk, individual intervention; b, factory population, all subjects, combined individual and mass intervention; c, employees, high risk, individual intervention; d, population, combined individual and mass intervention; e, population cohort, mass intervention; f, hospital, post myocardial infarction, individual intervention; g, hospital, high risk, individual intervention; h, population cohort, high risk, free living; i, hospital, patients with CHD; j, mental hospital patients; k, veterans hospital.

more stringent than the AHA "step 2" diet, in which the P/S is further increased to 1.4 and the cholesterol reduced below 200 mg/day.

Diet equivalent to "step 1" resulted in reductions in cholesterol from 0.2% to 4% in 9 studies, no change in one and an increase in 1% in another. Diets equivalent to or more intensive than "step 2" resulted in falls ranging from 6.5 to 15.5%. In reviewing these results, Ramsey et al. concluded that the small changes as a result of the "step 1" diet could not be attributed to lack of statistical power, changes in control groups (which did occur in several, see Section IV.D.3) or subject selection. These results contrast sharply with the assertions in many guidelines and reviews that cholesterol will fall by 10-25% in response to a "step 1" diet. Why, therefore, have the perceptions been so unrealistic? Sometimes there has been too much reliance on the results of short term experiments involving strictly supervised captive populations. There is also a tendency to extrapolate unjustifiably from studies using more rigorous diets (see Group 2 studies in Table 2) and even from the results of drug trials which tend to give larger and more consistent cholesterol lowering. One might also conclude that overinterpretation often stems from a sometimes almost evangelistic zeal to rid the world of all its heart disease problems by giving simple dietary advice.

The true worth of an intervention is measured only by the net difference between intervention and control groups. Those who advocate widespread public health measures based on simple dietary advice to lower blood cholesterol must assess the results of these intervention studies with the same rigorous evaluation as they would give to new clinical treatments. There has been a misguided tendency to accept the results of "responders" to diet and to ignore the non-responders as a minority of the public. Table 2 clearly demonstrates that, at least in the Group 1 studies, the mean effect of diet was close to zero so that the responders must have been balanced by an almost equal number of non-responders. If expected decreases in cholesterol concentrations in individuals in response to diet are considered as real and not due to random variation and are, moreover,
considered as equating with an improvement in health, then increases in concentration must also be considered harmful.

Finally, whereas, the studies using a "step 2" or a more stringent diet give consistently encouraging results, there are a number of problems associated with the design or interpretation of these studies that militate against direct extrapolation of the results to the general public (see IV.D.3).

C. Tenet 4: Reducing the Concentration of Plasma Cholesterol Reduces the Risk of CHD

1. Evidence from Intervention Trials: Drugs

The drug trials will be described briefly here since (a) they resulted in the strongest effects and (b) they have been used by many to suggest that the results would have been similar had diet been used to obtain a similar reduction in blood cholesterol concentration.

Some influential trials are discussed in detail here.

(a) Lipid Research Clinics Coronary Primary Prevention Trial (LRC-CPPT).\textsuperscript{132,133,134} This has probably been one of the most influential trials in terms of its impact on health policy. The subjects were 3806 males aged 35–59 years. They were apparently healthy but deemed to be at high risk of developing CHD by virtue of their high plasma total cholesterol concentrations which were in the top 5% of the distribution and greater than 6.85 mM. The subjects were generally unresponsive to diet and indeed any whose plasma cholesterol responded significantly to a cholesterol-lowering diet were excluded from the trial before randomization. The trial was conducted in the U.S.A. over a period of 7 years. The drug used was cholestyramine, a resin designed to sequester bile salts and prevent their reabsorption, thereby removing more cholesterol from the plasma by the liver. The experiment was placebo-controlled and double-blind. The end-points chosen were: total mortality, CHD mortality and/or definite non-fatal myocardial infarction.

Plasma total cholesterol was reduced by the drug by 8.3% (7.25 mM down to 6.65 mM) compared with the control group in which it fell 0.7% (7.22 mM down to 7.17 mM) \((P < 0.01)\). The cumulative 7-year incidence of fatal CHD and/or non-fatal myocardial infarction was 7% in the drug intervention group compared with 8.6% in the control, \(P < 0.05\). (Actually 1.6% less in the intervention group but 19% less if expressed as a percentage of a percentage, which is frequently done because it sounds much more impressive). There are many criticisms of this study. The validity of extrapolating results from a drug trial with men at high risk to dietary recommendations for a whole population is particularly questionable.

At the design stage of the trial, the authors stated that they would accept only results that were significant at a level of \(P < 0.01\) using a two-tailed test of significance.\textsuperscript{132} In the event (after the study had been designed and had commenced) they used a one-tailed test and accepted as significant results that differed at a level of \(P < 0.05\).\textsuperscript{133,134} Had the more appropriate two-tailed test been applied at \(P < 0.01\), the result for CHD mortality (which has been the biggest "positive" associated with this trial) would not have been significant.

Another possible confounding factor was the use of other drugs. Some patients were being prescribed \(\beta\)-blockers and the numbers were not evenly distributed between control and intervention groups. A properly conducted trial should not have included subjects prescribed other drugs.

