Role of Dietary Fat and Cholesterol in LDL Metabolism and Atherosclerosis

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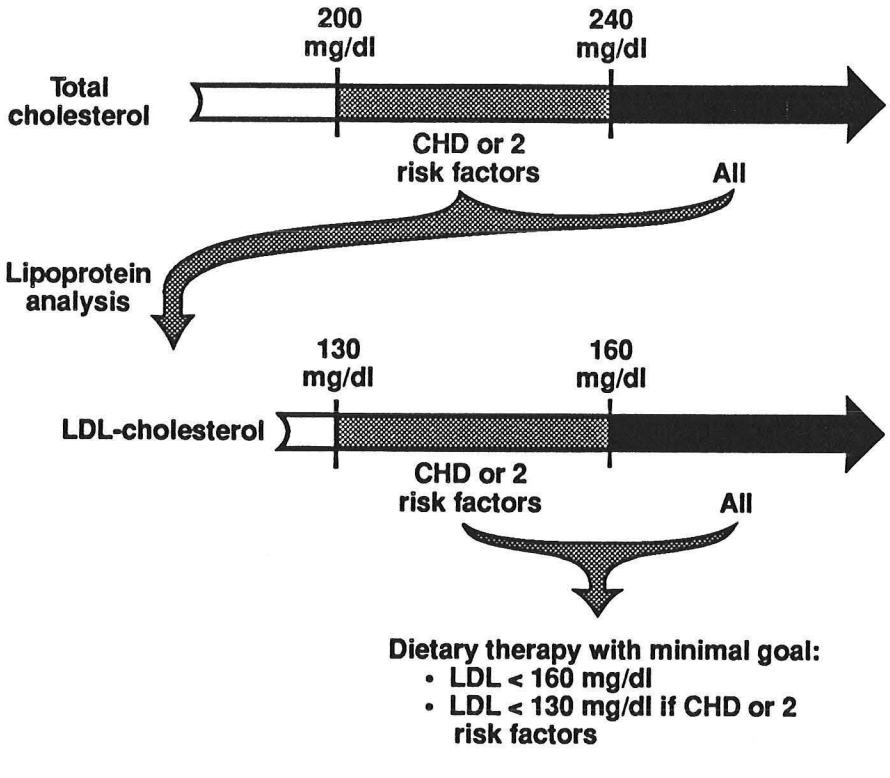
Introduction

Despite the fact that mortality rates from coronary heart disease(CHD) have markedly declined over the past two decades, CHD remains the leading cause of death in this country and accounts for more than 500,000 deaths per year(1). In 1985, faced with increasing evidence demonstrating the adverse health consequences associated with elevated blood cholesterol concentrations, the National Heart, Lung, and Blood Institute launched the National Cholesterol Education Program. In its initial report(2) entitled "lowering blood cholesterol to prevent heart disease," it was concluded that blood cholesterol concentrations are "undesirably high in most Americans, in large part because of our high dietary intake of calories, saturated fat and cholesterol." It was recommended that all

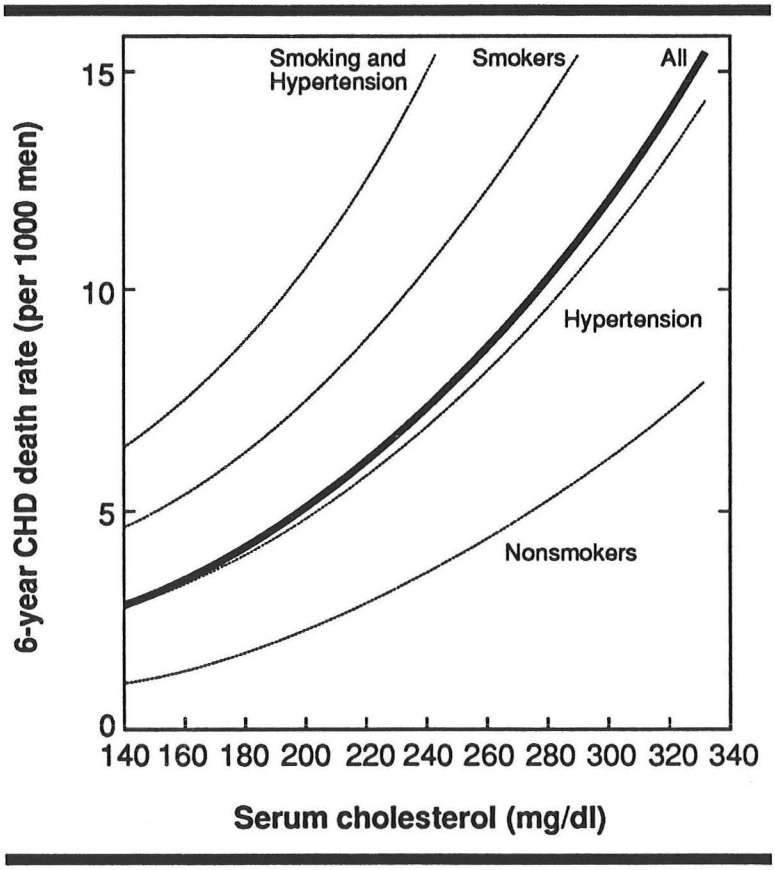
Figure 1. National Cholesterol Education Program guidelines. Adapted from reference 3.



