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6 Impact of Dietary Cholesterol and Fatty Acids on Serum Lipids and Lipoproteins in Man

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I. SUMMARY

The composition of the diet is an important determinant of serum lipid concentrations in man. An increase in dietary cholesterol causes an increment in serum total cholesterol, the excess cholesterol being located in both the low (LDL) and high density lipoproteins (HDL). Apparent differences in responsiveness are often due to chance fluctuations in lipoprotein levels; true differences do exist but are not large. Serum triglyceride concentrations are not systematically influenced by dietary cholesterol. Saturated fatty acids elevate LDL cholesterol when compared with isocaloric amounts of protein, carbohydrates, mono- or polyunsaturated fatty acids. This hypercholesterolaemic effect of saturated fatty acids holds essentially only for lauric, myristic and palmitic acid. Polyunsaturated fatty acids of the *n*–6 and *n*–3 series are about equally effective in reducing serum total cholesterol when compared with saturated fatty acids, but for *n*–3 fatty acids this is due largely to very low density lipoproteins (VLDL); LDL may even go up. Serum triglycerides are decreased, the decrease being more pronounced with *n*–3 than *n*–6 polyunsaturated fatty acids. Replacement of saturated by polyunsaturated fatty acids may lower HDL cholesterol concentrations when polyunsaturate intake exceeds about 15% of energy. Substitution of carbohydrates for isocaloric amounts of fat lowers both LDL and HDL cholesterol. The extent of cholesterol lowering depends on the fatty acid composition of the fat that is being replaced and on possible simultaneous changes in fatty acid composition. A decrease in the dietary fat: carbohydrate ratio also elevates serum triglyceride concentrations. Epidemiological observations are in agreement with the results of short-term, controlled dietary trials. Thus the effects of dietary lipids on serum lipids and lipoproteins described above appear to be permanent.

II. INTRODUCTION

The composition of the diet is an important factor in determining human serum lipoprotein concentrations. Increased concentrations of serum total and low density lipoprotein (LDL) cholesterol can cause atherosclerosis and ischaemic heart disease, and proper changes in the diet will delay manifestations of atherosclerosis. This applies to the entire population of Westernized countries, and especially to many hypercholesterolaemic patients, in whom lowering of LDL cholesterol will delay the occurrence of new ischaemic events.

When discussing the effects of dietary components on serum cholesterol levels, it is essential to discriminate between cholesterol carried by LDL, and that transported in the form of high density lipoproteins (HDL). There is abundant evidence that high concentrations of LDL, which carry 60–70% of total cholesterol in serum, cause atherosclerosis. Dietary intervention should thus aim at lowering of LDL cholesterol. High levels of HDL, on the other hand, might protect against atherosclerosis. Although it is not yet known whether increasing HDL levels through dietary change will lower the risk for coronary heart disease, it seems reasonable to aim at the formulation of diets that do not cause an undue decrease in HDL cholesterol. The role of subfractions as well as the compositions of LDL and HDL has not yet been clearly identified. This also holds for cholesterol and triglycerides in very low density lipoproteins (VLDL). We shall therefore largely focus this review on the effects of dietary lipids including cholesterol on serum total cholesterol, LDL cholesterol and HDL cholesterol. We will refer primarily to controlled studies in humans in which the test diets differed only with regard to the dietary component(s) under study. Epidemiological comparisons will also be considered, as they provide information on long-term effects as they occur under real-life conditions. An attempt is also made to describe the metabolic basis for the effects of dietary cholesterol and fatty acids on serum cholesterol concentrations.

III. EFFECTS OF DIETARY LIPIDS ON SERUM LIPOPROTEINS: SUITABLE AND UNSUITABLE EVIDENCE

Evaluation of the results of many published experiments on the effects of diet on serum lipoprotein metabolism is hampered by poor methodology. A good experimental design implies that possible time trends are taken into account by using control groups or crossover designs, and that dietary periods are long enough; 10–15 days being a minimum. Further, the number of subjects should be large enough so that diet-independent within-person

fluctuations in the concentrations of serum lipoproteins (Keys, 1967; Demacker *et al.*, 1985) are averaged out, and type II errors (i.e missing a real effect due to inadequate statistical power) are minimized. The mean effect of a certain diet factor can be estimated reasonably precisely with groups of 10–30 subjects. Obviously, food intake of experimental subjects has to be carefully controlled as lack of adherence makes the data useless. Therefore, most or all of the food should preferably be supplied. Chemical analysis of duplicate diets is to be recommended.

The test diets should only differ with regard to the components under study. In this respect, studies on the effects of macronutrients, unlike studies on micronutrients, carry particular difficulties. Fats or carbohydrates cannot be simply added to the diet: isocaloric amounts of other nutrients have to be left out in order to keep energy intake constant. If not, subjects will either gain or lose weight, which by itself could influence the concentrations of serum lipoproteins. The prerequisite that macronutrients under study must be substituted for isocaloric amounts of other macronutrients in the base diet implies that the observed effect is always the net effect of addition of some and omission of other macronutrients. As will be discussed below (cf. Table 3), replacement of dietary saturated fatty acids by polyunsaturated fatty acids effectively reduces serum cholesterol. Under specified conditions, two-thirds of the resulting drop in serum cholesterol is attributable to the saturated fatty acids removed, and one-third to the polyunsaturates added. Thus the impact of a given macronutrient on serum cholesterol should be considered in terms of a defined exchange of macronutrients. The issue is even more complicated for food items. The introduction of foodstuffs always will be associated with multiple changes in nutrient composition of the diet, unless such changes are carefully corrected for.

Many dietary trials have been performed with the use of liquid formula diets consisting of semipurified ingredients. Although caution is warranted in extrapolating the results, the composition of liquid formula diets can be controlled quite easily. On the other hand, when using test diets consisting of conventional, mixed, solid foods, it is very difficult to formulate diets differing only with regard to the components under study.

Controlled dietary trials generally last no more than a number of weeks, and therefore no information is obtained as to transient or retarded effects on serum lipoproteins. Such studies can fruitfully be complemented by epidemiological comparisons. Epidemiological data can also provide insight into the practicality of dietary recommendations. Thus the outcome of controlled trials should be compared with epidemiological data, it being appreciated that epidemiological data cannot provide information about the effect of a single dietary component, and that confounding factors can mask the anticipated relationships.

Numerous animal experiments have been carried out to unravel the effects of diet on serum cholesterol concentrations. The outcome has generated ideas to be tested in man, especially with regard to possible mechanisms underlying the effects of diet on cholesterol metabolism. However, the dietary effects on serum cholesterol in laboratory animals and humans differ quantitatively, and sometimes also qualitatively. Thus the animal data cannot be used to assess the impact of diet on serum cholesterol concentrations in man. This holds especially true for the effects of diet on separate serum lipoproteins, because most animals differ greatly from man with respect to the composition of lipoproteins and the distribution of cholesterol between serum lipoproteins (Chapman, 1980).

IV. DIETARY CHOLESTEROL

There is a persistent controversy about the effect of dietary cholesterol on the concentration of serum cholesterol in man (McGill, 1979). The conflicting results are partly due to poor design of the studies (Liebman, 1982), but individual differences in the sensitivity of serum cholesterol to dietary cholesterol have also been invoked (McGill, 1979; Katan *et al.*, 1986). In dietary trials cholesterol feeding is usually equivalent to whole egg or egg yolk feeding. One egg yolk contains about 250 mg of cholesterol and 5 g of fat, which is mainly monounsaturated. An increased consumption of eggs or egg yolks will replace some of the energy in the form of carbohydrates by an equivalent amount of fat and protein (Beynen and Katan, 1985a). In order to detect the effect of cholesterol *per se*, the control diet has to be balanced for the fat and protein in the eggs or egg yolks. In order to widen the theoretical base, effects of egg yolk cholesterol should be corroborated by studies using other sources of cholesterol.

A. Effect on Serum Total Cholesterol in Controlled Studies

The effect of dietary cholesterol on mean serum total cholesterol of groups of subjects can be predicted by the equation $\Delta\,\text{chol} = 1{\cdot}5\ (Z_2 - Z_1)$, where Δ chol is the change in serum cholesterol in mg/dl (1 mg/dl = 0·0259 mmol/l); Z_2 is the square root of the new cholesterol intake in mg/1000 kcal, and Z_1 the previous cholesterol intake (Grande *et al.*, 1965; Keys *et al.*, 1965b). This equation was based on low- and high-cholesterol diet comparisons, the diets containing various amounts and degrees of saturation of fat. Both crystalline cholesterol and egg yolk served as sources of cholesterol. The cholesterolaemic responses to the various levels of cholesterol intake were determined in five

different studies (Steiner and Domanski, 1941; Beveridge *et al.*, 1960; Connor *et al.*, 1961; Erickson *et al.*, 1964; Grande *et al.*, 1965).

In 1984, Keys re-evaluated the fomula for quantification of the dietary-cholesterol-induced change in serum cholesterol. For this purpose he used data concerning the serum cholesterol responses to dietary cholesterol in 20 controlled experiments in 10 different laboratories which had been reported after the first publication of the formula (Keys, 1984). Figure 1 illustrates the predicted (line) and observed (points) values for serum cholesterol changes produced by changing the cholesterol content of the diet. The observed values refer both to those on which the formula was based, and to those found after the formula had been established. It is clear that using the equation one can predict fairly well the group mean change in serum cholesterol concentration after a change in cholesterol intake. Figure 2 illustrates the relationship between predicted increase in serum cholesterol level and cholesterol consumption. It appears that at higher basal levels of

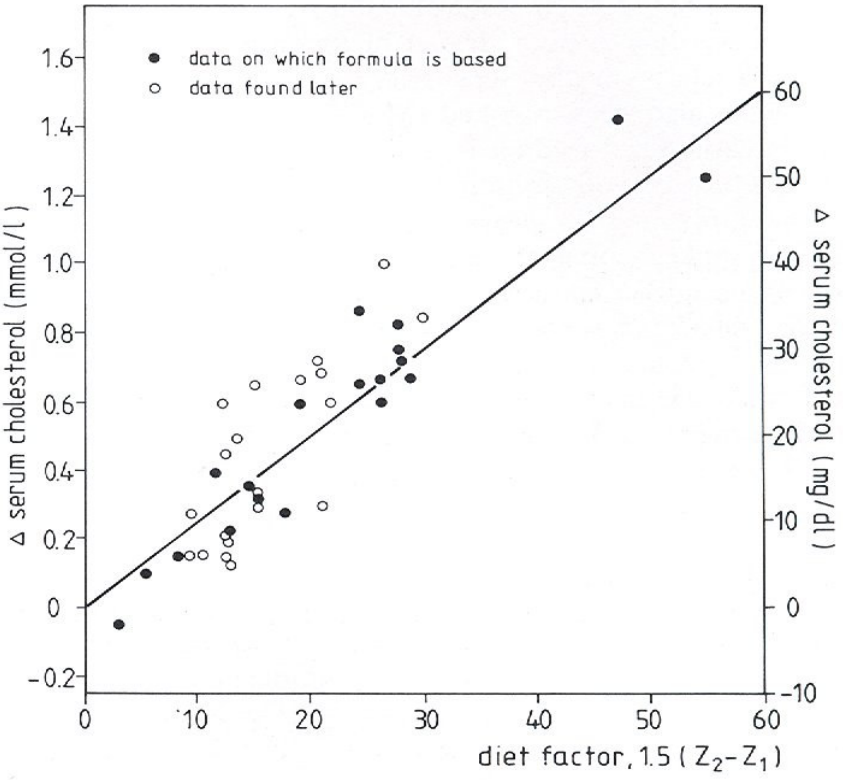

Fig. 1. Observed difference in serum cholesterol concentration after changes in cholesterol intake, and plotted against the diet factor 1·5 (Z_2-Z_1). Z is the square root of cholesterol intake, expressed as mg/1000kcal. The figure is based on Grande *et al.* (1965), Keys *et al.* (1965b) and Keys (1984).