(b) The Helsinki heart study.\textsuperscript{67} This was a randomized placebo-controlled intervention trial designed to reduce plasma cholesterol using the drug Gemfibrozil. The subjects were 4081 men aged 40–55 years apparently healthy but with average plasma total cholesterol between 7.4 and 7.5 mM. The trial lasted 5 years and the end-points were total deaths, fatal myocardial infarction, sudden cardiac death, unwitnessed death and non-fatal myocardial infarction.
The control and intervention groups were recommended to follow a diet that was designed to lower plasma cholesterol. However, whereas the mean plasma cholesterol of the intervention group was reduced by 7%, that of the control group actually rose by 3%. At the end of 5 years, 4.1% of the diet advice alone (control) group had suffered a fatal or non-fatal cardiac event compared with 2.7% of the drug treated group. The real difference is 1.4%. The results are presented to show that total (fatal plus non-fatal) heart disease was reduced by 34% (100–2.7/4.1 × 100). As Scott has pointed out, this is an example of how results can be presented to suggest that the outcome was more favourable than it actually was by expressing them as a percentage of a percentage i.e. 34% rather than 1.4%. Definite CHD deaths were presented as having been reduced by 25% (P < 0.02) which appears substantial. However, this only represented two fewer deaths. Total mortality was not significantly different: 2.1% for the control; 2.2% for the drug-treated group.

(c) WHO Clofibrate Trial. This study involved 15,745 men, aged 30–59 years, studied for a period of 5.3 years. Not only was there no significant difference between total CHD mortality in the control and experimental groups, but mortality from all causes was significantly higher in the experimental group and this excess mortality increased progressively with time. Concern about the toxic side-effects of Clofibrate has led to the progressive replacement of this drug by other agents.

2. Evidence from Intervention Trials: Surgery

The main evidence for the effectiveness of surgical intervention comes from the POSCH study, the limitations of which were discussed in detail in Section IV.A.4.

3. Evidence from Intervention Trials: Diet

(a) Study design. The motives for undertaking an intervention trial are likely to be mixed. The aim may be to test a scientific hypothesis (a purely scientific motive) or to ascertain whether the intervention will be an effective public health measure. In the former case, it would be advantageous to test the simplest possible hypothesis; for example, how modification of a single risk factor influences one specific end-point (e.g. myocardial infarction) so that there are few or no confounding variables. In the latter case, public health may be better served by intervening on many risk factors at a time, e.g. smoking, blood pressure, diet, weight reduction etc. (“multiple risk factor intervention trials” as distinct from “single risk factor trials”). It is inevitable that interpretation will be more difficult if more than one variable has been modified.

It is important to try to assess the degree of benefit obtained from modifying risk factors in an intervention trial. Research workers may elect to record all clinical events associated with CHD (for example, pains due to angina, irregularities in the electrocardiogram, as well as deaths from the disease), or all-cause mortality. In practice, age-adjusted mortality must remain the final arbiter of benefit because it removes all biases from the ascription of death and is also a reflection of total morbidity. Moreover, because CHD accounts for such a large proportion of deaths in many countries, a benefit in relation to CHD mortality should be reflected in total mortality. However, because deaths occur more rarely than non-fatal coronary events, it is necessary to study large numbers of subjects over long periods of time to be sure of being able to measure statistically significant differences between groups, if death is chosen as an end-point.

Intervention trials may also be divided into “primary” and “secondary” (Table 3). Primary trials have generally aimed to prevent the end-points of CHD (e.g. angina, myocardial infarction, death) in high risk people who, nevertheless, are free of symptoms. Secondary trials aim to prevent the occurrence of a second heart attack or similar event.
TABLE 3. Dietary Impact on CHD Morbidity and Mortality in Major Intervention Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Duration (years)</th>
<th>Non-fatal</th>
<th>Non-fatal</th>
<th>CHD events</th>
<th>CHD deaths</th>
<th>Total deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
<td>Single risk factor, primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Finnish mental hospitals</td>
<td>922</td>
<td>9</td>
<td>35</td>
<td>19</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Los Angeles Veterans</td>
<td>846</td>
<td>8</td>
<td>44</td>
<td>36</td>
<td>70</td>
<td>48</td>
</tr>
<tr>
<td>Single risk factor, secondary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leren</td>
<td>412</td>
<td>11</td>
<td>NR</td>
<td>NR</td>
<td>94</td>
<td>79</td>
</tr>
<tr>
<td>Multiple risk factor, primary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHO European Trial</td>
<td>60,881</td>
<td>4-6</td>
<td>561</td>
<td>505</td>
<td>450</td>
<td>428</td>
</tr>
<tr>
<td>Gothenburg</td>
<td>30,000</td>
<td>12</td>
<td>489</td>
<td>501</td>
<td>461</td>
<td>462</td>
</tr>
<tr>
<td>MRFIT</td>
<td>12,866</td>
<td>7</td>
<td>NR</td>
<td>NR</td>
<td>124</td>
<td>115</td>
</tr>
<tr>
<td>Oslo</td>
<td>1232</td>
<td>5</td>
<td>22</td>
<td>13</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Multiple risk factor, secondary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DART</td>
<td>2033</td>
<td>2</td>
<td>47</td>
<td>35</td>
<td>97</td>
<td>97</td>
</tr>
</tbody>
</table>

C, control; T, treated; NR, not recorded; m, results for men only; f, group given advice on, fat modification only.