Americans(with the exception of children under two years of age) be advised to modestly reduce their intake of total fat, saturated fat and cholesterol. In a second major report(3) entitled " Report of the National Cholesterol Education Program Expert panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults," specific guidelines were provided to assist in the management of hypercholesterolemia as outlined in abbreviated form in Fig. 1. First, it is recommended that blood cholesterol be measured in all adults at least once every five years. Based on these measurements, individuals are classified as having desirable (< 200 mg/dl), borderline-high (200-239 mg/dl) or high (≥ 240 mg/dl) blood cholesterol levels. These cutoff points are based on the risk associated with various levels of serum cholesterol as determined in the Multiple Risk Factor

Intervention Trial(4) in which 6-year mortality from CHD was determined in 361,662 men aged 35-57(Fig. 2) These data show a continuous increase in CHD mortality with increasing serum cholesterol levels that holds true for all ages and for various risk factor subgroups (nonhypertensive nonsmokers, nonhypertensive smokers, hypertensive nonsmokers, and hypertensive smokers). Individuals classified as having high blood cholesterol levels should have lipoprotein levels measured. In addition, persons with borderline-high levels who also have definite coronary heart disease or two other risk factors for CHD (including male sex), should also undergo lipoprotein analysis. Individuals who undergo lipoprotein analysis are then classified as having desirable (<130 mg/dl),

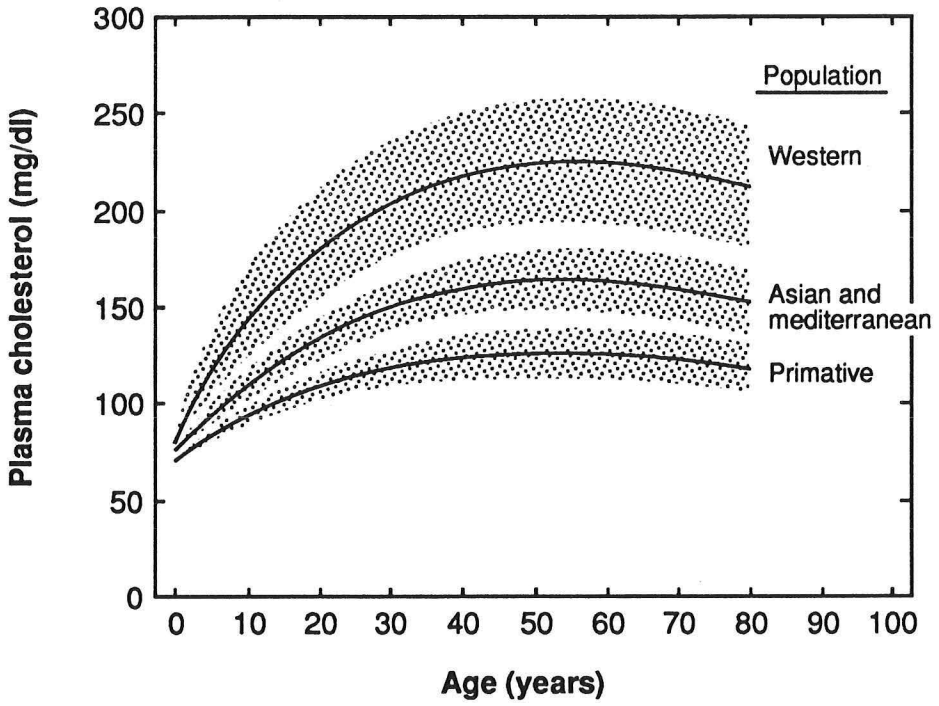
Figure 2. Multiple Risk Factor Intervention Trial. Adapted from reference 4.



borderline-high (130-159 mg/dl) or high (≥ 160 mg/dl) LDL cholesterol levels. Persons with high LDL cholesterol levels plus those with borderline-high levels and the presence of definite CHD or at least two other risk factors for CHD are candidates for medical advice and intervention using dietary modification as the initial mode of treatment. Minimal goals of therapy are an LDL cholesterol < 160 mg/dl or < 130 mg/dl if CHD or two risk factors for CHD are present. Using this algorithm, approximately one-third of the adult population, or 60 million people, are candidates for intervention(5). Thus, in addition to the general recommendation for all Americans to modestly reduce dietary cholesterol and saturated fat, intensive dietary therapy aimed at lowering plasma LDL cholesterol

below specific target levels is recommended for the one-third of adult Americans identified as being at particularly high risk for CHD by the algorithm outlined above. Individuals unable to achieve their target LDL levels would be considered for drug therapy although at somewhat higher cutoff points. The number of persons who would be candidates for drug therapy could be quite large and will depend on the success of diet therapy. In the following section I will review some of the epidemiologic and clinical trial data linking diets rich in cholesterol and saturated fat with elevated plasma cholesterol levels and increased mortality from CHD. Subsequent sections will review data on how dietary cholesterol and fat regulate plasma LDL concentrations and how elevated LDL concentrations may, in turn, lead to the initiation and progression of atherosclerosis.

Figure 3. Adapted from references 10-21.