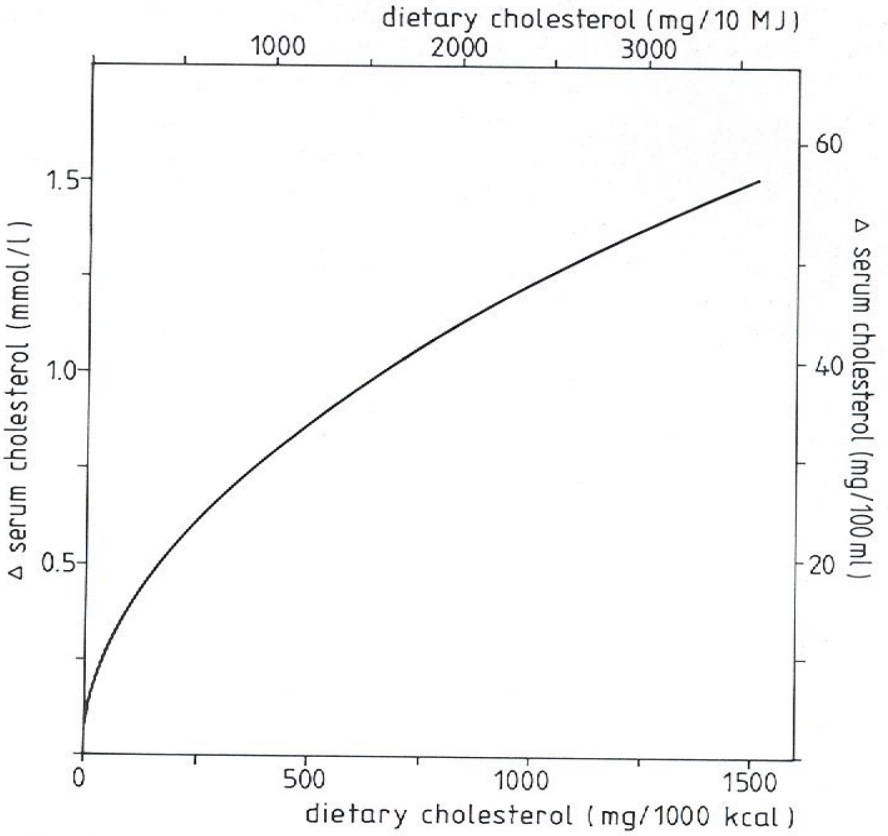

Fig. 2. Predicted change in serum total cholesterol concentration when cholesterol intake is changed from zero to the amount specified on the *x*-axis. The shape of the curve was calculated with the use of the formula of Keys *et al.* (1965b).

cholesterol intake the responsiveness of serum cholesterol to a given increase in dietary cholesterol is less pronounced than at lower basal intakes. However, there is no plateau at which dietary cholesterol no longer influences serum cholesterol.

1. Time Course of Serum Cholesterol Changes

The full effect on serum cholesterol of increased intakes of cholesterol is probably expressed within two to three weeks (Grande *et al.*, 1965, Connor *et al.*, 1961; Tan *et al.*, 1980; Katan *et al.*, 1986). Cholesterol loading studies in which subjects were challenged with 750–1500 mg of cholesterol per day, provided in the form of egg yolk, have demonstrated that after one day serum total cholesterol was not changed (Sodhi *et al.*, 1979; Katan and Beynen, 1983). After two days the mean increase in the concentration of serum cholesterol was 5%, and after 10 days it was 11% (Katan and Beynen, 1983). Thus serum cholesterol is influenced by the cumulative cholesterol

content of the diet in the previous two weeks and not that of the previous meal.

2. Hypo- and Hyperresponders

In many studies on the effect of dietary cholesterol on serum cholesterol in man a striking variability in the cholesterolaemic response between subjects has been noted (Keys *et al.*, 1965c; McGill, 1979). Although the concept of hypo- and hyperresponders became widely accepted, the reproducibility of individual differences in response has only recently been established. In repeated trials we have demonstrated that modest, stable differences in responsiveness of serum cholesterol to dietary cholesterol do exist in man (Fig. 3). From these experiments it is also clear that one will always find subjects who appear hyperresponsive in one experiment and hyporesponsive in another (Beynen and Katan, 1985b; Katan *et al.*, 1986). The wide scatter of responses seen in single experiments and in clinical settings is largely due to irreproducible chance fluctuations (Katan *et al.*, 1986). After correction for intraindividual fluctuations of serum cholesterol, the true width of the responsiveness distribution upon an increase in cholesterol intake from about 100 to 750 mg/day was found to be rather small. Assuming that the distribution is Gaussian, then 16% of the subjects would have a cholesterolaemic response of either less than half of the mean response or more than 150% of the mean. Only about 2% would show no increase at all (Katan *et al.*, 1986). A lack of responsiveness to dietary change should be ascribed

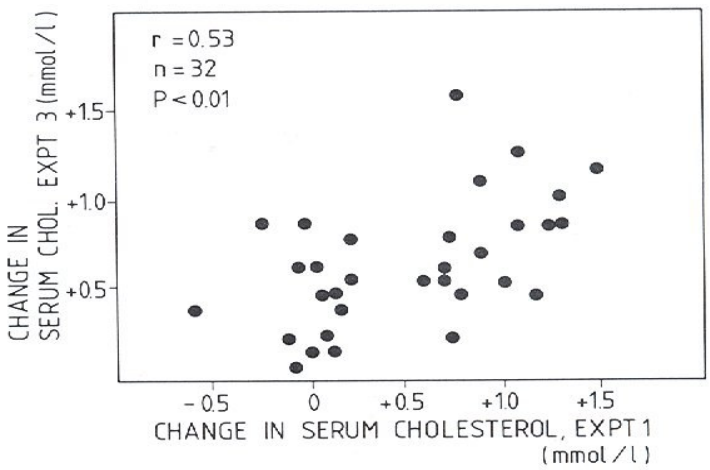

Fig. 3. Relationship between the individual responses of serum cholesterol to a change in dietary cholesterol observed in two different experiments. In experiment 1 the volunteers successively consumed 10 and 55 mg of cholesterol/MJ; in experiment three these values were 15 and 85 mg/MJ. Reproduced with permission from Beynen *et al.* (1985).

to poor adherence or to chance before the "hyporesponder" concept is invoked.

B. Effect on LDL and HDL Cholesterol in Controlled Studies

Table 1 summarizes the effects of dietary cholesterol on LDL and HDL cholesterol in various studies. Apart from one study (Ginsberg *et al.*, 1981), all studies have shown an increase in serum total cholesterol which was associated with an increment in LDL cholesterol. As a rough guide, it can be concluded that about 85% of the absolute increase in cholesterol found in serum after cholesterol feeding will reside in the LDL fraction.

In most studies dietary cholesterol also caused an increase in the concentration of HDL cholesterol. In vegetarians, however, Sacks *et al.* (1984) found that the ingestion of one egg per day reduced HDL cholesterol by 5%. Fisher *et al.* (1983) observed a decrease in HDL cholesterol after feeding cystalline cholesterol in a liquid fomula diet containing coconut fat as fat source (Table 1). No change in HDL cholesterol was seen when the liquid diet contained corn oil (Fisher *et al.*, 1983). Zanni *et al.* (1987) found an increase in HDL cholesterol with diets containing cholesterol and 31% of calories as corn oil (Table 1), but not with lard-containing diets.

Egg yolk is a convenient source of cholesterol for dietary trials. However, without correcting for the relatively large amount of fat that comes with it,

TABLE 1

Effect of dietary cholesterol as the only variable on serum total, LDL and HDL cholesterol, and triglyceride concentrations in controlled experiments in man employing diets low and high in cholesterol

	Dietary cholesterol (mg/1000 kcal)			Change in serum cholesterol (%)			Change in serum triglycerides (%)
Reference	*n*	Low	High	Total	LDL	HDL	
Katan *et al.*, 1986	94	49	234	+10		+ 4	
Nestel *et al.*, 1982	6	86	600	+ 9		+21	
Packard *et al.*, 1983	7	109	889	+29	+41	+18	−18
Ginsberg *et al.*, 1981	5	150	500	− 1	+ 1	− 8	+18
Applebaum-Bowden *et al.*, 1984	9	53	400	+ 6	+10	− 0	+ 3
Sacks *et al.*, 1984	17	55	218	+ 4	+11	− 5	+ 4
Nestel, 1986	6	79	392	+ 5	+ 4	+ 6	+18
Schonfeld *et al.*, 1982	11	120	300	+11	+15	+ 6	− 7
Fisher *et al.*, 1983	9	0	400	+ 6	+ 7	−10	+23
Zanni *et al.*, 1987	9	74	495	+16	+22	+28	− 2

the observed effects may be confounded. From the practical point of view the effects of egg yolk feeding are relevant because the general public is concerned with food items rather than nutrients. The addition of between three and six whole eggs or egg yolks to the diet of healthy subjects has repeatedly been shown to increase both LDL and HDL cholesterol concentrations (Mahley *et al.*, 1978; Mistry *et al.*, 1981; Porter *et al.*, 1977; Flynn *et al.*, 1979; Applebaum-Bowden *et al.*, 1979; Tan *et al.*, 1980; Flaim *et al.*, 1981; Oh and Miller, 1985; Beynen and Katan, 1985a). In a study with 34 subjects who habitually ate at least 1 egg per day, we observed that cessation of egg consumption, which reduced dietary cholesterol from 323 to 110 mg/1000 kcal, resulted in a decrease of serum total and HDL cholesterol by 5 and 3%, respectively (Beynen and Katan, 1985b). These effects may have been anticipated on the basis of the controlled (Table 1) and non-controlled cholesterol feeding studies in the laboratory.

C. Effect on Serum Triglycerides

Table 1 shows that increased intakes of cholesterol have no systematic effect on serum triglyceride concentrations. In egg feeding studies a decrease in fasting triglyceride and VLDL concentrations has been observed (Beynen and Katan, 1985a; Mistry *et al.*, 1981; Bronsgeest-Schoute *et al.*, 1979). However, it cannot be excluded that these effects had been caused by the fat in the egg yolks resulting in a decrease in the intake of carbohydrates.

D. Induction of β-VLDL and HDL_c

Cholesterol might cause the appearance in animals of lipoproteins not normally measurable (Mahley, 1978). These particles are β-VLDL, a β-migrating lipoprotein enriched with apoprotein E that floats in the VLDL region, and HDL_c, an HDL particle that is rich in cholesteryl esters and apoliprotein E, and that, unlike normal HDL, is precipitated by heparin-manganese reagent. The experimental evidence for induction of such particles by dietary cholesterol in humans is meagre.

Nestel *et al.* (1982) reported that in three out of six subjects tested cholesterol feeding caused an increase in the number of VLDL particles that bound to heparin on heparin-sepharose columns. This was taken as evidence for the induction of β-VLDL. Mistry *et al.* (1981) observed β-migrating VLDL on agarose gel electrophoresis in seven out of 14 subjects after egg yolk consumption. However, VLDL apo E levels have shown to be unaffected by dietary cholesterol (Fisher *et al.*, 1983).

The idea that cholesterol feeding induces the formation of HDL_c in humans

is based on a study of Mahley *et al.* (1978), who showed that egg feeding resulted in the appearance of HDL particles with increased ability to displace LDL from the apo B,E receptor in normal human fibroblasts in culture. These data have been interpreted as evidence for the induction of HDL_c. However, apo E concentrations in the HDL density region are not altered by cholesterol feeding (Fisher *et al.*, 1983; Beynen and Katan, 1985a). Likewise, dietary cholesterol does not influence whole serum concentrations of apo E (Schonfeld *et al.*, 1982; Nestel, 1986; Zanni *et al.*, 1987; Nestel *et al.*, 1982).