(b) Primary prevention trials, single risk factor—the Los Angeles Veterans study.48 Although in a randomized, double-blind controlled trial, the substantially higher number of deaths from causes other than CHD in this 8-year study gave concern that there may be harm associated with the experimental diet, even though the difference was not statistically significant. This study showed a significant reduction in coronary events in the group given a diet with increased P/S, which was seen only in those with an initially high plasma cholesterol concentration that subsequently fell. There was no significant effect on total mortality. The number of subjects was small (846 total) and the published paper gives abundant evidence of poor adherence to the experimental diet. The authors calculated “adherence” to the diet as being the number of meals taken in the study dining hall as a percentage of the maximum number that could have been taken. On this basis, “adherence” was less than 10% in as many as 82 out of 422 controls (19%) and 120 out of 424 in the experimental group, (28%). Most seriously, the average age of the men was as high as 65 at the beginning of the trial.

(c) The Finnish mental hospitals trial.246 This trial is often quoted as giving strong evidence (1) for the feasibility of lowering cholesterol by dietary means and (2) for the concept that, by so doing, CHD mortality can be lowered significantly. The study started in 1958 and involved the populations of two Finnish Mental Hospitals, designated “N” and “K”. During the first 6 years patients in hospital “K” continued their usual diet while those in hospital “N” received a modified diet similar to that employed in the Los Angeles Veterans trial.48 After 6 years the diets used in the two hospitals were reversed. There were significant reductions in mean plasma cholesterol concentrations in patients during consumption of the experimental diets and these changes were reversed when the diet was switched back to the control regimen. The main dietary changes were the substitution of ordinary milk (probably full-fat pasteurized, but this was not specified) by an emulsion of soyabean oil in skimmed milk and the replacement of butter and ordinary margarine with a polyunsaturated margarine. The P/S of the experimental diet was 6-fold higher than that of the control diet. During the experimental diet period, the mean plasma cholesterol concentration decreased by 16.5% but the triacylglycerol levels remained unchanged.

Three categories of CHD were recorded, namely: coronary death, major ECG change and/or coronary death and intermediate and/or major ECG change or coronary death. The different categories are cumulative with the second a subset of the third and the first a sub-set of the second and third. Analysis of individual categories failed to demonstrate any significant effect on coronary death. The paper failed to comment on this point, yet it is an important observation in view of the massive change in the P/S and the relatively long duration of the study.
A major problem with this trial was that the population studied was continually changing throughout the 12 years of the study. Some patients were discharged to other institutions and there were new admissions. The patient population was “rejuvenated” by discarding the six oldest annual cohorts and admitting six newer annual cohorts at the younger end of the age range. This was done to eliminate the effect that age might have in the comparison of various parameters during the study, which is a fairly common practice. A one-tailed test of significance was used and no “Z” scores were given. Therefore, it is impossible to know if the results would have been significant using the more powerful two-tailed test.

This was one of the few intervention studies to include women as well as men although the generally favourable results for men did not apply to women, a point not generally emphasized.

(d) Primary prevention trials, multiple risk factor. Scientifically and from the point of view of understanding the effects of dietary fat, these are all unsatisfactory because, when changes do occur upon intervention, it is difficult to ascertain precisely the cause of those changes. They are also unsatisfactory from public health point of view. Changing several risk factors that are known to act cumulatively would be expected to produce a very positive effect, whereas the benefits demonstrated by these trials were marginal (Table 3).

(e) WHO collaborative trial. With 365,000 man-years of observation, this has been the largest study reported to date. It was conducted in factory populations in four European countries: Belgium, Italy, Poland and the U.K. (44 control and 44 intervention factories). The subjects were 60,881 men aged between 40 and 59 years. Although this was a randomized trial, it is not always appreciated that it was the factories rather than the individuals that were randomized. The trial lasted for 6 years.

The men in the intervention factories received advice about diet and the importance of not smoking, about increasing their physical activity and reducing their weight and were given information about the risks of high blood pressure. Those with a mean systolic blood pressure above 160 mm Hg were given hypotensive drug therapy. Although screening examinations were offered to all intervention subjects they were given only to a random 10% of control subjects. The end-points recorded in this study were total mortality, fatal CHD and non-fatal myocardial infarction.

The summary of this entire trial gives a list of percentage reductions in the various end-points, which appear to be encouraging. Yet the summary does not point out that none of the results are statistically significant. Since many readers will not read further than the summary, the impression gained is likely to be quite misleading. However, the overall poor result conceals quite large differences in outcome between the centres. Thus, in Belgium, there was a substantial reduction in risk factors with a commensurate reduction in incidence of CHD of 24% whilst in the U.K., little change in risk factors or mortality occurred.

The summary paper is quite confused about the extent to which the results demonstrate tangible benefits. For example, the authors state in the discussion that: “These benefits are large enough to be of great public health importance in relation to the small costs of intervention but they do not achieve the conventional level of significance and, therefore, by themselves, they constitute only moderate evidence that intervention is effective.” Yet later in the discussion, the authors claim: “This trial has yielded strong experimental evidence that among ordinary middle-aged men, advice on risk factor control is effective to the extent that it is accepted, and it appears to be safe”. These statements are hardly compatible and the expression “only moderate evidence” is meaningless.

(f) The Goteborg multifactor primary prevention trial. This randomized controlled trial involved 30,000 male subjects aged 47–55 years and extended over 12 years. The mean plasma cholesterol concentration at the start of the study was 6.8 mm, whilst the average diastolic blood pressure was greater than 115 mm Hg. The men smoked on average more
than 15 cigarettes per day. Subjects were educated about dietary and smoking habits and were given anti-hypertensive drug treatment. End-points were total mortality, deaths from myocardial infarction and stroke, as well as incidence of non-fatal myocardial infarction.