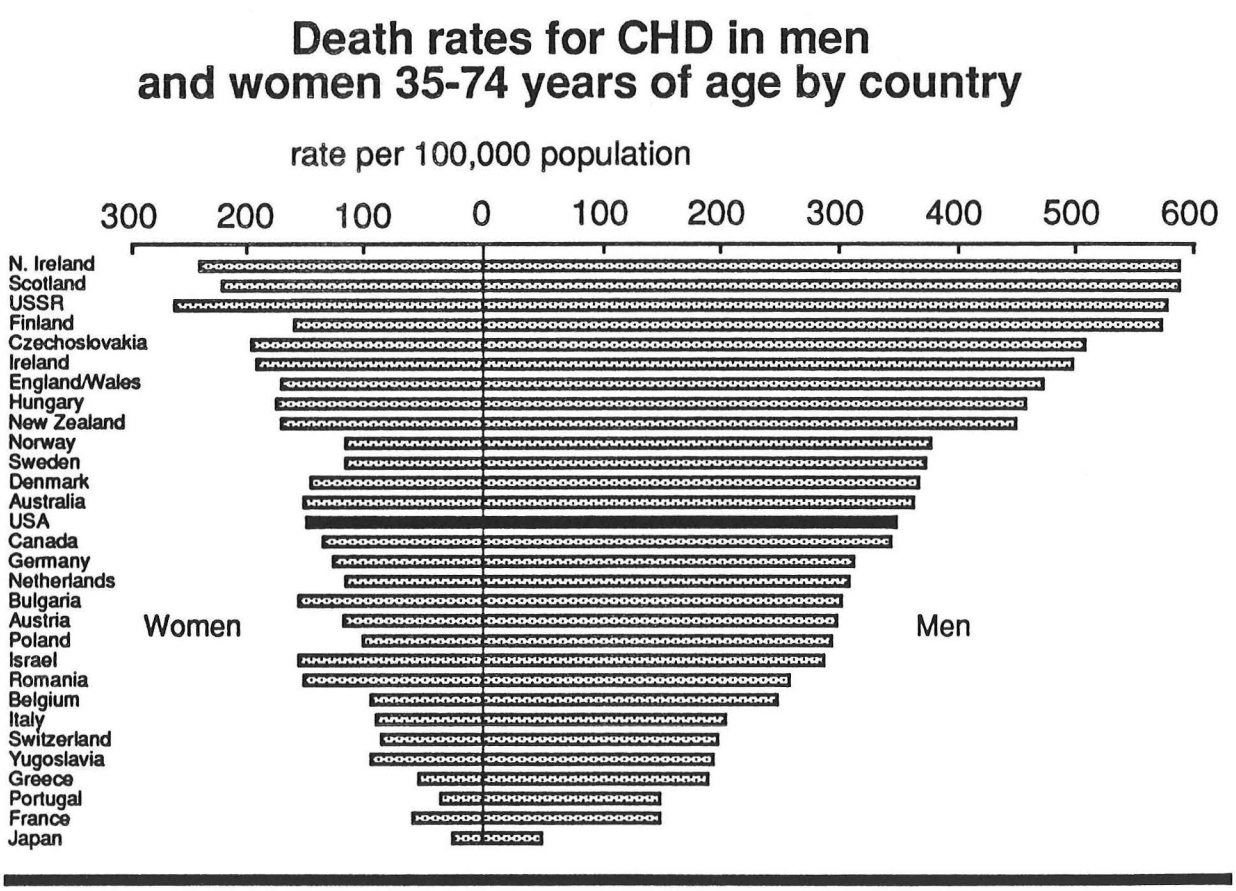


Epidemiologic data linking diet, plasma cholesterol and CHD

Systematic comparisons of populations show large differences in diet, plasma cholesterol concentrations and mortality from CHD(6-9). As illustrated in Fig. 3, mean plasma cholesterol levels are identical at birth(65-75 mg/dl) in populations from around the world(10-13). However, within the first few years of life, plasma cholesterol concentrations begin to diverge among different populations and these differences become even more pronounced by middle age. At one extreme are Western populations where intake of saturated fat and cholesterol is high. In these populations mean plasma cholesterol concentrations rise to levels of 200-250 mg/dl and CHD is prevalent(4, 14). At the other extreme are certain well-studied groups who because of their location and poverty subsist on a near vegetarian diet containing virtually no saturated fat or cholesterol(15-

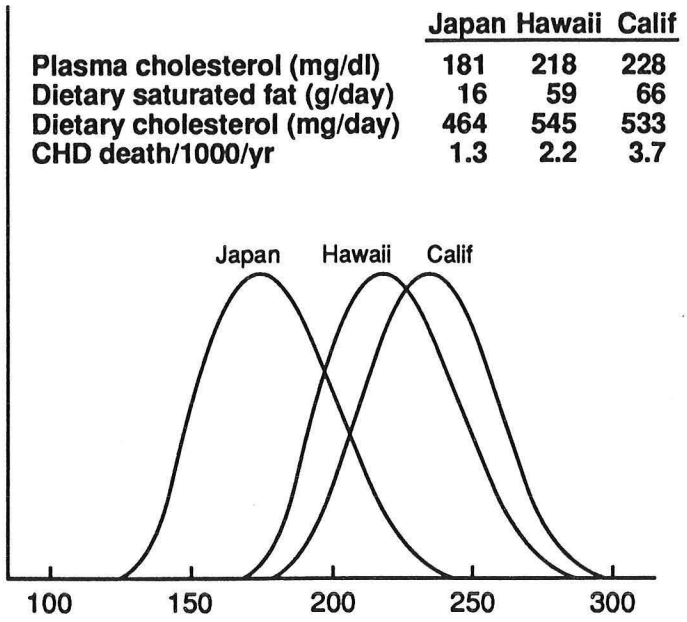
18). In these groups mean plasma cholesterol concentrations rise to only 110-120 mg/dl and CHD does not exist so far as can be determined. The majority of people in the world including most of those living in Asia and in countries surrounding the Mediterranean consume a diet containing about half the saturated fat and cholesterol present in Western diets. In these populations mean plasma cholesterol levels rise into the 160-180 mg/dl range and, as shown in Fig. 4, the incidence of clinical CHD is approximately one-fifth to one-tenth of that in the West(9, 19-21). Thus, in international cross-sectional comparisons, death from CHD correlates strongly with plasma cholesterol concentrations which, in turn, correlate strongly with intake of saturated fat and cholesterol. Such correlations