E. Epidemiological Data on Dietary Cholesterol and Serum Cholesterol

Apart from one study with a limited number of subjects (Connor and Connor, 1972), we are not aware of controlled, long-term studies with dietary cholesterol as the only variable. Thus the possibility of long-term adaptive effects not detected in short-term experiments cannot be fully discarded. Epidemiological studies may provide information on this point. Comparison of different populations entails a considerable risk that observed relationships between dietary cholesterol and serum cholesterol reflect the effect of saturated fat intake. Dietary saturated fatty acids are generally associated with cholesterol. However, within populations fat intakes do not differ as much as do cholesterol intakes (Beaton *et al.*, 1979). Thus a positive relationship between cholesterol intake and serum cholesterol within a population might indicate that the cholesterol-induced increase in serum cholesterol does not disappear after long-term exposure to high cholesterol intakes. Whether or not there is partial adaptation of serum cholesterol levels after cholesterol feeding remains unknown. Since epidemiological studies show relationships which are not necessarily causative, the data should be interpreted with reserve.

In several studies significant within-population correlations were found between cholesterol intake and serum cholesterol independent of other nutrients. In 6-year old children ($n = 84$), Crawford *et al.* (1981) found positive correlations for consumption of eggs ($r = 0{\cdot}54$) and dietary cholesterol ($r = 0{\cdot}45$). Similar correlations were reported by Knuiman *et al.* (1983) for young boys in Finland and in the Netherlands. Within a population of Tarahumara Indians ($n = 103$) Connor *et al.* (1978) observed a very strong relationship between dietary and serum cholesterol ($r = 0{\cdot}898$). These studies suggest that the effect of dietary cholesterol seen in controlled studies is not transient. This is supported by data of Vorster *et al.* (1987). Workers on an egg farm with long-term high intakes of cholesterol (about 1200 mg/day) had higher levels of serum cholesterol than control subjects with low intakes of

cholesterol (about 140 mg/day) but otherwise similar diet; the difference agreed quite well with that predicted from short-term experiments using Keys' formula.

F. Animal Experiments

Dietary cholesterol will increase serum total cholesterol in all common species of laboratory animals if given high enough loads. The extent of the increase depends on the animal species, and is determined by the flexibility of compensatory mechanisms such as enhanced excretion of bile acids and neutral steroids and depression of cholesterol synthesis, as well as by the efficiency of cholesterol absorption. There are qualitative and quantitative differences in the flexibility of compensatory mechanisms among different species, including man (Dietschy and Wilson, 1970; Beynen, 1988). Thus laboratory animals and humans differ with respect to the response of serum total cholesterol to dietary cholesterol in quantitative terms.

An increase in cholesterol intake from 250 to 1000 mg/day by a 70-kg man is equivalent to an increase from about 3·5 to 14 mg/day/kg body weight. In cholesterol feeding studies with animals the loads generally range from 0 to 250 mg/day/kg body weight. Unlike humans in cholesterol feeding trials, in most studies laboratory animals never reached a new steady-state because compensatory mechanisms are overwhelmed by these relatively huge amounts. As a result, large amounts of cholesterol accumulate in the body, especially in the liver, and pathological conditions develop (Beynen *et al.*, 1986). This in turn may lead to biased results.

The extremely high cholesterol loads used in animal studies should be considered in any attempt to extrapolate the outcome to man. The abnormal lipoproteins, β-VLDL and HDL_c, that occur in animal models (Mahley, 1978) are seen at cholesterol loads that are not realistic for man. This may explain why there is as yet no solid evidence that β-VLDL and HDL_c are formed after cholesterol feeding of humans.

G. Underlying Mechanisms

The increase in serum total cholesterol after cholesterol feeding of humans is due mostly to an increase in LDL cholesterol concentration. In addition, there may be an increase in HDL cholesterol that is small in absolute terms. In this section we attempt to describe the effect of dietary cholesterol on lipoprotein metabolism in molecular terms. Figure 4 presents a simplified scheme of the general pathways of cholesterol metabolism, which will be used to illustrate the metabolic basis for the hypercholesterolaemic action

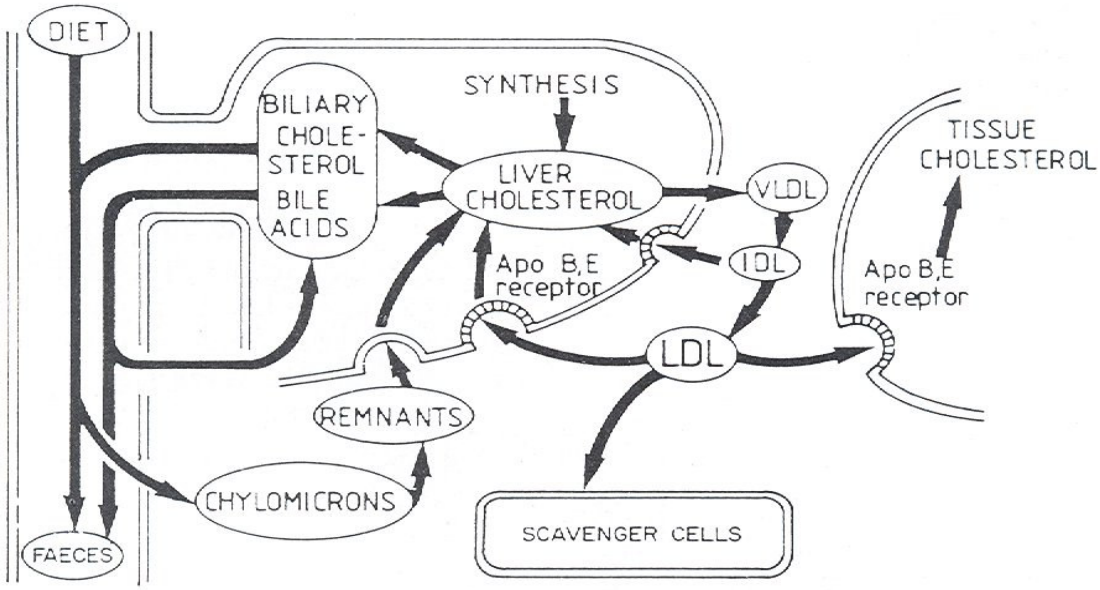

Fig. 4. Schematic presentation of cholesterol metabolism.

of dietary cholesterol.

In man the responses to increased amounts of ingested cholesterol are generally a diminished synthesis of cholesterol in the body and an enhanced excretion of neutral steroids (non-absorbed cholesterol) in the faeces (Dietschy and Wilson, 1970). Apparently, inhibition of cholesterol synthesis cannot prevent the genesis of a new steady-state in which serum cholesterol is increased. Upon an increase in cholesterol consumption more dietary cholesterol carried by the chylomicron remnants enters the liver. The liver is probably very efficient at removing chylomicron remnants from the plasma. The excessive uptake of cholesterol tends to cause an increase in liver cholesterol pools. The expanded cholesterol pools may theoretically trigger various compensatory mechanisms, namely an increased transport of cholesterol into bile, enhanced conversion of cholesterol into bile acids which also enter bile, inhibition of hepatic cholesterol synthesis, down-regulation of the LDL (apo B,E) receptor, and an increased output of apoprotein B-containing lipoprotein particles. Only the latter two metabolic responses lead to elevation of serum cholesterol concentrations.

There is some evidence that increased rates of transport of hepatic cholesterol into bile occur after cholesterol feeding, but this endogenous cholesterol is probably re-absorbed in the intestine (Kudchodkar, 1986). Thus this effect cannot be regarded an effective compensatory mechanism. It has been well documented that humans, in clear contrast to rats, do not respond to cholesterol loading by increasing bile acid production and

excretion (Beynen *et al.*, 1987).

The human liver responds to excessive uptake of cholesterol by secretion of cholesterol into the blood. The increased output of cholesterol by the liver would explain the observed increase in LDL production after cholesterol feeding (Packard *et al.*, 1983). LDL may be secreted as such into the plasma from the liver, but in essence it appears initially as its precursors VLDL and intermediate density (IDL) lipoproteins. Nestel and Billington (1983) have indeed shown that in man cholesterol feeding causes an increase in IDL-apoprotein B production. This may also explain the dietary-cholesterol-induced increase in HDL cholesterol, because precursors are formed from excess surface material during catabolism of VDL and IDL. As the triglyceride core of chylomicrons and VLDL is depleted, these particles shrink and surface material, primarily phospholipids and free cholesterol, is transferred to HDL.

Subsequently, the number of LDL receptors will decrease through down-regulation (Brown and Goldstein, 1986), as shown in blood mononuclear cells (Mistry *et al.*, 1981; Applebaum-Bowden *et al.*, 1984). In fact, the reduction in LDL receptors on peripheral cells from fasting blood after egg consumption is remarkably large, and cannot be accounted for by the quite modest changes in LDL; other, possibly post-prandial, lipoproteins are perhaps involved. As a result the receptor-mediated fractional clearance of LDL decreases (Packard *et al.*, 1983) but the absolute amount of LDL cholesterol delivered to the cells by the receptor pathway increases somewhat because the concentration of substrate (LDL) is increased. The rise in LDL production will also increase LDL clearance by the receptor-independent scavenger pathway (Packard *et al.*, 1983). In this way a new equilibrium is reached in which LDL production again equals LDL catabolism.

V. DIETARY FATTY ACIDS

In general, the replacement of saturated by polyunsaturated fatty acids in the diet is the single most powerful intervention to lower plasma cholesterol levels in man. However, this statement needs qualification. Among saturated as well as polyunsaturated fatty acids, individual fatty acids exert different effects on serum cholesterol. The cholesterolaemic effect of saturated fatty acids when compared to polyunsaturated fatty acids may also depend on the absolute amount of fat in the diet. In this section we refer to various studies which have contributed to the delineation of the relationships between dietary fats and serum cholesterol.

A. General Effects of Fat Type on Serum Total Cholesterol in Controlled Studies

It has been reported repeatedly that fats rich in saturated fatty acids raise serum cholesterol while oils rich in polyunsaturated fatty acids lower it. Keys *et al.* (1957, 1965a) integrated the results of various studies with human subjects, and were able to predict the cholesterolaemic effect of dietary fats by an equation. Gram for gram, saturated fatty acids are twice as potent in elevating serum cholesterol as polyunsaturated fatty acids are in bringing about a decrease. Monounsaturated fatty acids were considered neutral; that is, isocaloric substitution of carbohydrates for monounsaturated fatty acids had no effect on serum cholesterol (Keys *et al.*, 1958). Obviously, replacement of saturated by monounsaturated fatty acids will cause a decrease in serum cholesterol; this important concept is illustrated in Table 3. Keys *et al.* (1965d) later refined their predictions in that the cholesterol-elevating effect of saturated fatty acids is limited to fatty acids containing 12 (lauric acid), 14 (myristic acid) or 16 (palmitic acid) carbon atoms.

Table 2 compares a series of fats and their effects on serum cholesterol in man as predicted by the formula of Keys *et al.* (1965d). According to this equation the dietary-fat-induced change in serum cholesterol, Δ chol in mg/dl, is given by $1.2\ (2\Delta S - \Delta P)$ where S and P are percentages of total calories provided by glycerides of the saturated fatty acids lauric, myristic and palmitic acid and by polyunsaturated fatty acids, respectively. The table shows increases in serum total cholesterol occurring upon substitution of the indicated fats for safflower oil. Thus at a level of fat intake of 100 g per day, of which half consists of butter, the consumption of safflower oil at the expense of butter would cause a decrease in serum cholesterol by 35·6 mg/dl (0·92 mmol/l). It should be stressed that calculations such as in Table 2 only hold for group means and not for individual subjects. There can be a considerable between-person variation in the response of serum cholesterol to a change in dietary fat type (Ahrens *et al.*, 1957; Jacobs *et al.*, 1983; Katan *et al.*, 1988b).

Table 2 illustrates that coconut fat is as cholesterolaemic as butter. Thus the fatty acid composition of a fat, rather than its origin from plants or animals, determines the level of serum cholesterol.