Although intervention in all three risk factors was apparently effective in reducing blood cholesterol, blood pressure and smoking, similar reductions occurred in the control group so that at the end of the trial there were no significant differences between the groups. This is similar to what occurred in the North Karelia Study.\textsuperscript{185,186,202,203} Clearly a natural decrease had been taking place in all three risk factors in the male population of Goteborg. Total mortality, stroke and CHD incidence did not differ significantly between intervention and control groups. Moreover total CHD incidence did not decline in Goteborg during the 12 year course of the study, despite the decline in the three major risk factors.

In regard to this last statement, this paper\textsuperscript{257} nicely illustrates the confusion that enters the authors' discussion when the results they obtain are not what they expected. Thus, they stated: "We have not been able to detect any general decline in CHD incidence in Sweden or in Goteborg in spite of downward trends in CHD risk factors during the 1970s". The reader might justifiably conclude that the influence of "conventional risk factors" on CHD is rather small. It is all the more extraordinary, therefore, to find the authors stating, further along in the discussion: "Thus, findings so far indicate that if a substantial effect on risk factor levels in intervention groups as opposed to control groups can be achieved, effects on CHD incidence and mortality can be expected". There appears to be no justification for this statement whatsoever from the results presented.

An interesting feature of the study that is easy to overlook, is that it covered all males born in the city during certain years. The intervention sample, therefore, contained men of all social classes, employed and unemployed, health conscious as well as those with no particular care for their health. One result of this strategy was a rather large group of "non-participants" in the intervention group (25%). This group contained a higher proportion of men of lower socio-economic status and more alcoholics. Their mortality and morbidity were significantly higher than in the rest of the group. This finding is in line with findings in other countries that the incidence of CHD is higher in lower socio-economic groups.

Another feature of this publication that is also easy to overlook is an editorial comment tucked away at the end of the paper (p. 288) by Professor Rose, a London-based epidemiologist. He comments that "the results of the trial are important but not in the way the investigators had hoped". He goes on to explain that the reason why they are important is that they demonstrate that: (1) Behaviour and risk factors in whole populations can change rapidly. (2) The high risk approach (illustrated by this trial, he says) is weak while a population strategy is effective.

These conclusions of Rose are quite unjustified. (a) The investigators had, apparently, no influence over what was happening to their "control" group and, therefore, it cannot be claimed that this trial demonstrates any truths about a population based strategy; (b) no evidence has been put forward that the decline in risk factors seen in Goteborg over the period of the study had anything to do with a planned population-based strategy, and (c) the decline in risk factors was not associated with improvements in the disease, so that the arguments are entirely academic.

\textit{(g) The Oslo study.}\textsuperscript{101} Although this was a randomized controlled dual risk factor intervention trial, the design was not double or single blinded. The number of subjects was considerably smaller than other trials. The subjects were 1232 apparently healthy men, 40–49 years old, in the upper quartile of the plasma cholesterol distribution with values between 7.5 and 9.8 mm. Interventions were: counselling to reduce smoking and allocation of the intervention group to a diet in which total fat had been reduced from 44% to 28% of calories and saturated fatty acids from 18% to 8% of calories. The P/S ratio was raised from 0.39 to 1.01. The study is held up as one of the best demonstrations that changing dietary fat can lower cholesterol significantly and show marked improvements in CHD risk. (Plasma total cholesterol was reduced 13% and coronary events were significantly
reduced in the intervention group but not overall CHD mortality.) However, there are reasons for being rather cautious about this optimistic interpretation of the trials' results, especially in regard to their universal applicability.

The study design was basically good and the statistics used were appropriate to the type of study. There was exceptionally good compliance and a low drop-out rate. However, the study was not blinded and there is evidence for unintentional changes being made by subjects in the control group. Only the men in the intervention group were asked about their eating and smoking habits at each follow-up period but no further detail was given about what this involved in either qualitative or quantitative terms. It is poor practice to treat the control group differently from the intervention group, other than in the specific interventions imposed. It was at least as important for the investigators to have checked on whether the controls, perhaps being aware of the implications of lifestyle for risk of CHD, had started to modify their behaviour, as it was for them to have checked on the compliance, or otherwise of the intervention subjects with the interventions. The fact that HDL concentrations showed a significant increase over the baseline value at 4-year follow-up might suggest that some environmental factor had changed during the course of the study.

An important feature of the trial was that men who were judged, after they had completed a diet questionnaire, to have been on a diet that might have been expected to result in low blood lipid concentrations, were excluded from the study. We know, however, that these men must have been unresponsive to diet, since they had already been chosen as having exceptionally high blood lipid concentrations, which must, therefore, have been caused by factors other than diet. Therefore, the study group consisted of men who were more likely to respond to diet than the total sample from which the group was selected. While this does not invalidate the study, and is indeed a common practice in such studies, it has to be borne in mind when interpreting the apparently encouraging results of the study and the use of those results to make recommendation to the general public: the subjects are not representative of the general population.

From a nutritionist's viewpoint, a weakness of the study was that there was insufficient information presented about the dietary advice given and no explanation of how the "food score" (which was used in the questionnaire to evaluate the cholesterolaemic potential of the men's normal diet) was calculated. It is not apparent whether these data are available in other publications. In the two tables presenting dietary data, the first tells the reader only that a change in the use of a particular food item occurred in a certain proportion of the subjects: not the extent of change in individuals. Thus we know that 80% of the subjects in the highest quintile of the intervention group used butter before the trial and only 1.6% after 5 years but we do not know how much of the foodstuff was used. Data for nutrient intakes are presented after 4 years on the trial but there is no information on what happened during the intermediate period.