Figure 4. WHO statistics, 1985.



can also be demonstrated among individuals having the same genetic background as most clearly shown in the Japanese emigration studies. As illustrated in Fig. 5, a stepwise increase in the intake of saturated fat and cholesterol, plasma cholesterol concentrations and death from CHD was found among Japanese men living in Japan, Hawaii and California(8). Other risk factors such as smoking, hypertension and diabetes did not show this Japan-Hawaii-California gradient. A similar gradient in the intake of saturated fat and cholesterol, plasma cholesterol levels and CHD mortality is seen when comparing rural chinese, chinese living in Beijing and chinese living in Hong Kong.

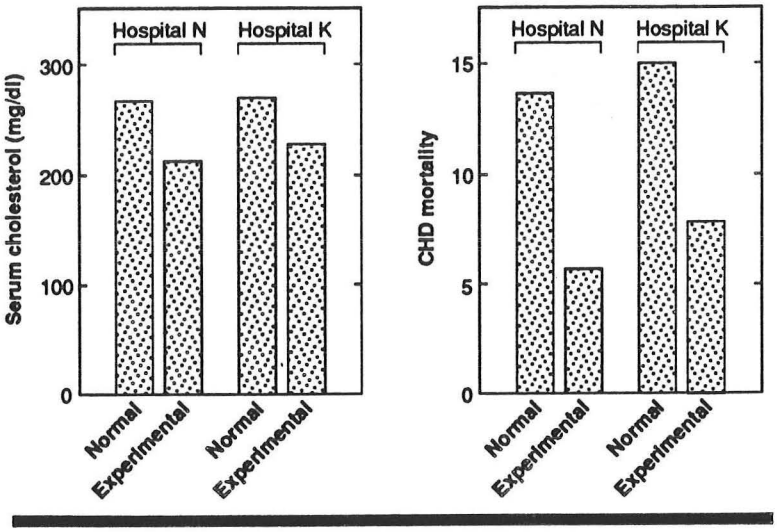
Figure 5. Adapted from reference 8.



Since factors other than diet that might influence CHD risk are not constant in these epidemiologic studies, randomized controlled intervention trials have been undertaken to determine if a reduction in mortality from CHD can be achieved by diet modification. There has been at least six single factor randomized controlled trials of the effect of dietary intervention on serum cholesterol and the incidence of CHD(22-27). None of these trials convincingly showed an effect of diet on mortality from CHD. However, unlike the epidemiologic data, where large differences in plasma cholesterol maintained over a lifetime are related to the incidence of mortality from CHD, these clinical trials are only able to assess whether minor reductions in plasma cholesterol maintained for three to seven years decreases CHD. It is now apparent that these trials were far too small to study the effects on major endpoints such as CHD mortality and it has been determined that a single factor diet-heart study adequate to provide definitive evidence would not be feasible because of considerations of size, duration, adherence, confounding factors, and cost (> 1 billion dollars).

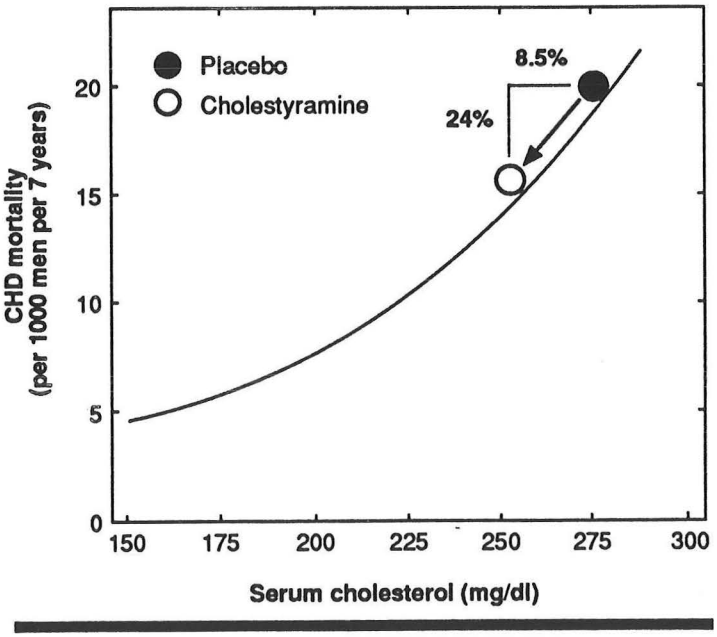
Although not a randomized trial in free-living people, the Finnish Mental Hospital Study did show a significant effect of diet modification on plasma cholesterol concentrations and mortality from CHD(28). This trial involved more than 4000 patients in two separate hospitals. During the first six-year period, the cholesterol and saturated fat content of the diet was reduced in one hospital while the other hospital remained on the normal diet and served as the control. In the second six-year period, the first hospital was returned to the normal diet and the second hospital was placed on the experimental diet. In each hospital, plasma cholesterol concentrations were reduced by about 15% and mortality from CHD by greater than 50% during the six-year period on the experimental diet compared to the six-year period on the normal diet(Fig. 6).