Upon the isocaloric replacement of dietary fats, the omission determines the resulting serum cholesterol change as much as does the addition. Keys' formula indicates that two-thirds of the lowering of serum cholesterol seen after consumption of polyunsaturated fatty acids at the expense of saturated fatty acids is attributable to the saturated fatty acids removed, and only one-third to the polyunsaturates added. When exchanging foods changes in cholesterol intake (ΔZ, expressed as square root of mg dietary cholesterol

TABLE 2
Theoretical effects of various fats on serum cholesterol in subjects consuming 100 g of fat in a diet providing 2250 kcal and 400 mg cholesterol per day as predicted by the formula of Keys *et al.* (1965d)

Dietary fat	Fatty acids (g/100 g product)		Increase in serum cholesterol upon substitution per day of 50 g of a particular fat for 50 g of safflower oil	
	Saturated (C12–C16)	Polyunsaturated	(mg/dl)	(mmol/l)
Safflower oil	6·3	74·5	0	0
Linseed oil	5·3	66·0	1·5	0·04
Sunflower oil[a]	5·9	65·7	2·0	0·05
Soyabean oil	10·4	57·9	5·9	0·15
Corn oil	10·9	58·7	5·9	0·15
Rape seed oil[b]	6·4	33·3	9·9	0·26
Peanut oil	9·6	32·0	11·7	0·30
Cotton seed oil	23·5	51·9	13·6	0·35
Olive oil	11·0	8·4	18·1	0·47
Lard (pork)	25·3	11·2	25·6	0·66
Cocoa butter	25·5	3·0	26·3	0·68
Beef tallow	29·5	4·0	29·4	0·76
Palm oil	44·6	9·3	33·9	0·88
Butter fat	39·0	3·7	35·6	0·92
Coconut fat	69·6	1·8	47·7	1·24
Palm kernel oil	71·5	1·6	48·7	1·26

[a]Linoleic acid content over 60%.
[b]Erucic acid content, zero.
The effects on serum cholesterol of cholesterol in beef tallow (109 mg/100 g), lard (95 mg/100 g) and butter fat (256 mg/100 g) are accounted for, but this does not affect the rank order of cholesterolaemic effects of the fats. Fatty acid compositions of fats are based on data provided by the Consumer and Food Economics Institute (1979).

per 1000 kcal) also have to be accounted for. The following Keys' formula can be used: $\Delta \text{chol (mg/dl)} = 1{\cdot}2(2\Delta S - \Delta P) + 1{\cdot}5\Delta Z$ (Keys *et al.*, 1965d). This formula does not involve carbohydrates, proteins and monounsaturated fatty acids because these macronutrients are defined as neutral. However, when these nutrients are substituted for isocaloric amounts of saturated fatty acids, serum cholesterol will decrease.

Table 3 illustrates how the effect on serum cholesterol of changes in diet is the net effect of addition and omission of foodstuffs. Olive oil is rich in the monounsaturated oleic acid, as are new types of rape seed and other seed oils. When added to the diet at the expense of butter, serum cholesterol drops (Table 3). When added at the expense of such polyunsaturated fats as safflower, soyabean and corn oil, serum cholesterol will increase (Table 2).

TABLE 3

The effect on serum cholesterol of added foodstuffs, when different foods are replaced while energy intake is kept constant. The predicted change in serum cholesterol was calculated from Keys' formula (Keys *et al.*, 1965d). The basal diet provided 10 MJ (2400 kcal) per day

	Composition of the resulting diet				Predicted serum cholesterol change (mmol/l)
	Fatty acids (% of energy)			Cholesterol (mg/MJ)	
	Total	Saturated	Polyunsaturated		
None (basal diet)	38	15	6·5	24	—
25g of olive oil, isocalorically replacing butter	38	11	7·0	17	−0·4
25g of olive oil, isocalorically replacing potatoes and bread	47	16	7·1	24	0·0
Two eggs, isocalorically replacing cheese and meat	37	13	6·7	71	+0·1
Two eggs, isocalorically replacing toast and jam	42	16	6·8	76	+0·4

Replacement of potatoes and bread by isocaloric amounts of olive oil does not affect serum cholesterol (Table 3). Likewise, the effect of cholesterol consumption in the form of eggs depends on the overall change in the diet. Keys' formula predicts that if an amount of cholesterol equivalent to two eggs a day (500 mg/day) is added to a moderate-cholesterol diet (24 mg of cholesterol/MJ; 240 mg/day) without changes in protein or fat intake, the serum cholesterol level will rise by on average 0·29 mmol/l (11·3 mg/dl) or about 6%. The extent of the serum cholesterol change, upon consumption of whole eggs, however, depends on what foods the eggs replace in the diet (Table 3).

1. Long-term Controlled Trials

There are no long-term controlled trials in which the type of fat was the only dietary variable. Apart from an increase in polyunsaturated fatty acids

at the expense of saturated fatty acids, the intake of cholesterol was also lower in the experimental diet of the studies of Leren (1970), Dayton *et al.* (1969) and Miettinen *et al.* (1972). In these studies the control and experimental diets were fed to patients with myocardial infarction, men living in a home for military veterans, and patients of mental hospitals, respectively. The subjects received the diets for periods of up to six years. On the experimental diets serum cholesterol concentrations dropped by 12 to 15%, and remained at the lowered concentration throughout the entire experimental period. A small portion of the lowering in serum cholesterol might be due to the decrease in cholesterol intake. Nevertheless, these long-term trials strongly suggest that the hypocholesterolaemic effect of dietary polyunsaturated fatty acids is stable over long periods of time.

B. Saturated Fatty Acids and Serum Total Cholesterol

1. Fatty Acids with 4 to 10 Carbons

In coconut and palm kernel oil and butter fat, fatty acids with up to 10 carbons make up about 12% of the saturated fatty acids. Normal mixed diets provide some 1–10 g/day, depending on dairy intake (Katan *et al.*, 1984). A mixture of saturated fatty acids with chain lengths ranging from C6 to C10 produced lower serum cholesterol levels than butter fat but higher levels than corn oil (Hashim *et al.*, 1960). When compared with safflower oil, a mixture of caprylic (C8:0) and capric acid (C10:0) did not affect serum cholesterol concentrations (Uzawa *et al.*, 1964). Beveridge *et al.* (1959) reported that the incorporation of a mixture of short- and medium-chain fatty acids equivalent to 30% of total calories into a fat-free formula diet, at the expense of carbohydrates, did not alter serum cholesterol. Substitution of butyric acid for isocaloric amounts of carbohydrates did not influence serum cholesterol (Keys, 1958; cited by Keys *et al.*, 1965d). Thus these limited studies would suggest that fatty acids with 4 to 10 carbons have no effect on serum total cholesterol relative to carbohydrates or oleic acid.

2. Lauric, Myristic and Palmitic Acid

Daily intakes of C12:0, C14:0 and C16:0 range from 30 to 60 g, with C16:0 providing the bulk. There is controversy about the relative effects on serum cholesterol of lauric, myristic and palmitic acid. The formula established by Keys *et al.* (1965d) does not discriminate between these fatty acids. On the basis of experiments with a series of natural fats, Hegsted *et al.* (1965) concluded that palmitic acid has a much lesser hypercholesterolaemic effect than does myristic acid. In addition, lauric acid was found not to have a specific effect on serum cholesterol. In a subsequent study with semisynthetic

oils, McGandy *et al.* (1970) observed that myristic acid and palmitic acid were equally active in elevating serum cholesterol, their effect being greater than that of lauric acid. Using synthetic triglycerides, Vergroesen and De Boer (1971) demonstrated that lauric and myristic acid had similar cholesterol-elevating effects, irrespective of whether the experimental fats contained high amounts of either oleic or linoleic acid. Both lauric and myristic acid were more hypercholesterolaemic than palmitic acid.

3. Stearic Acid

Daily intake of C18:0 ranges from 10 to 20 g. Based on studies with readily available natural oils, including cocoa butter which contains about 35% of total fatty acids as stearic acid, Keys *et al.* (1965d) assigned a neutral role to stearic acid. Grande *et al.* (1970) have performed well-controlled studies using mixtures of natural fats which confirmed the lack of effect of stearic acid. Relative to palmitic acid, stearic acid was found to have a cholesterol-lowering effect. Using natural fats, Hegsted *et al.* (1965) arrived at the conclusion that stearic acid, like short-chain fatty acids, had no specific effect on serum cholesterol. Bonanome and Grundy (1988) recently confirmed this finding in a well-controlled trial.

If one uses natural oils it cannot be excluded that they contain an unknown factor responsible for the observed cholesterolaemic effect. When semisynthetic fats were utilized, stearic acid was found to be almost as hypercholesterolaemic as palmitic acid (McGandy *et al.*, 1970). This corroborates work of Vergroesen and De Boer (1971), who also reported that palmitic and stearic acid were equally effective in elevating serum cholesterol concentrations.

It thus appears that there is controversy as to the cholesterolaemic effect of stearic acid itself, and further studies are required on this point. However, stearic acid in the form in which it usually occurs in foods appears to be less hypercholesterolaemic than other saturated fatty acids.

C. Monounsaturated Fatty Acids and Serum Total Cholesterol

Monounsaturated fatty acids are fatty acids with one double bond. Double bonds cause bends in the carbon chain, which interfere with crystal formation and keep the fat liquid, producing an oil. C18:1 is the most abundant monounsaturated fatty acid in the diet; mixed diets provide some 20 to 80 g/day. Most of this occurs as the *cis*- isomer, oleic acid. Oleic acid is found abundantly in various plant oils, such as olive oil, rape seed oil and new types of sunflower and safflower oil, the relative percentage being about 65–85%.

Trans octadecanoic acid (elaidic acid), the geometric *trans* isomer of oleic acid, is formed during hydrogenation of oils. The hydrogenation process results in geometrical and positional changes in the double bonds of unsaturated fatty acids as well as in a reduction of the number of double bonds. Partially hydrogenated vegetable oils form a major source of dietary fat in various Westernized countries. Margarines and shortenings consisting of hydrogenated oils may contain up to 50% of *trans* fatty acids, much of it in the form of elaidic acid. Daily intakes are typically 0 to 5 g/day.

1. Oleic Acid

On the basis of studies in which various readily available natural fats were fed at levels ranging from about 20 to 40% of the calories, it has been concluded that oleic acid has no cholesterolaemic effect different from that of equivalent amounts of carbohydrates (Keys *et al.*, 1965a; Hegsted *et al.*, 1965). Using various synthetic fats containing either 70% of linoleic or oleic acid, serum cholesterol concentrations were found to be consistently lower with linoleic acid, irrespective of whether the rest of the dietary fat consisted of lauric, myristic, palmitic or stearic acid (Vergroesen and De Boer, 1971). This cholesterol-elevating effect of oleic acid, compared with linoleic acid, agrees with the equations of Keys *et al.* (1965a) and Hegsted *et al.* (1965).

Mattson and Grundy (1985) used liquid formula diets containing 40% of calories of fat either in the form of high-linoleic or high-oleic safflower oil. When compared with a diet rich in palmitic acid, both diets resulted in equal lowerings of serum cholesterol. This could be due to the high content of linoleic acid in both the low- and high-oleic acid diet. As will be discussed below, the cholesterol-lowering action of linoleic acid reaches a plateau at high levels of intake.

2. Elaidic Acid

Conclusive results as to specific effects of dietary *trans* fatty acids can only be obtained by using their *cis* counterparts as controls and leaving the rest of the fatty acids unchanged—no mean test. Vergroesen (1972) reported that elaidic acid at a level of 40% of calories caused slightly higher concentrations than did oleic acid, this effect being independent of the amount of linoleic acid in the diet. In other studies elaidic acid was also found to be slightly hypercholesterolaemic (Anderson *et al.*, 1961; McOsker *et al.*, 1962). However, in a later study by Mattson *et al.* (1975) no such effect was seen. It appears that *trans* fatty acids, even at high levels of intake, do not have a major effect on serum cholesterol. The hypercholesterolaemic effect of elaidic acid has been found to be markedly smaller than that of equal amounts of a mixture of lauric and myristic acid (Vergroesen, 1972; Vergroesen and Gottenbos, 1975).