The dietary advice was not the same for all men in the trial. Thus, for subjects with a high total cholesterol only, the dietary change consisted of a reduction in saturated fatty acid intake and a slight increase in the intake of polyunsaturated fatty acids. For those with elevated triacylglycerols (whether or not they were overweight) a reduction of total energy was also recommended. Despite this, the results of these subgroups were combined at the end of the study.

After 4 years, the intervention subjects had reduced their energy intakes from total fat and saturated fatty acids, increased their energy intake from polyunsaturated fatty acids and carbohydrates and increased the P/S of the diet. Despite the fact that total energy intakes in the two groups were not significantly different, the men in the intervention group had lost weight significantly after 4 years.

Two possible explanations may be envisaged. Either, the figures for energy intakes of the two groups at 4 years follow-up did not represent the overall energy intakes over the 4 year period. If so, this puts the other nutrient data into some doubt. Alternatively, other lifestyle factors that had not been measured may have changed; for example exercise levels. The weight reduction in the intervention group could partly explain the significant decrease
in total cholesterol independently of dietary fat. If this argument, that exercise levels increased, is correct, then the trial unwittingly incorporated another "risk factor" which further clouds the precise interpretation of the results.

Despite these criticisms, the study achieved a very significant reduction in plasma cholesterol in the intervention group (13%) especially as the diet was not strictly supervised. The significance for the general population, however, must remain in doubt as these were men with extremely high initial plasma cholesterol concentrations who had been selected as being more responsive to diet than the general population.

Another interpretational problem in this trial is that both smoking and diet were altered, making less certain the relative importance of these two risk factors. However, the authors concluded (using Cox's proportional hazards model) that the reduction in smoking (-45%) would have had less benefit than reduction in plasma cholesterol, a conclusion that seems to differ from several other studies. One possible explanation is that smoking is not a less powerful risk factor but that once a person has smoked, a level of damage has been done, which is not significantly influenced by later changes in smoking habits. Nevertheless, the authors were still forced to conclude that: "If this had been a dietary trial only, the difference in myocardial infarction incidence would probably not have reached statistical significance".

As regards end-points, there was a significant decrease in the rate of sudden death and in total coronary events in the intervention group, but the decrease in cardiovascular mortality was not significant mainly because fatal myocardial infarction was higher in the intervention group.

This trial has been quoted by adherents to the lipid hypothesis as providing conclusive evidence for the feasibility of practical and substantial cholesterol lowering by dietary advice and for the benefits that accrue in terms of reduced coronary disease. However, another interpretation is that a relative lack of benefit in terms of cardiovascular or total mortality was achieved despite a big reduction in fat (44 to 28% of energy) and the big increase in P/S (0.39 to 1.01).

(h) The U.S. Multiple Risk Factor Intervention Trial (MRFIT). This American study ranks as one of the largest, most complicated and most demanding medical experiments ever performed on a group of living human beings. It cost over $115 million, involved examining about 360,000 men, lasted 10 years and involved 250 research workers in 28 medical centres. After initial screening, the study group consisted of 12,866 men aged between 35 and 57 years in high risk categories for the three risk factors deemed to be the most important for intervention, namely elevated plasma cholesterol (>7.6 mmol/l), high blood pressure (diastolic > 90 mmHg) and a smoking rate of, on average, more than 30 cigarettes per day. The interventions involved advice to:

(1) Reduce saturated fatty acid intake to less than 10% of calories (later changed to less than 8%).
(2) Reduce dietary cholesterol intake to less than 300 mg/day (later changed to less than 250 mg/d).
(3) Increase PUFA to provide 10% of calories.
(4) Reduce smoking.

Anti-hypertensive drugs were administered. End-points were CHD and total mortality.

Marmot and Mann described the results as "disappointing", a very mild description of a trial that did not advance knowledge in any significant way. Whereas in the intervention group, changes in the risk factors went in the expected direction, the changes in the control group were also substantial, resulting in small differences between the two groups. Differences between the groups in terms of CHD or total deaths were insignificant. Over the study period, CHD mortality in the USA had declined by 25%. This trial cannot, therefore, be used to demonstrate that a reduction in plasma cholesterol or other risk factors will bring about a reduction in CHD incidence or mortality.

The results described in the 1982 paper referred to a follow-up period of about 6 years. In a paper published in 1990, the authors claimed that 3 years after intervention ceased
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(10.5 years follow-up), there was evidence of a benefit, namely a 10.6% reduction in CHD mortality and a 7.7% reduction in deaths from all causes. This was widely heralded as a vindication of the firmly-held belief that dietary fat modification could reap rewards provided that one was sufficiently persistent. However, this is yet another example of withholding from the headlines the information that the changes were still not statistically significant and, therefore, strictly speaking, not changes at all.