Figure 6. Finnish Mental Hospital Study. Adapted from reference 28.



The Oslo Primary Prevention Study examined the effect of diet and smoking advice on the incidence of CHD in healthy normotensive men over 5 years of followup(29). The dietary advise achieved a 50% net reduction in the intake of saturated fat and cholesterol in the intervention group and mean plasma cholesterol concentrations during the trial were 13% lower in the intervention group. The corresponding net reduction for self-reported smoking cessation was 7%. At the end of the trial, death from CHD was reduced by 55% and all cause mortality was reduced by 33%. It is difficult in multi-factor trials to determine the contribution of each factor to outcome; nevertheless, in this study multivariate

Figure 7. The Lipid Research Clinics Primary Prevention Trial. Adapted from references 30-31.



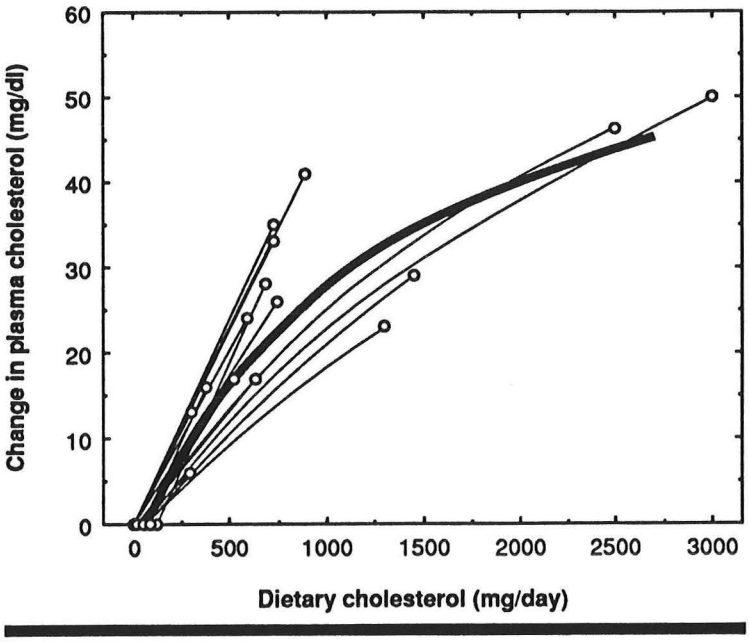
analysis suggested that the cigarette factor accounted for at most 25% of the reduction in CHD mortality. This trial was also significant in that it demonstrated that diet modification could bring about a sustained reduction in plasma cholesterol concentrations in free living men.

Finally, although not designed to test the efficacy of cholesterol-lowering diets, the results of the Lipid Research Clinics Primary Prevention Trial provide the most conclusive evidence on the potential for primary prevention of CHD through reduction of elevated plasma cholesterol concentrations(30, 31). This seven year trial in 3,806 men demonstrated that the incidence of death from CHD could be reduced in middle-aged men by means of cholestyramine treatment. As shown in Fig. 7, cholestyramine treatment produced a 12.6% greater reduction in LDL cholesterol in the treatment group than in the placebo group. Those achieving this modest reduction in LDL cholesterol levels had a 24% reduction in CHD mortality compared to the placebo group.

Effect of dietary cholesterol on LDL metabolism

Epidemiological studies in human populations have suggested a strong correlation between the intake of cholesterol in the diet and CHD. Although some studies suggest that dietary cholesterol may play a role in atherosclerosis independent of its effects on blood cholesterol(32, 33), the link between dietary cholesterol and atherosclerotic lesions is thought to be primarily related to its hypercholesterolemic effects. The effect of dietary cholesterol on plasma cholesterol concentrations has been demonstrated repeatedly in metabolic ward studies. Fig. 8, which summarizes the results of many of these studies, shows mean changes in plasma cholesterol concentrations when various amounts of