D. Polyunsaturated Fatty Acids and Serum Total Cholesterol

1. n–6 Fatty Acids

Linoleic acid (C18:2, *n*–6) is the most abundant fatty acid of the *n*–6 series. Mixed diets typically provide some 10 to 20 g/day. Linoleic acid occurs at high concentrations in various plant oils such as sunflower oil (65%), maize oil (60%), soyabean oil (55%) and sesame oil (40%). In soft margarines up to 60% of the fatty acids can be linoleic acid.

γ-Linolenic acid (C18:3, *n*–6) is synthesized in the body from linoleic acid by desaturation. Compared to the usual dietary lipids this fatty acid is rare; mixed diets provide some 0·1 g/day. Although present in the membrane phospholipids of mammals and in breast milk, it is only in the red meat of herbivores that it occurs in appreciable amounts. Two principal genera of plants contain relatively large amounts of γ-linolenic acid (up to 20% of total fatty acids). These plants are the evening primroses and the currants, in particular the blackcurrant.

Arachidonic acid (C20:4, *n*–6) is synthesized in the human body from linoleic acid. It is present at low concentrations in membranes of animal tissues; mixed diets provide 0·2 to 0·4 g/day.

(a) Linoleic acid. For most dietary fats the term polyunsaturated fatty acids in the Keys' formula refers to linoleic acid. This indicates that linoleic acid has a specific cholesterol lowering activity. Confirmation has been provided by studies using diets containing synthetic fats, and formulated so that the effects of linoleic acid could be compared directly with those of lauric, palmitic, oleic and stearic acid (Thomasson *et al.*, 1967; Vergroesen and De Boer, 1971). Compared with these fatty acids linoleic acid was hypocholesterolaemic. However, unlike what would be expected on the basis of the Keys' formula, this effect became a plateau at high intakes. According to Vergroesen (1972) and Brown (1971) no further reduction of serum cholesterol concentrations is seen at linoleic acid intakes higher than about 15% of total calories.

(b) γ-Linolenic acid. Horrobin and Manku (1983) conducted a placebo-controlled, double-blind crossover study in which patients with atopic eczema were treated with daily doses of 2, 4 or 6 g of evening primrose oil, containing about 10% of γ-linolenic acid and 70% of linoleic acid. After 12 weeks, serum cholesterol levels had dropped in a dose-dependent manner, the decreases being 14, 17 and 22%, respectively. This study suggested that evening primrose oil contains a potent hypocholesterolaemic factor, perhaps γ-linolenic acid. The nature of the placebo was not given (Horrobin and Manku, 1983), but it probably was liquid paraffin (Wright and Burton, 1982). In a study without a control group, Chaintreuil *et al.* (1984) showed that 2 g

of γ-linolenic acid per day, but not 0·5 g, given in the form of evening primrose oil, lowered serum cholesterol concentrations in diabetics by about 13%. Boberg *et al.* (1986) did not detect an effect on serum cholesterol of 2 g of evening primrose oil daily in hypertriglyceridaemic patients. These authors employed a double-blind crossover design with olive oil as placebo. Thus the cholesterol-lowering activity of γ-linolenic acid remains in doubt.

(c) Arachidonic acid. Worne and Smith (1959) have measured the response of serum cholesterol in volunteers after supplementing their diet with 4 g daily of either ethyl linoleate or methyl arachidonate. After 90 days, arachidonate had reduced serum cholesterol concentrations by about 20% compared with linoleate. Kingsbury *et al.* (1961) reported that ethyl arachidonate reduced serum cholesterol more effectively than cod-liver oil concentrate. Thus arachidonic acid might have a potent hypocholesterolaemic activity.

2. n–3 Fatty Acids

n–3 Fatty acids are characterized by having their first double bond located at the number 3 carbon counted from the methyl end of the fatty acid chain. Two types of *n*–3 fatty acids are found in human foods. α-Linolenic acid (C18:3 *n*–3) is present at high concentrations in linseed oil (50%), at lower concentrations in soyabean and rape seed oil (10%), and in trace amounts in many plant foods. Daily intake in man is typically some 1 to 3 grams. The other type are the very-long-chain *n*–3 fatty acids with 5 or 6 double bonds. These are typically found in fish oils, which are relatively rich in eicosapentaenoic acid (C20:5, *n*–3) and docosahexaenoic acid (C22:6, *n*–3). Typical consumption of these fatty acids in man is 0·1 to 0·8, and 0·1 to 1 g/day, respectively. It is uncertain whether these latter two fatty acids can be formed in appreciable amounts from dietary C18:3, *n*–3 in man. There are few or no natural foods that provide large amounts of *n*–3 fatty acids; as a result, daily intakes are an order of magnitude lower than those of *n*–6 fatty acids. This appears to make the effects of *n*–3 fatty acids on lipids and lipoproteins of purely academic interest, because it does not help to know that C20:5, *n*–3 affects blood lipids if you cannot get more than a gram per day from natural sources. However, natural sources can be supplemented by nutrient supplements, which are nowadays consumed by many healthy people in vast amounts. Also, the fats and oils industry is expert at producing edible fats of any composition that appeals to the customer. The problems involved in manufacturing stable, safe and tasty margarines high in C20:5, *n*–3 and C22:6, *n*–3 are huge, but not too huge for modern science and technology. Thus, effects of *n*–3 fatty acids merit detailed attention.

Ethyl linolenate and linoleate at intakes of 4 g per day reduced serum

cholesterol to about the same extent (Worne and Smith, 1959). Singer *et al.* (1986) have compared the effects of sunflower and linseed oil, which essentially entails comparing linoleic and α-linolenic acid. The cholesterol-lowering activity of both oils was found to be similar.

The effects of various fish oils on serum lipids in man were first investigated about three decades ago (Bronte-Stewart *et al.*, 1956; Keys *et al.*, 1957; Malmros and Wigand, 1957; Ahrens *et al.*, 1959). The general finding was that fish oils produce as low levels of total serum cholesterol as vegetable oils rich in polyunsaturated *n*–6 fatty acids. More recent data are summarized in Table 6. Obviously, the effects of fish oil on total cholesterol are somewhat equivocal. However, few of these studies entailed the specific exchange of very-long-chain *n*–3 fatty acids against equal amounts of other fatty acids that one would like to see, and in several cases cholesterol intakes were also not well equalized across dietary treatments. Harris *et al.* (1983) have compared *n*–6 and *n*–3 fatty acids. The test diets provided about 20% of total fatty acids in the form of either *n*–6 or *n*–3 fatty acids. When compared to the diet rich in *n*–6 fatty acids, the diet containing *n*–3 fatty acids reduced serum total cholesterol. The major difference between the diets was in the amounts of *n*–6 and *n*–3 fatty acids, but the diet rich in *n*–6 fatty acids also contained more stearic acid and fewer monounsaturated fatty acids (C16:1, *n*–7; C20:1, *n*–9; C22:1, *n*–11). Thus it cannot be concluded that, gram for gram, *n*–3 fatty acids more effectively reduced serum cholesterol concentrations than their *n*–6 counterparts. Effects of fish oil on serum lipoproteins will be discussed below.

E. Fat Type and LDL and HDL Cholesterol in Controlled Studies

1. Fats Rich in n–6 *Fatty Acids*

Table 4 summarizes the results of studies in which the effects of increased intakes of *n*–6 polyunsaturated fatty acids on LDL and HDL cholesterol concentrations were measured. We assume that in these studies the increased consumption of polyunsaturated fatty acids was effected at the expense of saturated fatty acids while monounsaturated fatty acids were kept constant. However, since various authors reported the dietary P/S ratios but not the fatty acid composition of the experimental diets, our assumption may not be correct. It cannot be excluded that P/S ratios were raised by lowering the intake of saturated fatty acids at the expense of monounsaturated fatty acids. As stressed above, such a change in dietary fatty acid composition will also lead to cholesterol lowering.

As would be anticipated, increasing the consumption of polyunsaturated fatty acids results in a decrease in LDL cholesterol (Table 4). In keeping

TABLE 4

Effect of increased proportions of polyunsaturated fatty acids in the diet on serum total, LDL and HDL cholesterol, and triglyceride concentrations at a constant total fat intake in controlled experiments in man

		P/S ratio of diet		Amount of dietary fat (% of energy)			Change in serum cholesterol (%)			
				Total	Polyunsaturated					Change in serum triglycerides
Reference	*n*	Low	High		Low	High	Total	LDL	HDL	%
Weisweiler *et al.*, 1985	22	0·2	1·0	35	3	13	−11	−13	+ 4	−20
Kuusi *et al.*, 1985	39/39	0·4	0·9	24	5	8	− 8	− 9	− 4	−16
Schwandt *et al.*, 1982	30	0·3	1·0	37	6	12	−11	−14	0	−14
Jackson *et al.*, 1984	6	0·4	1·0	40	7	13	− 6	− 9	−10	+ 6
Jones *et al.*, 1987	15/16	0·3	1·0	39	6	11	− 3		+ 5	+18
Brussaard *et al.*, 1980	14/15	0·2	1·7	40	3	19	−10	−15	− 1	−20
Katan *et al.*, 1988a	47	0·2	1·9	45	5	21	−16	−21	− 7	−13
Vessby *et al.*, 1980	30	0·2	2·0	44	4	20	−12	− 8	− 5	−13
Schaefer *et al.*, 1981	11	0·2	2·0	40			−11	− 9	−16	− 7
Zanni *et al.*, 1987	19	0·6	2·1	31	3	17	−12	−14	−17	−11
Chait *et al.*, 1974	23	0·2	2·4	40			−16	−10	0	−39
Fisher *et al.*, 1983	9	0·1	2·7	31	3	14	−32	−36	−21	−32
Harris *et al.*, 1983	7	0·5	3·4	40	7	21	− 9	− 9	+ 2	− 1
Shepherd *et al.*, 1980	8	0·3	4·0	40			−23	−22	−20	−14
Becker *et al.*, 1983	12	0·2	4·4	40	4	20	− 8	−13	+ 9	− 6
Mattson and Grundy, 1985	12	0·2	6·5	40	4	28	−16	−18	−13	− 7
Vega *et al.*, 1982	10	0·2	6·5	40			−25	−26	−15	−23
Turner *et al.*, 1981	15	0·2	8·0	40			−16	−19	−14	− 1

P = polyunsaturated fatty acids: S = saturated fatty acids.

with an early report of Nichaman *et al.* (1967), it appears that polyunsaturated fatty acids, when compared to saturated fatty acids, also lower HDL cholesterol levels. Increasing the proportion of energy provided by polyunsaturated fatty acids from 5 to 20% causes a decrease in HDL cholesterol of about 5%. However, some of the experimental diets had extremely high P/S ratios which cannot, or only laboriously, be accommodated into a natural mixed solid diet. The lowering of HDL cholesterol seen on such experimental, extreme high P/S diets appears to be less or absent at a dietary P/S ratio of 1·0 (Table 4), which is the ratio generally recommended to the general public.

2. Fats Rich in Oleic Acid

An increase in dietary monounsaturated fatty acids at the expense of saturated fatty acids causes a decrease in serum total cholesterol concentrations. Mattson and Grundy (1985) suggested that, unlike polyunsaturated fatty acids, monounsaturated fatty acids leave HDL cholesterol unchanged. They fed human subjects liquid formula diets with either palm oil, high-oleic safflower oil or high-linoleic safflower oil. Both the high-oleic and high-linoleic safflower oil caused a reduction of LDL cholesterol by about 19%, when compared with palm oil. However, the oleic acid-rich safflower oil tended to leave HDL cholesterol unchanged, whereas linoleic acid reduced it by about 13%. Possibly, such an effect is also obtained with olive oil instead of high-oleic safflower oil (Zoppi *et al.*, 1985; Sirtori *et al.*, 1986).