(i) Secondary prevention trials—the Oslo diet–heart study. In 1970, the Oslo diet–heart study group reported the results of an 11 year study. Intermediate results were published after 5 years. A group of 412 men, aged 30–64 years, were randomized 1–2 years after a first myocardial infarction. The experimental group were allocated to a diet containing 39% of calories as fat (104 g/day). Saturated, monounsaturated and polyunsaturated fatty acids accounted for 8.5%, 10.1% and 20.7% of energy respectively, giving a P/S of 2.4. Plasma cholesterol fell from an initial value of about 7.8 mmol/l to 6.3 mmol/l after 3 months and remained steady for the next 5 years. But by the eleventh year, it had crept up to 7.1 mmol/l, despite encouragement to remain on the diet.

No indication was given about adherence to the diet and indeed, although this was a diet–heart study, the quality of the nutritional data would not have warranted publication in a nutrition journal. The authors stated that: ‘‘The surviving controls were informed that a reduced fat intake possibly might be beneficial but they received no detailed dietary instructions’’. This yet again reveals the confusion between advice given to lower total fat intake and advice to exchange saturated with polyunsaturated fatty acids.

After 5 years, the incidence of fatal and non-fatal myocardial infarction was found to be significantly reduced (P = 0.05) but sudden death was uninfluenced. After 11 years, death from all causes had occurred in 101 of the experimental group and 108 controls (no significant difference). There was a significantly reduced myocardial infarction mortality (32 experimental; 57 control, P = 0.04) whilst the total number of coronary deaths (fatal myocardial infarction plus sudden death) was not significantly changed (79 experimental; 94 control, P = 0.09).

Although intervention was designed only to lower plasma cholesterol, other risk factors were also measured. Mortality from reinfarction was calculated according to age, plasma cholesterol, blood pressure, body weight and smoking habits and there was a highly significant interaction between them.

(j) The South Wales Diet and Re-infarction Trial (DART). This randomized controlled prospective intervention trial is related to the Caerphilly Heart Study described in Section IV.A.5 and studied 2033 men under the age of 70 who had suffered one acute myocardial infarction. The hypotheses examined were that re-infarction and mortality can be reduced in men who have survived one myocardial infarction by increasing the intake of dietary fibre and/or fatty fish and of decreasing the total fat intake. The end-points measured were: total mortality, CHD deaths and further non-fatal myocardial infarctions. The analysis was confined to events and deaths occurring within two years of entry into the trial.

The interventions involved advice designed to:
(1) reduce fat intake to 30% of total energy and to increase the P/S to 1.0,
(2) provide at least two weekly portions (200–400 g) of fatty fish (mackerel, herring, pilchard, sardine, salmon or trout),
(3) increase the intake of cereal fibre by 18 g daily.

A reduction of fat intake to 32% of energy was achieved in the experimental group but did not quite reach the 30% of energy hoped for by the researchers. The P/S was nearly doubled from 0.4 to 0.78. Consumption of eicosapentaenoic acid increased from 0.7 to 2.3 g per day. Cereal fibre intake doubled from 9 to 18 g/day.

There were no significant differences as a result of advice to modify fat or fibre but a highly significant reduction (P < 0.01) in CHD deaths and a significant reduction in total mortality (P < 0.05) as a result of advice to increase fatty fish consumption.

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The study was extremely well designed. It was factorial in nature so that men were randomized independently to receive advice or no advice on each of the three factors, resulting in eight different subgroups. This design enabled three interventions to be studied simultaneously so as to give evidence of each factor independently of the effects of the other two. However, this makes the assumption that there is no interaction between the different factors which seems quite unlikely. The randomization was extremely effective, as illustrated by Tables 3-6 in Burr et al.\textsuperscript{34} and this distinguishes the study from many others that have either been poorly randomized or not at all.

In view of the carefulness of the study design and execution, it is surprising to find no reference to the statistical methods used. This detracts severely from the value of the publications since the results of such studies often stand or fall by the rigour and appropriateness of the statistical evaluations used.

The authors commented on the disappointingly low fall in plasma cholesterol of the fat advice group and speculate that it may have been due to the small dietary difference achieved. However, although the reduction in total fat may have been disappointingly modest, the increase in P/S was not inconsiderable and provided a far higher ratio than in the U.K. population as a whole and in most Western countries. If useful changes in plasma cholesterol cannot be achieved with a diet that contains only 32% calories as fat and a P/S of nearly 0.8, then it must be concluded that this sort of dietary advice is not very helpful.

The effect of fatty fish was mainly on deaths from CHD. Re-infarctions that were not fatal were little affected. The evidence, therefore, suggests that intake of fatty fish reduced deaths and the severity of attacks but not the likelihood of attacks. The authors speculated that the beneficial effects might have been due to an effect on blood clotting through a mechanism involving EPA and the specific eicosanoids produced from it. Several factors would have changed by introducing the fish diet, however, and more research is needed to pinpoint the mechanisms involved.

The study provides further support for the argument, developed throughout this review, that factors influencing plasma cholesterol are of secondary rather than primary importance in CHD and that the true mechanisms are still not clear. It suggests also that research devoted more to the thrombotic effects rather than to atherosclerosis may be more productive.

V. CONCLUDING REMARKS

A. Deficiencies of the Lipid Hypothesis

In conclusion, the lipid hypothesis fails on several counts.

(1) There is insufficient correspondence in vascular pathology between animal models and man; myocardial infarction cannot be consistently reproduced in animals.

(2) Although progression to CHD in patients with FH is held to be the best evidence for the lipid hypothesis, the pathology is not comparable.

(3) International epidemiological evidence is flawed by confounding factors and selection biases. Some obvious inconsistencies have been ignored. For example, the very low rate of CHD in France, despite comparatively high SFA consumption, and the declining rates in Germany during a period when butter consumption was actually increasing.