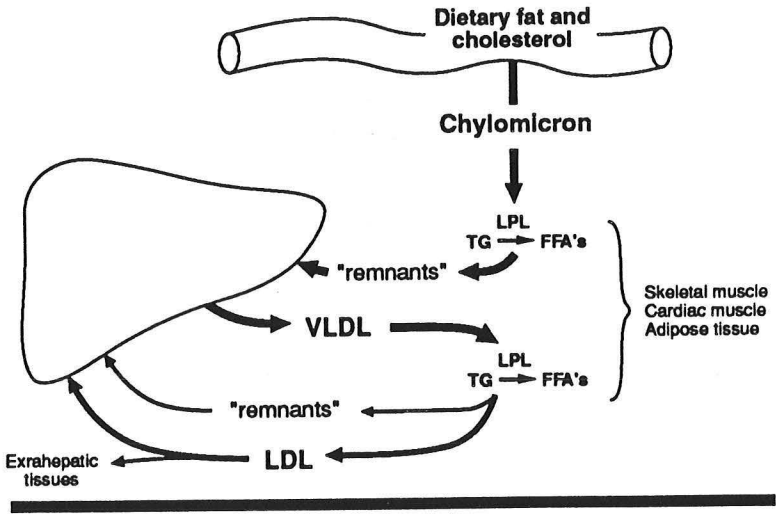
Figure 8. Summary of results from several metabolic ward studies showing the effect on plasma cholesterol levels of adding various amounts of cholesterol to low cholesterol diets. Adapted from references 34-39.



cholesterol are added to low cholesterol diets under metabolic ward conditions(34-39). Over the range of dietary cholesterol intakes commonly seen in humans (0-500 mg/day), mean plasma cholesterol concentrations increase linearly as increasing amounts of cholesterol are added to the diet. On average, the rise in plasma cholesterol concentrations is about 5 mg/dl for each 100 mg of cholesterol that is added to the diet. Above 500 mg/day, increasing the amount of cholesterol in the diet has a variable but generally smaller effect on plasma cholesterol concentrations. The average cholesterol intake in Western diets is 400-500 mg/day. Thus, the addition of even large amounts of cholesterol to an ad libitum Western diet may produce relatively little change in plasma cholesterol concentrations. On the other hand, reducing dietary cholesterol from the usual 400-500 mg/day to 200-250 mg/day would lower plasma cholesterol concentrations by 10-12 mg/dl and eliminating cholesterol from the diet would lower plasma cholesterol by 20-25 mg/dl. In addition to the rigorously controlled metabolic ward studies described above, several cholesterol feeding experiments have been performed in outpatients and in some of these studies the changes in plasma cholesterol concentrations in response to dietary cholesterol were nonsignificant or were less than those predicted from the metabolic ward studies. In many of these studies cholesterol was added to diets already containing significant amounts of cholesterol and as pointed out above, doubling or tripling of the amount of dietary cholesterol will not necessarily increase plasma cholesterol levels if the initial amount of dietary cholesterol is already substantial. In addition, documentation of compliance is more difficult in free-living individuals. As would be expected, plasma cholesterol levels fall significantly when agents that interfere with cholesterol absorption are administered(40-42).

Dietary cholesterol-induced changes in plasma cholesterol levels are due mainly to changes in LDL cholesterol. Dietary cholesterol is not incorporated directly into LDL but rather increases plasma LDL concentrations indirectly through its effects on cholesterol metabolism in the liver. The relationship between whole body cholesterol balance and LDL metabolism is illustrated in Fig. 9. Cholesterol absorbed from the diet, along with the large amount of triglyceride present in the diet, is incorporated into triglyceride-rich chylomicron particles by the enterocyte. Chylomicrons are transported to peripheral capillaries where the triglyceride is hydrolyzed by the enzyme lipoprotein lipase liberating free fatty acids which are taken up by adipocytes and muscle cells. After removal of most of the triglyceride, the chylomicron remnant, which still contains all of the dietary cholesterol, is cleared rapidly in the liver via a specific, high capacity chylomicron remnant receptor pathway that recognizes apoprotein E(43-45). Like the small intestine, the liver also secretes triglyceride-rich particles (called very low density lipoproteins or VLDL), which are also transported to peripheral capillaries where VLDL triglyceride undergoes the same fate as chylomicron triglyceride. After removal of most of the triglyceride, the VLDL remnants circulate back to the liver where they undergo one of two fates(46). Some of the VLDL remnants are cleared rapidly by the liver through interaction of apoprotein E on the surface of the remnant with lipoprotein receptors on hepatocytes. Most of the VLDL remnants, however, are acted on by the enzyme hepatic lipase, which removes all remaining triglyceride resulting in the formation of cholesterol-rich LDL particles. Apoprotein B is the sole protein of LDL and is the ligand that is recognized by the LDL receptor(47). LDL is cleared from plasma by receptor dependent and receptor

Figure 9.



independent pathways. Under normal conditions, about three-fourths of LDL turnover is mediated by LDL receptors, the vast majority of which are located in the liver, and the remainder by receptor independent pathways(48, 49). Receptor dependent LDL uptake in the liver is saturable and can be regulated by dietary and pharmacologic manipulations. Thus, plasma LDL concentrations are determined largely by events in the liver since the liver is the source (via VLDL) of LDL, is involved in the conversion of VLDL to LDL and accounts for most of LDL clearance from the plasma via the LDL receptor pathway.