3. Fats Rich in n–*3 Fatty Acids*

(a) Fish oils. Fish oil concentrates are nowadays commercially available in the form of gelatin capsules. Such concentrates usually have defined contents of eicosapentaenoic and docosahexaenoic acid, and contain added vitamin E to protect against lipid peroxidation. The availability of *n*–3 fatty acid concentrates has made the pursuance of the lipidaemic effects of these fatty acids much easier.

It is clear from Table 5 that intakes of *n*–3 fatty acids up to 5 g/day do not systematically affect serum total cholesterol concentrations. However, both LDL and HDL cholesterol concentrations may be increased under these conditions. Some of the studies in Table 5 were not strictly controlled. Furthermore, the effect of increased intakes of fish oil on serum cholesterol may be dependent on the types of foodstuffs that are displaced from the diet. Relatively low intakes of fish oil concentrate (up to 15 g/day; up to 4 g of eicosapentaenoic acid/day) were ineffective in lowering serum cholesterol in studies based on a placebo-controlled, double-blind design with (Simons *et al.*, 1985; Boberg *et al.*, 1986) or without (Sanders *et al.*, 1985; Harris *et al.*, 1987) a crossover sequence. Very high intakes of fish oil (up to 50 g/day) may lower serum cholesterol (Harris *et al.*, 1983; Nestel *et al.*, 1984;

TABLE 5
Effect of ingestion of fish oil concentrates in capsule form on serum lipids in human subjects

Reference	Approximate intake of *n*–3 fatty acids (g/day)			Change in serum lipids (%)			
				Cholesterol			Triglycerides
	EPA	DHA	*n*	Total	LDL	HDL	
Bronsgeest-Schoute *et al.*, 1981	0·4	0·7	9	+ 2		+ 2	−15
Bronsgeest-Schoute *et al.*, 1981	0·7	1·2	9	+ 3		+ 6	− 4
Bronsgeest-Schoute *et al.*, 1981	1·3	2·1	10	+ 3		− 2	− 1
Bronsgeest-Schoute *et al.*, 1981	2·6	4·2	11	− 1		+ 5	−39
Nestel *et al.*, 1984	10	7	6/7	−15	+10	−18	−70
Hamazaki *et al.*, 1984	1·6	1·0	12	−10			−31
Saynor *et al.*, 1984	3·2	2·3	107	− 1		+10	−37
Simons *et al.*, 1985	1·1	0·7	13	− 2	+ 5	+ 5	−17
Simons *et al.*, 1985	2·9	1·9	15/12	−12	+ 9	+ 7	−48
Sanders *et al.*, 1985	2·9	2·0	11	+ 3		+13	−26
Popp-Snijders *et al.*, 1986	1·9	1·1	7	0			−42
Boberg *et al.*, 1986	1·8	1·2	14	0	+12	+10	−31
Rylance *et al.*, 1986	3·6	2·4	28	+ 7		+10	−35
Verheugt *et al.*, 1986	1·8	1·2	5	−15		+11	
Von Schacky *et al.*, 1985	1·9	2·8	6	+ 2		0	−30
Hay *et al.*, 1982	3·5	2·0	13	+ 1		+18	−26
Harris *et al.*, 1987	3·5	2·0	8	0	+34	0	−44

EPA = eicosapentaenoic acid: DHA = docosahexaenoic acid.

Illingworth *et al.*, 1984; Phillipson *et al.*, 1985) but the extent of this effect depends on the type of fat being removed from the diet. If fish oil consumption essentially pushes saturated fatty acids out of the diet, the cholesterol-lowering effect is more pronounced than when the intake of *n*–6 polyunsaturated fatty acids is reduced (Harris *et al.*, 1983; Phillipson *et al.*, 1985).

Hidden underneath these fairly minor and sometimes opposing effects on total cholesterol, important shifts in cholesterol between lipoproteins are taking place. Fish oil causes a marked drop in serum triglycerides (see below) and thus, presumably, in VLDL cholesterol. When fish oil lowers total cholesterol, this will often be due largely to VLDL. Simultaneously, however, there occurs a marked increase in LDL cholesterol (Table 5) and in LDL

apo B. This reciprocal effect on VLDL and LDL is well known from drug treatment of hypertriglyceridaemia. In view of the well-established atherogenic effect of LDL, any increase of LDL should be deemed undesirable, even if VLDL and total cholesterol fall.

Effects of C20:5, *n*–3 and C22:6, *n*–3 on HDL are not well defined. The rise that is often seen after fish oil ingestion could be an aspecific result of the increased fat:carbohydrate ratio in the diet (see below).

Thus, fish oils may cause deleterious changes in lipoprotein pattern, and recommendations for use of fish oil capsules in healthy subjects should be deferred until the effects of these preparations are better defined.

(b) Whole fish. Fish consumption is associated with the intake of *n*–3 fatty acids. An increased intake of fish has to displace other foodstuffs from the diet so as to maintain a constant caloric intake. Obviously, the extent of this displacement depends on the amount and type of fish. Low-fat fish, so-called white fish, such as perch, cod and pike, provide less fat on a gram for gram basis than do fatty fish such as mackerel, salmon and herring. This also holds for the unique fatty acids in fish oil: the very-long-chain polyunsaturated *n*–3 fatty acids. In contrast, white fish contain more protein on a weight basis.

The lipidaemic effect of fish consumption depends on the nature of the foodstuffs that are replaced. Fehily *et al.* (1983) asked male office workers, who habitually consumed fatty fish less than once a week, to eat at least 100 g portions of fatty fish (mackerel, kippers, herrings, sardines, pilchards, salmon or trout) at least twice a week. On a gram for gram basis fish consumption caused a decrease in the "spontaneous" intake of meat and cheese, whereas egg consumption was not affected. The hypolipidaemic effect of fish consumption would be greater in those individuals who consistently replaced saturated-fat rich foodstuffs (beef, cheese) than in those who replaced vegetable oils or carbohydrate-rich products (bread, potatoes).

Table 6 summarizes the results of studies in which the effect of consumption of whole fatty fish on serum lipids was measured. Apart from the studies of Von Lossonczy *et al.* (1978) and Van Houwelingen *et al.* (1987), the studies presented in Table 6 were not strictly controlled. It is thus difficult to ascertain what part of the observed effect is due to specific lipidaemic properties of the components of the fish. Von Lossonczy *et al.* (1978) compared the intake of 200 g daily of mackerel with 150 g of cheese in a double crossover design, but even in this study the intake of macronutrients during both dietary periods were not identical. One could even argue that this study largely demonstrates the hypolipidaemic effect of removal from the diet of saturated fatty acids in the form of cheese. Van Houwelingen *et al.* (1987) studied the effects of supplementation of the diet with either

mackerel or meat paste. Most studies presented in Table 6 were conducted without a control group so that corrections for possible time trends could not be done. However, from the practical point of view it is important to note that very high intakes of fatty fish will reliably lower serum triglycerides, and reduce serum total and LDL cholesterol, but increase HDL cholesterol concentrations. It is uncertain whether the increase in HDL which was often seen is due to something in the fish, or to a reduction in carbohydrate intake as a result of a forced high fish consumption (see below).

F. Fat Type and Serum Triglycerides

Compared with saturated fatty acids, *n*–6 polyunsaturated fatty acids lower serum triglycerides (Table 4). *n*–3 Fatty acids have the same effect, but are probably much more potent (Tables 5 and 6). However, no direct comparisons are available to conclude unequivocally whether *n*–6 or *n*–3 fatty acids lower serum triglycerides more effectively.

G. Fat:Carbohydrate Ratio and LDL and HDL Cholesterol

Changes in amount of dietary fat imply a change in the amount of carbohydrates in the opposite direction, as dietary protein and alcohol can be kept constant. The impact on serum cholesterol of a decrease in fat intake depends on the fatty acid composition of the high-fat diet and on changes in fatty acid composition concomitant with the decrease in absolute fat intake. Replacement of saturated fatty acids by carbohydrates lowers serum cholesterol, whereas replacement of polyunsaturated fatty acids may not. A

TABLE 6
Effect of consumption of whole fatty fish on serum lipids in human subjects

	Fish intake			Change in serum lipids (%)			
	Type	Amount (g/day)		Cholesterol			Triglycerides
Reference			*n*	Total	LDL	HDL	
Fehily *et al.*, 1983	Mixed	45	118	+ 1	+ 3	+ 3	− 6
Van Houwelingen *et al.*, 1987	Mackerel	135	40/42	0			− 2
Von Lossonczy *et al.*, 1978	Mackerel	200	42	− 8		+ 6	−34
Singer *et al.*, 1983	Mackerel	±280	15	− 7	− 7	+ 9	−43
Singer *et al.*, 1983	Herring	±280	15	− 3	− 8	0	−13
Bradlow *et al.*, 1983	Mixed	±350	8	−15			−33
Singer *et al.*, 1985	Mackerel	±280	16	−20	−17	+16	−69
Singer *et al.*, 1985	Herring	±280	16	−15	−13	+24	−65

decrease in absolute fat intake associated with an increase in saturation of dietary fatty acids may cause an increment of serum cholesterol.

These points were made abundantly clear by Hegsted *et al.* (1965) and by Keys *et al.* (1957, 1965a, 1965d) in two classical series of experiments. Less well known are the studies of Vergroesen *et al.* (1970), who fed liquid formula diets varying in the amount and type of fat to monks. The fats used were glyceryl trilaurate (90% glyceryl trilaurate + 10% safflower oil) olive oil and safflower oil. Each fat was given at levels of 20, 35 and 50% of calories. The diets were fed to groups of 6 to 9 volunteers for a period of 6 weeks. At a fat intake of 35% of calories serum cholesterol concentrations were highest with trilaurate and lowest with safflower oil (Table 7). Lowering of fat intake reduced serum cholesterol clearly only if the fatty acid composition of the fat was simultaneously changed towards a higher degree of unsaturation. If safflower oil was the fat source, the replacement of fat calories by carbohydrate calories did not lower serum cholesterol.

The interrelated effect of fat type and amount of fat has also been demonstrated in more recent studies employing diets based on regular foodstuffs. Brussaard *et al.* (1980) have shown that a decrease in fat intake from 40 to 30% of energy caused a decrease in serum total cholesterol when saturated and monounsaturated fatty acids were replaced by carbohydrates and polyunsaturated fatty acids. However, when fat in the form of polyunsaturated fatty acids was replaced by carbohydrates there was no lowering of serum cholesterol. Likewise, Lewis *et al.* (1981) showed that a high-fat diet (40% of calories from fat) containing 12·8% of energy derived from linoleic acid was as effective in lowering serum total cholesterol as a low-fat (27 en % fat; 8·7 en % linoleic acid) diet.

TABLE 7

Effect of type and amount of dietary fat on serum total cholesterol in man. Formula diets were fed to healthy inhabitants of a monastery

Dietary fat	Energy % of fat	Serum cholesterol (mmol/l)		
		initial	final	change
Glyceryl trilaurate	20	5·23	5·23	0·00
Olive oil	20	5·13	4·53	−0·60
Safflower oil	20	5·13	4·25	−0·88
Glyceryl trilaurate	35	5·26	5·21	−0·05
Olive oil	35	5·02	4·45	−0·57
Safflower oil	35	5·28	4·14	−1·14
Glyceryl trilaurate	50	5·05	5·31	+0·26
Olive oil	50	5·05	4·82	−0·23
Safflower oil	50	5·31	4·25	−1·06

After Vergroesen *et al.* (1970).

Table 8 shows that a decrease in fat intake is invariably associated with a decrease in HDL cholesterol levels. Epidemiological studies are in perfect accordance with this (see below). The effect appears to be due to the increased carbohydrate consumption, because Lithell *et al.* (1985), in a very intriguing study, showed that replacing protein by carbohydrates also caused HDL to fall and VLDL to rise. On the basis of both controlled experiments and epidemiological studies, Katan (1984) arrived at the rough guide that replacement of 10% of energy as fat by an equivalent amount of carbohydrate will lower HDL cholesterol by about 0·10 mmol/l. Thus, although low-fat diets often lower serum total and LDL cholesterol levels, such diets also produce a decrease in HDL cholesterol. The degree of LDL lowering depends on the simultaneous change in fatty acid composition of the diet.