(4) Within-countries, there is little support for an involvement of dietary lipids. Neither regional nor social class differences can be explained. In the U.K., the Asian population is at greater risk despite lower blood pressure, lower plasma cholesterol, and less smoking.

(5) Downward trends in CHD mortality (regardless of race, region or work status) in some countries cannot be explained by changes in either quantity or quality of dietary lipids, which do not follow an appropriate time sequence. There has been no corresponding downward trend in CHD incidence.
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(6) Neither the greater risk in men than in women, nor the enhanced risk of women after the menopause, nor the consistent falls over the past few decades in women compared with more erratic changes in men can be explained.

(7) Plasma total cholesterol is a weak predictor of CHD compared with haemostatic factors and less than half the risk of CHD is accounted for by known "risk factors". At least three factors that most strongly predict CHD risk cannot be modified anyway: close family history of heart disease; being male; and increasing age.

(8) Extrapolations from drug trials designed to lower blood lipids in high risk individuals to dietary advice for whole populations are unwarranted.

(9) The ultimate test of the lipid hypothesis is to demonstrate experimentally that lowering plasma LDL-cholesterol by dietary means, lowers the risk of CHD and reduces both CHD and total mortality (since, in developing countries, CHD deaths account for a high proportion of total mortality). Dietary changes that are likely to be acceptable to whole populations as distinct from high risk individuals, have minimal impact on plasma total cholesterol. Only very stringent diets achieve useful reductions.

While some studies have demonstrated statistically significant improvements in CHD morbidity, any improvements in CHD mortality have been small and there is no evidence for improvement in total mortality. Indeed, there is growing evidence that non-cardiovascular mortality may actually be increased, sometimes from other illnesses, sometimes from causes unrelated to illness. These have been brushed aside by those convinced by the rightness of the population dietary strategy but there is no rational reason to ignore such evidence and it should not lightly be dismissed. A recent report of a multifactorial primary prevention trial in Finland noted that while significant reductions in total CHD risk occurred during the first 5 years of intervention, after a further 10 years, total deaths in the intervention group were 67 and in the control group, 46. Of these, cardiac deaths were 34 (intervention) and 14 (control) and violent deaths 13 (intervention) and 1 (control). An editorial in the same issue of the journal reveals the mental contortions undergone by those who want to explain the results in terms of conventional wisdom. There is not a hint that the theory may be wrong and needs to be discarded.

B. Other Potential Research Areas

1. A Role for Modified LDL

There can be no doubt that lipids are involved in the progression of CHD both in its atherosclerotic and thrombotic phases; there is a wealth of evidence, some of which has been reviewed here. There is also evidence for lipid involvement in other components of heart disease, such as cardiac arrhythmias, which has not been reviewed here. The points of contention from this reviewer’s viewpoint are (1) whether plasma LDL has a causal role as distinct from an exacerbating role; and (2) whether dietary lipids have a primary role in either causation or exacerbation of the disease. I have concentrated here on the second aspect, namely dietary lipids but have mentioned in passing that the strongly held view for a causal role for LDL can be challenged.

Thus, haemodynamic stress can induce atherosclerosis in vessels of animals with very low LDL concentrations, suggesting that a high concentration of LDL is not a prerequisite for lesion development. The earliest lesions are small translucent mounds that gradually increase in size and contain little lipid, although they do contain LDL. The concentration of LDL in the interstitial fluid is over twice that in the plasma. The accumulation of LDL, therefore, seems secondary to some earlier or more primary process. Moreover, the concentration of Lp(a) in the gelatinous lesions accounts for most of the immobilized apoB-containing lipoproteins whereas in the plasma it is present at only about one-tenth of the concentration of LDL. Lp(a) is a variant form of LDL whose apoprotein is structurally related to plasminogen and binds to plasminogen binding sites on fibrin. It is a stronger predictor of CHD risk than LDL and may provide an important link between the atherosclerotic and thrombotic phases of the disease.
Research so far has revealed little influence of diet on its concentration in plasma but this is clearly an area where more investigation is needed.

Emphasis has shifted in recent years away from the atherogenic potential of LDL itself toward an understanding of the role of modified LDL and its uptake by the scavenger receptors of macrophages to form foam cells. LDL may be modified by a number of mechanisms, but *in vivo*, the most likely modifications to have practical importance are glycosylation of the apoprotein and peroxidation of the PUFA, which may then damage the apoprotein. Ideas gained from work at the cellular level find some support in epidemiology. Thus, Gey et al. studied 16 populations in Europe representing regions of high (Finland, Scotland), medium (Denmark, Northern Ireland, Israel) and low (Switzerland, Southern Italy, Southern France, Catalonia, Spain) CHD incidence. There was an 8-fold difference in CHD mortality between Glasgow, Scotland and Catalonia, Spain but no difference in mean plasma cholesterol. Cholesterol could not, therefore, explain the difference. The authors noted that when all 33 countries of the MONICA Study worldwide were considered, the influence of cholesterol is even weaker than in the 16 European populations studied here.

Plasma \( \alpha \)-tocopherol concentration, however, explained 63% of the variance in CHD mortality between these populations and this increased to 74% when other antioxidant vitamins were added and 87% when "classical risk factors" were also added.