Cholesterol is an essential structural component of cell membranes and serves as a precursor for steroid products such as bile acids and steroid hormones. Thus, all tissues have ongoing requirements for cholesterol. However, cholesterol is not a necessary component of the diet since the body can synthesize all the cholesterol that it needs from dietary carbohydrate and fat. In the absence of dietary cholesterol, total body cholesterol synthesis amounts to about 1 gram per day and, in a steady-state, an equivalent amount of cholesterol is eliminated from the body largely as biliary cholesterol and bile acids. The addition of increasing amounts of cholesterol to the diet ultimately leads to an increase in liver cholesterol which, in turn, can have profound effects on the synthesis and catabolism of LDL(16, 48, 50-54). Approximately 30-50% of dietary cholesterol is absorbed by the small intestine and in any given individual the fraction of cholesterol that is absorbed remains constant over a wide range of cholesterol intakes. Absorbed cholesterol is delivered quantitatively to the liver via the chylomicron remnant receptor which, in contrast to the LDL receptor pathway, is not regulated by cellular cholesterol. As cholesterol accumulates in the liver, the liver responds by reducing de novo cholesterol synthesis rates and, if necessary, by suppressing the LDL receptor pathway. Both of these compensatory changes protect the liver from becoming further overloaded with cholesterol. Unfortunately, however, suppression of hepatic LDL receptors leads to an increase in circulating LDL levels. In addition, dietary cholesterol increases the rate at which LDL is produced(53) thereby further increasing plasma LDL concentrations.

A remarkable feature of all cholesterol feeding studies, including metabolic ward studies, is the marked variability of response to dietary cholesterol(55-57). Thus, in response to a given amount of dietary cholesterol, some individuals will have a marked increase in plasma cholesterol concentrations while others will have little change and most will have an intermediate response. As an example,

Figure 10. Individual variation in response to dietary cholesterol. Redrawn from reference 57.

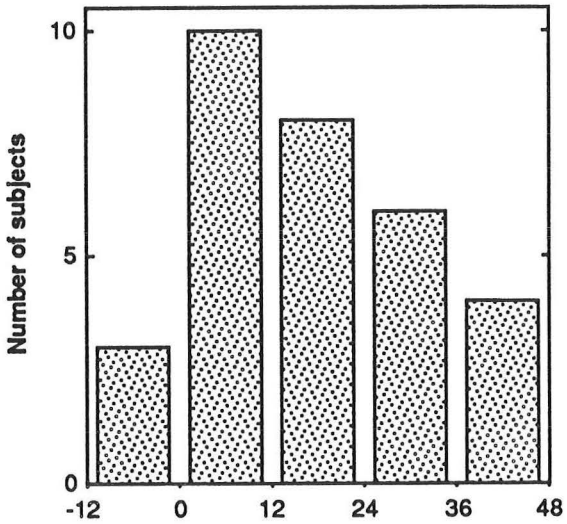
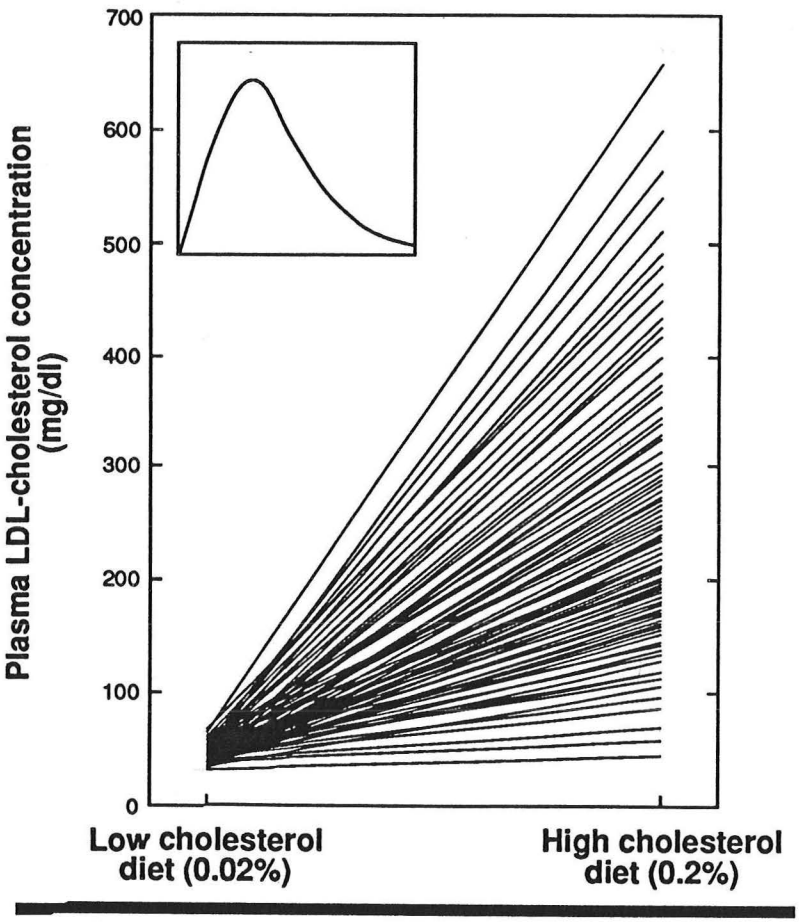


Fig. 10 shows the serum cholesterol response in 32 subjects when dietary cholesterol was increased from ~120 mg/day to ~700 mg/day(57). Several individuals showed no response to the dietary cholesterol, others showed a marked response of over 35 mg/dl and most showed intermediate responses. Since all subjects were on the same diets, and since other environmental influences were similar (exercise, alcohol intake, etc.), these differences in response to dietary cholesterol presumably represent (to a large degree) variations at gene loci involved in the absorption or metabolism of cholesterol. In addition, since individual responses to dietary cholesterol are normally distributed(55-57), the genetic effect is assumed to be polygenic, ie., caused by the contribution of several genes with small additive effects. (Although certain single gene defects, like mutations in the LDL receptor gene, may have large effects on lipoprotein levels, the frequency of these genes is small and they account for only a small fraction of the variability in the population at large.)