Whether the extent of HDL lowering is influenced by the type of dietary fat that is replaced, is not yet clear. Grundy (1986a) compared the effects of high-fat liquid formula diets which were either rich in saturated or monounsaturated fatty acids and a low-fat formula diet. The high-fat diets contained 40% of total calories as fatty acids, and the low-fat diet only 20%. The low-fat and high-monounsaturated diets contained mixtures of two types of safflower oil, one high in oleic acid, and the other rich in linoleic acid. The high-saturated diet contained a mixture of coconut fat and both safflower oils. Replacement of saturated fatty acids by carbohydrates resulted in a decrease in both LDL and HDL cholesterol (Table 8). Replacement of saturated by monounsaturated fatty acids also lowered LDL cholesterol but HDL cholesterol was left unchanged (Grundy, 1986a). The important point

TABLE 8

Effect of a decrease in the dietary fat:carbohydrate ratio on serum total, LDL and HDL cholesterol, and triglyceride concentrations in controlled human trials

Reference	*n*	Amount of dietary fat (% of energy)		Change in serum cholesterol (%)			Change in serum triglycerides (%)
		High	Low	Total	LDL	HDL	
Hulley *et al.*, 1972	13	50	39	+ 7	+ 8	−16	+43
Brussaard *et al.*, 1980	15/15	40	30	+ 2		− 6	+20
Lewis *et al.*, 1981	12	40	27	− 5	− 4	− 6	+ 8
Cortese *et al.*, 1983	8	45	25	−13	−17	−17	0
Enholm *et al.*, 1982	54	36	24	−21	−23	−21	−12
Brussaard *et al.*, 1982	16/17	31	21	− 5	− 8	−10	+61
Grundy, 1986	7	40	20	− 7	− 9	−30	+36
Jones *et al.*, 1987	15	39	20	− 6		−12	+47
Huff and Nestel, 1982	6	47	18	− 3		−10	+53
Kashyap *et al.*, 1982	9	65	15	− 3	− 1	−20	+45

here is that replacing saturated fatty acids by carbohydrates lowers HDL cholesterol, whereas replacing saturated fatty acids by monounsaturated fatty acids did not. Both dietary changes however do lower LDL cholesterol. That monounsaturated fatty acids selectively lower LDL cholesterol while leaving HDL unchanged has also been shown by Mensink and Katan (1987). In this study natural solid mixed diets either rich in complex carbohydrates or olive oil were used. Figure 5 also illustrates that serum total cholesterol fell on both diets to the same extent. The effects of the various classes of polyunsaturated fatty acids on HDL have not yet been entirely clarified.

H. Fat:Carbohydrate Ratio and Serum Triglycerides

Table 8 and Fig. 5 show that a decrease in the dietary fat:carbohydrate ratio generally results in marked elevations of serum triglycerides. Similar results were published by other investigators (Andersén and Hellström, 1980; Ruderman *et al.*, 1971; Witztum and Schonfeld, 1978). Apart from the studies of Jones *et al.* (1987), Brussaard *et al.* (1980, 1982) and Mensink and Katan (1987), the data in Table 8 refer to studies of males. Women do show a less pronounced increase in serum triglycerides upon the replacement of fat by carbohydrates than men (Beveridge *et al.*, 1964; Mensink and Katan, 1987), possibly because they have higher lipoprotein lipase activities. It has been argued that carbohydrate-induced hypertriglyceridaemia reaches a maximum within a few weeks, and then diminishes again (Mancini *et al.*, 1973; Beveridge *et al.*, 1964). However in studies of healthy volunteers lasting 3–4 months there was no sign of such a transient effect (Ahrens *et al.*, 1961; Brussaard *et al.*, 1982; Jones *et al.*, 1987). The data of Antonis and Bersohn (1961) are often quoted to assert that carbohydrate-induced hypertriglyceridaemia is transient. Closer analysis, however, revealed that serum triglycerides returned to baseline only in those subjects whose baseline levels where already high through a high intake of butter (Brussaard *et al.*, 1982).

I. Epidemiological Data on Dietary Fatty Acids and Serum Cholesterol

The effect of saturated fats on serum total cholesterol concentrations seen in controlled trials has also been demonstrated in epidemiological studies. Studies within homogeneous populations tend to produce equivocal results, probably because the range of intakes is too small and the variability of intakes too large (Jacobs *et al.*, 1979). Still, weak but significant correlations between dietary fatty acids and total cholesterol do turn up fairly regularly (Shekelle *et al.*, 1981).

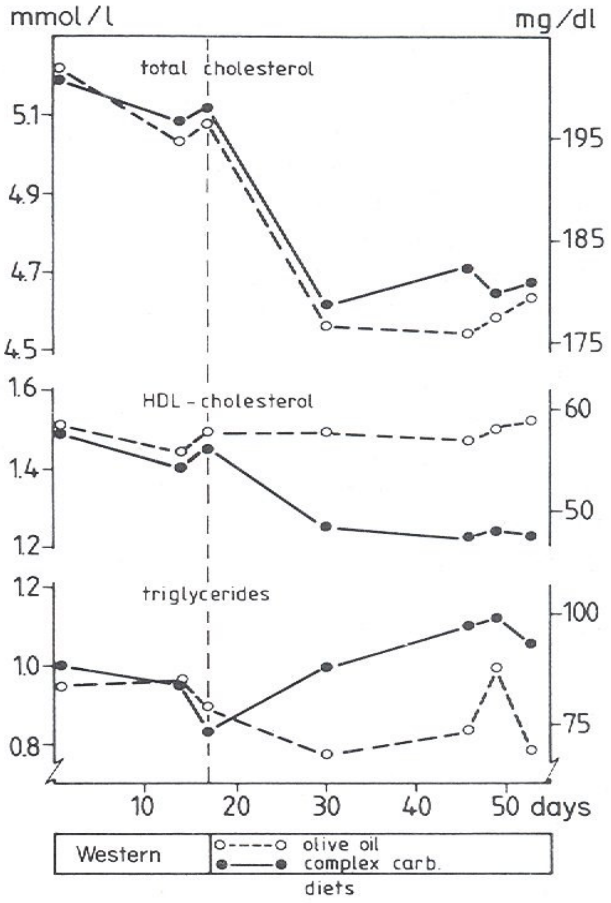

Fig. 5. Mean serum total and HDL cholesterol and triglyceride concentrations in 48 subjects receiving a Western-type diet followed by an experimental diet either rich in olive oil or carbohydrates. The experimental diets essentially differed only with respect to their fat:carbohydrate ratios. The carbohydrate-rich diet contained 62% of calories as carbohydrates and 9% as monounsaturated fatty acids. The olive-oil rich diet provided 46% of calories as carbohydrates and 24% as monounsaturates. Figure reproduced with permission from Mensink and Katan (1987).

Dietary differences are more pronounced between populations, and here correlations are much stronger. In the Seven Countries Study of Keys (1970) there was a strong positive correlation ($r = 0{\cdot}89$, $n = 14$) between the average intake of saturated fatty acids and serum total cholesterol concentrations of various populations. As would be anticipated, the association between total fat intake and serum cholesterol was much weaker (Keys, 1970). The variance of serum total cholesterol is probably due largely to LDL cholesterol.

The effect of the fat:carbohydrate ratio on HDL is clearly evident even within populations (Katan, 1984), but again it is brought out more clearly

if populations with contrasting dietary habits are compared. Knuiman *et al.* (1987) have performed a series of epidemiological studies on the relationship between diet and HDL cholesterol. Most of these studies involved young boys so as to reduce the influence of confounding factors on HDL cholesterol concentrations such as smoking, drinking, obesity, drug use and physical activity (Knuiman and West, 1983). A clear and consistent positive relation between dietary fat:carbohydrate ratio and HDL cholesterol was found between, and in several cases also within populations (Knuiman *et al.*, 1987). On the other hand, the dietary fat:carbohydrate ratio is negatively associated with fasting serum triglyceride concentrations (Sullivan *et al.*, 1987).

The epidemiological observations thus support the outcome of the controlled studies described above: saturated fatty acids increase serum total cholesterol, and low fat:carbohydrate ratios decrease HDL cholesterol and elevate serum triglycerides. The important point is that most experimentally observed, diet-induced effects on serum lipids are confirmed by observations on normal healthy populations eating self-selected mixed diets; thus these effects are permanent, and they can be achieved by diets that have been eaten by large numbers of people for a long time.

J. Animal Experiments

In essence, the type and amount of dietary fat influence serum total cholesterol concentrations similarly in animals and humans. Table 9 shows the effects of various dietary fats on serum cholesterol levels in rabbits. When comparing Tables 2 and 9 it follows that rabbits respond in a similar way to humans, at least in qualitative terms. Quantitatively, animal data concerning dietary

TABLE 9
Effect of various fats on serum cholesterol in rabbits

Dietary fat	Change in serum cholesterol upon substitution of an indicated fat for soyabean oil (mmol/l)
Soyabean oil	0·00
Corn oil	−0·21
Cotton seed oil	+0·88
Peanut oil	+0·70
Olive oil	+2·12
Beef tallow	+2·82
Butter fat	+4·14
Coconut fat	+7·64

Based on studies reported by Carroll (1971). The diets of the rabbits consisted of (g/100g): casein, 30; dextrose, 44; fat, 15; salt mixture, 5; celluflour, 5; vitamin mixture, 1. The experiment lasted 2 weeks. The rabbits, weighing about 1·2 kg, would have consumed about 10 g of fat per day.

fatty acids and serum total cholesterol cannot be extrapolated to man. Likewise, dietary effects on serum lipoproteins cannot be extrapolated because lipoprotein profiles differ between most laboratory animals and humans (Chapman, 1980). For the subject of this chapter the proper study of mankind is man.

K. Underlying Mechanisms

At a constant level of total fat intake, the replacement of saturated fatty acids by polyunsaturated fatty acids causes a reduction in LDL cholesterol. If the replacement is extreme there is also a decrease in HDL cholesterol. The impact of decreasing fat intake on serum cholesterol concentrations depends on the fatty acid composition of the initial high-fat diet but also on possible simultaneous changes in dietary fatty acid composition. However, irrespective of changes in qualitative fat intake, HDL cholesterol will be lowered upon replacement of dietary fat by carbohydrates. In this section we attempt to describe the effects of dietary fat on lipoprotein metabolism in molecular terms.

1. Polyunsaturated Fatty Acids

Although the replacement of dietary saturated by polyunsaturated fatty acids is a very effective way to lower serum cholesterol, the mechanism of this effect is still unclear. Several mechanisms of action have been proposed to explain the hypocholesterolaemic effect of polyunsaturated fatty acids, but there is considerable controversy. There are two competing theories to explain the lowering of serum LDL cholesterol induced by feeding polyunsaturated fatty acids. One theory proposes increased LDL catabolism and the other decreased formation of LDL. Both mechanisms necessarily have effects on other aspects of cholesterol metabolism so that a new steady-state can be reached.