This was a cross-sectional epidemiological study that showed strong statistical association between antioxidant nutrients and CHD mortality. It does not prove that having a high level of antioxidant defence will protect against heart disease any more than statistical associations between plasma cholesterol and CHD in other studies prove that high cholesterol *causes* CHD. Moreover, it was not a dietary study and cannot make any predictions about the effects of these nutrients in the diet. Dietary intervention studies are needed to clarify this point.

If subsequent studies confirm the role of antioxidant nutrients in the diet, the emphasis in dietary guidelines may need to shift away from emphasis on the modification of fat towards an emphasis on the consumption of fruit and vegetables rich in antioxidant nutrients. This does not in any way mean that advice to modify fat may not be beneficial for many *individuals*, especially those with clinical problems of overweight, diabetes mellitus or very severe hyperlipoproteinaemia.

2. Deprivation in Early Life: Metabolic Programming

There are two lines of evidence that an adverse environment during early life could have important effects on later risk of cardiovascular disease. First, height, which is largely determined by growth in early childhood, has been shown to be related to cardiovascular mortality in studies in three populations. Barker and his colleagues have collected data from all 212 local authority areas in England and Wales and shown that the geographical pattern of death rates from all cardiovascular diseases closely resembles that of neonatal mortality (deaths before one month of age) in the 1920s. The geographical distribution of maternal mortality was also closely similar to neonatal mortality and these studies are clearly beginning to establish a geographical association between high death rates from cardiovascular diseases, poor fetal growth and poor maternal physique and health. In addition to these associations which point to the importance of the intrauterine environment, the distribution of CHD, but not stroke, is related to high infant mortality between birth and one year and to an adverse environment in infancy as well as fetal life.

The same relationships are now being shown in individuals as well as populations. The records of 5654 men born in Hertfordshire (a prosperous area of the U.K. with a relatively low current rate of CHD) in 1911–30 have been traced. Among those whose weights were 8 kg or less at one year, CHD death rates were around three times greater than those who attained 12 kg or more. In between there was a strong and graded relationship which spanned more than 60 years.
Again, these are statistical associations only. Plausible biological mechanisms need to be found if these epidemiological relationships are to be developed into satisfactory theories about the development of CHD. It is plausible that the development of the vascular system and blood pressure in later life could be influenced by adverse inter-uterine conditions. Blood pressure and risk of hypertension in 50 year old men and women for whom very detailed clinical records at birth were traceable, was found to be strongly predicted by a combination of placental and birth weight. Systolic and diastolic pressures rose as placental weight increased and fell as birthweight increased. Highest pressures occurred among people who were small babies with large placentas. Reduced blood flow to the trunk in favour of the brain induced in a fetus that is small in relation to its placenta could have irreversible consequences, perhaps by influencing arterial structure. There is evidence that haemodynamic load in early life can alter the structure and compliance of larger arteries and this may provide a link with the views of Stehbens on the role of haemodynamic factors. Such metabolic and physiological programming at vulnerable periods in early life, producing a physiology that is ill-equipped to cope with affluent conditions later in life may apply to other processes such as the antioxidant defence system, the blood lipoproteins and the insulin sensitivity of tissues. In any case, these new avenues for research offer some hope of escaping from the impasse into which over-emphasis on the lipid hypothesis has driven this area of science.

3. Biomechanical Approaches

Insufficient attention has been given to Stehbens' views on the role of mechanical stress, resulting from turbulent flow, in the development of arterial lesions. Research needs to be concentrated on the underlying reasons why the arteries in some individuals are more susceptible to the effects of turbulence than in others. Differences in the structure of the proteins of the elastic tissues need closer examination. There is scope here for integrated research by biophysicists, bioengineers, physiologists, biochemists and cardiologists (with input, in the author's view from those with an interest in chaos theory).

C. Endpiece

From a nutritionist's point of view, there can be no argument 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 reduction or modification may not be beneficial for some individuals. However, this is quite a different matter from claiming, as did a BBC Television programme in March 1992, that “a fatty diet is the reason that one in five people in this country (U.K.) will die of a heart attack”. (Note that the claim is “the reason”, not “a reason”.) The programme's presenters further claimed that this statement had been agreed by “experts” after years of debate and conflicting information.

The arguments and discussion of the scientific evidence presented in this review will not convince those “experts” who have already made up their minds, for whatever reason, be it truly scientific or political, that a fatty diet is the cause of CHD. However, I hope that some readers, who were, perhaps, unaware that the lipid hypothesis had any shortcomings, will have been persuaded that the relationships between the fats we eat and the likelihood that we may die from a heart attack is by no means as simple as these simplistic statements imply. The fact that over 50% of CHD mortality is unexplained by any of the frequently described environmental factors, let alone by dietary fat consumption, and that morbidity and mortality changes have not followed similar patterns should make scientists sceptical of such over-generalizations. It should also give them a desire to probe more deeply into the true biological mechanisms underlying the upsurge and decline in CHD during this century. As long ago as 1951, one author wisely stated: “I have no doubt that we are grossly oversimplifying the problem of both the aetiology 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 40 years, this advice still holds good.
Acknowledgements—The author wishes to express his appreciation to all the many friends and colleagues with whom he has had helpful exchange of ideas, especially Margaret Ashwell, David J. P. Barker, Kurt G. Berger, Nazeli Borlak, Gerard Hornstra, David Kritchevsky, George J. Miller, Alexander McNair, Ray H. Rosenman, Tom A. B. Sanders and William E. Stehbens.

(Received 31 March 1992)

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