Spritz and Mishkel (1969), and later Nakamura *et al.* (1980), have proposed that polyunsaturated fatty acids alter lipoprotein structure and thereby increase their catabolism. Experimental results of Kuksis *et al.* (1982) do not support this proposal. Other workers (Pownall *et al.*, 1980; Baudet *et al.*, 1984) have suggested that dietary polyunsaturated fatty acids alter the structure of LDL, and thereby increase its rate of catabolism by receptor-mediated and/or receptor-independent pathways. Spady and Dietschy (1985) have shown that LDL cholesterol lowering in hamsters fed polyunsaturated fat (safflower oil) is associated with an increase in hepatic receptor-mediated uptake of LDL when compared with hamsters fed saturated fat (hydrogenated coconut fat). Both mechanisms (Spritz and Mishkel, 1969; Spady and

Dietschy, 1985) imply that LDL turnover is increased. This should be reflected by an increase in the excretion of bile acids and/or neutral steroids. However, although some authors have indeed reported enhanced excretion of steroids with the faeces in subjects consuming polyunsaturated fatty acids (Wood *et al.*, 1966; Nestel *et al.*, 1975), others were unable to demonstrate such an effect (Brussaard *et al.*, 1983; Glatz *et al.*, 1985). Most studies have been performed under steady-state conditions when a lower, constant level of serum cholesterol had been reached. Theoretically, it is possible that polyunsaturated fatty acids cause a rapid, transient rise in steroid excretion. There is some experimental evidence for such a rapid and transient effect (Nestel *et al.*, 1973, 1974). Serum cholesterol is then decreased and settles at the new level while the rate of steroid excretion has fallen towards the initial value. Thus in the new steady-state no changes in steroid excretion after feeding polyunsaturated fatty acids would be detected. It should be realized that a persistent, enhanced excretion of steroids has to be associated with increased rates of endogenous cholesterol synthesis in order to prevent the body from cholesterol depletion.

It is difficult to see that the cholesterol-lowering effect of polyunsaturated fatty acids is explained by a transient rise in the faecal excretion of steroids. Cholestyramine, a bile-acid binding resin, causes an increase in the output of bile acids and a decrease in the level of serum cholesterol (Grundy, 1986b). However, when the use of the drug is stopped, both the rate of bile acid excretion and serum cholesterol concentration return to baseline values. Thus a temporary rise in bile acid excretion does not lead to a permanently lowered concentration of serum cholesterol.

Polyunsaturated fatty acids of both the *n*–6 and *n*–3 series effectively lower serum triglyceride concentrations. Fish oil concentrate has been shown to depress the synthesis of VLDL (Nestel *et al.*, 1984; Sanders *et al.*, 1985). Cortese *et al.* (1983) have determined the turnover of VLDL and LDL apoprotein B in subjects consuming diets containing 40% of energy as fat which were either high in saturated (P/S ratio = 0·12) or *n*–6 polyunsaturated fatty acids (P/S ratio = 3·8). When subjects were transferred from the low to the high-P/S diet VLDL turnover dropped by 31%, and LDL turnover by 23%. Nakamura *et al.* (1980) have shown that serum triglyceride production is diminished on a diet containing safflower oil, when compared with coconut fat. Turner *et al.* (1981) and Shepherd *et al.* (1980) reported that LDL apoprotein B synthesis was reduced by 9 and 5%, respectively, on a high P/S diet compared with a low P/S diet. High intakes of fish oil also inhibit LDL synthesis (Illingworth *et al.*, 1984).

Depressed VLDL synthesis may be responsible for the observed reduction of serum triglycerides in subjects on diets rich in polyunsaturated fatty acids. Since VLDL is a precursor of LDL, inhibition of VLDL synthesis after

feeding polyunsaturated fatty acids would also explain the observed reduction of the rate of LDL synthesis. The diminished synthesis of VLDL agrees with observations that polyunsaturated fatty acids, in contrast to saturated fatty acids, are channelled away from the production of VLDL triglycerides (Nestel and Barter, 1971; Chait *et al.*, 1974). If part of dietary polyunsaturated fatty acids do not go into VLDL, they have to go somewhere else. Beynen and Katan (1985c, 1986) have put forward the suggestion that replacement of saturated by polyunsaturated fatty acids in the diet may lower LDL concentrations because the liver preferentially converts polyunsaturated fatty acids into ketone bodies instead of into VLDL-triglycerides. In isolated rat liver systems linoleic acid has been shown to induce significantly higher rates of ketone body synthesis and lower output of triglycerides than palmitic acid (Kohout *et al.*, 1971; Nestel and Steinberg, 1963). The strong hypotriglyceridaemic effect of fish oils may be explained by the same mechanism. The livers of rats fed fish oil have been shown to secrete significantly less VLDL and in addition secreted more ketone bodies than livers from rats fed safflower oil (Wong *et al.*, 1984).

Thus, unlike saturated fatty acids, polyunsaturated fatty acids are transported to the tissues for oxidation without leaving a trail of lipoprotein remnants in the form of LDL, the main carrier of cholesterol in serum. Figure 6 depicts this concept. This hypothesis tacitly assumes that a decrease in VLDL output by the liver is associated with a decrease in cholesterol secretion. Indeed it is likely that cholesterol is required as an essential structural component of the VLDL surface. The cholesterol esters in the VLDL core are probably acquired from HDL in serum, in exchange for VLDL triglycerides (Chajek and Fielding, 1978). A decrease in flux of VLDL from the liver would also lower the amount of cholesterol esters acquired by VLDL, which in turn would lower the flux of cholesterol esters into the LDL pool. In any event, the mechanism proposed requires that cholesterol turnover is decreased. This in turn should be reflected by a decrease in cholesterol excretion in the form of neutral steroids and/or bile acids. As mentioned above, such effects have not been documented.

The opposing theories to explain LDL lowering by dietary polyunsaturated fatty acids are both supported by experimental evidence. However, the anticipated effects on steroid excretion have not been demonstrated. Possibly, this lies in the fact that the measurement of faecal steroid excretions is extremely difficult, and therefore not accurate enough to detect small changes. In any case, further work is necessary to decide which mechanism is responsible for the serum cholesterol-lowering effect of polyunsaturated fatty acids.

2. Fat:Carbohydrate Ratio

Replacement of saturated fatty acids by isocaloric amounts of carbohydrates causes a reduction of LDL and HDL cholesterol concentrations. The capacity

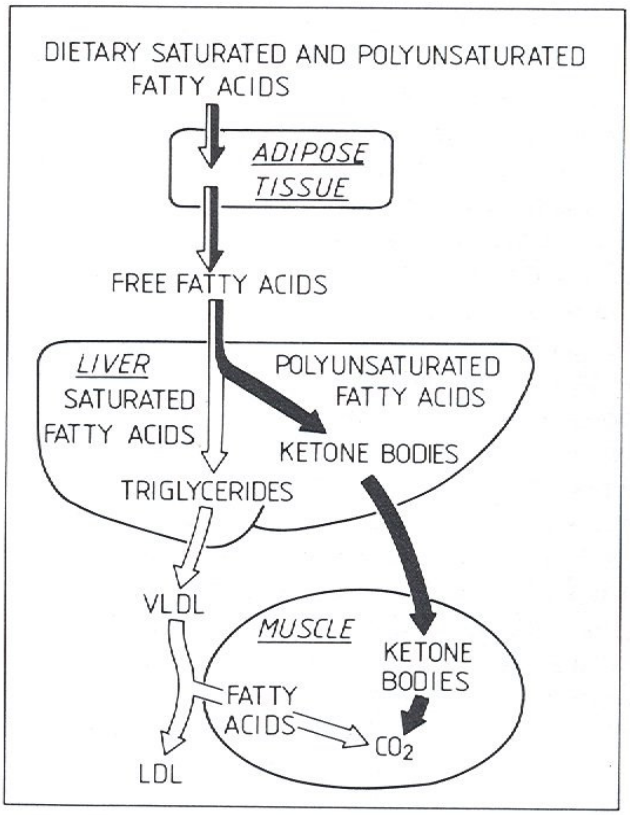

Fig. 6. Hypothetical pathways for the production of VLDL and ketone bodies from saturated and polyunsaturated fatty acids in the fasting state. In man, in the post-absorptive state the fatty acyl moiety of newly synthesized serum triglycerides is predominantly derived from serum fatty acids (Barter *et al.*, 1972), which in turn originate from the adipose tissue. The fatty acid composition of the adipose tissue of humans reflects that of the diet (Beynen *et al.*, 1980). After a meal, dietary fatty acids are temporarily stored in a rapidly turning-over pool in the adipose tissue (Hirsch *et al.*, 1960), and in the fasting state they enter the hepatic pathways of either triglyceride formation (esterification) or β-oxidation followed by ketogenesis. Figure reproduced with permission from Beynen and Katan (1985c).

of man to convert glucose into fatty acids—essentially palmitic and stearic acid (Gellhorn and Marks, 1961)—is probably very limited (Björntorp and Sjöström, 1978; Hoffman *et al.*, 1980; Acheson *et al.*, 1987). Ingested glucose is primarily stored in the form of glycogen (Björntorp and Sjöström, 1978). This implies that replacement of saturated fatty acids by carbohydrates could cause a reduced supply of fatty acids for esterification in the liver. As explained above, this in turn could result in LDL cholesterol lowering. Polyunsaturated fatty acids may not be used preferentially as substrates for the pathway of esterification. Thus replacement of polyunsaturated fatty acids by carbohydrates does not necessarily lower LDL cholesterol.

Increased consumption of carbohydrates at the expense of fat calories will lower HDL cholesterol concentrations. This is probably related to the fact that HDLs are involved in the catabolism of triglyceride-rich lipoproteins (chylomicrons and VLDLs) as both activators and products. Chylomicrons obtain from HDL the apoprotein C-II, which is essential for the breakdown of triglyceride-rich particles by lipoprotein lipase. On the other hand, as chylomicrons and VLDL are broken down by lipoprotein lipase, they lose apoprotein A-I and other surface materials, which are transferred to the HDL density range and give rise to mature HDL particles through the further action of lecithin cholesterol acyltransferase (Tall and Small, 1980).

Replacement of dietary fat by carbohydrates leads to a decrease of chylomicron production: a chylomicron flux is replaced by a glucose flux, and in man the increase in VLDL flux is probably limited, as argued above. This decreased supply of triglyceride-rich particles as substrates for lipoprotein lipase will lead to a lower rate of production of surface remnants, especially apoprotein A-1, and thus of HDL. In fact, the activity of lipoprotein lipase also falls when fat in the diet is replaced by carbohydrate (Lithell *et al.*, 1982). Whether this is a reaction to the decreased supply of substrate (chylomicrons, VLDL) or activator (HDL) is not clear. These combined effects might be responsible for the carbohydrate-induced hypertriglyceridaemia.

VI. INTERACTION OF DIETARY FATTY ACIDS WITH DIETARY CHOLESTEROL

It is clear that the type of dietary fat has a major, and the amount of dietary cholesterol a relatively minor impact on serum cholesterol concentrations. This final section aims to determine whether the type of dietary fat can modify the rise in serum cholesterol induced by dietary cholesterol. In order to solve this question only experiments should be considered which used diets varying both in the amount of cholesterol and fatty acid composition. Preferably, these experiments should be designed as a 4×4 Latin square. The beautiful National Diet–Heart Study (1968) fulfilled this and all other conditions mentioned in Section III, and thus settles the matter. Table 10 shows that the dietary cholesterol-induced increase in serum cholesterol was somewhat smaller on a diet high in polyunsaturated fatty acids than on a diet high in saturated fatty acids. Thus there is an interaction of dietary fat type with dietary cholesterol. Other studies with small numbers of subjects and/or less optimal experimental designs had either similar (Schonfeld *et al.*, 1982; Fisher *et al.*, 1983; Nestel, 1977; Oh and Monaco, 1985; Connor *et al.*, 1964; Brown, 1971) or opposite results (Anderson *et al.*, 1976; Hegsted *et al.*, 1965; Nestel *et al.*, 1975; Zanni *et al.*, 1987; McNamara *et al.*, 1987).

TABLE 10

Effects on serum cholesterol of cholesterol incorporated into diets rich in either saturated or polyunsaturated fatty acids

Reference	*n*	P/S ratio of diets		Dietary polyunsaturated fatty acids (% of energy)		Dietary cholesterol (mg/1000 kcal)		Cholesterol-induced change in serum cholesterol (%)	
		Low	High	Low P/S	High P/S	Low	High	Low P/S	High P/S
National Diet–Heart Study, 1968	113	0·5	1·8	7	15	43	150	+7	+2
National Diet–Heart Study, 1968	87	0·1	0·9	3	10	88	206	+6	+4

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