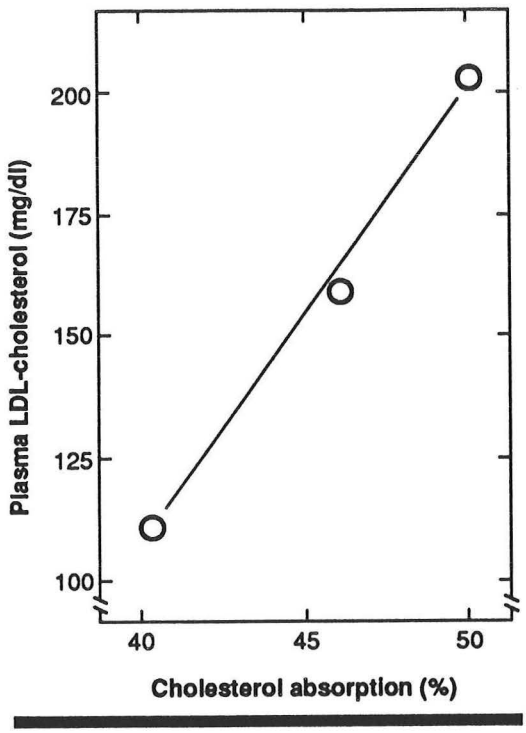
A number of family and twin studies have examined the contribution of heredity and environment to blood cholesterol and lipoprotein variability. These studies indicate that genetic factors account for most of the variation in plasma cholesterol and lipoprotein concentrations in Western populations where everyone consumes a similar diet(58, 59). However, these genetic factors appear to be strongly interactive with the environment, especially the composition of the habitual diet. Thus, much of the variability in plasma cholesterol levels may be due to genetically determined differences in responsiveness to environmental factors such as a high cholesterol, high saturated fat diet. Variability of response to dietary cholesterol occurs in all species that have been examined; however, this phenomena has been studied most extensively in non-human primates. As an example, Fig.11 shows plasma LDL cholesterol concentrations in 78 cynomolgus monkeys when fed low and high cholesterol diets. Mean plasma LDL cholesterol concentrations are about 50 mg/dl on the low cholesterol diet and rise to over

Figure 11. Variability of response to dietary cholesterol in 78 Cynomolgus monkeys.



200 mg/dl on the high cholesterol diet. More striking is the enormous variability in response to dietary cholesterol with LDL cholesterol concentrations on the high cholesterol diet varying over a ten-fold range. Since all animals were treated identically and consumed the same diets, this variability in response to dietary cholesterol is due largely to genetically determined differences. This genetic effect is presumably polygenic since responsiveness to dietary cholesterol is normally distributed(insert). As expected, when maintained on the high cholesterol diet for prolonged periods of time, animals with high plasma LDL concentrations develop much more extensive atherosclerosis than animals with low LDL levels(60). The genetic differences that account for variable responsiveness to dietary cholesterol are not known. Recent studies have been unable to show any differences in the regulation of the LDL receptor pathway or de novo cholesterol synthesis between high and low responding monkeys. The most consistent difference between high and low responding monkeys that has been documented to date is enhanced absorption of dietary cholesterol in the high compared to the low responding animals(61, 62). Similarly, as shown in Fig. 12, cholesterol absorption efficiency has been shown to correlate with plasma cholesterol and LDL concentrations in humans(52, 63).

Figure 12. Relationship between cholesterol absorption efficiency and Plasma LDL-cholesterol in Finnish men.

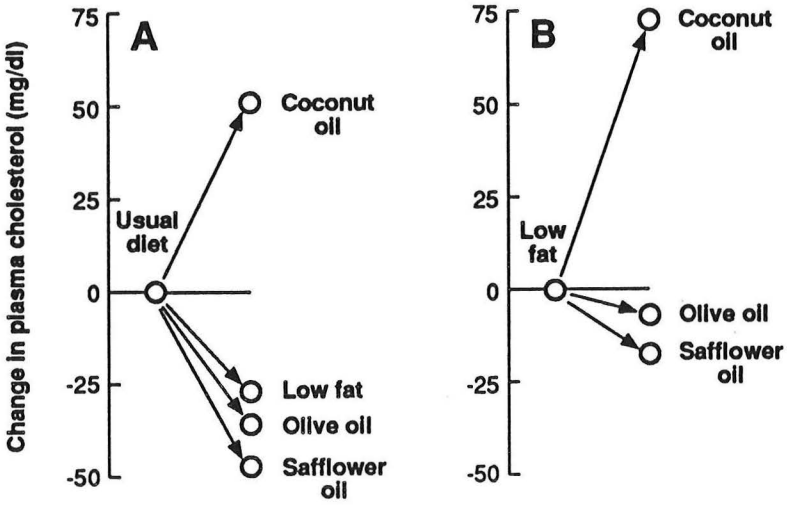


Effect of dietary fats on LDL metabolism

The amount and, more importantly, the kind of fat in the diet have well-documented effects on plasma lipid levels. Fats are divided into major classes based on the type of fatty acids that predominate, ie., saturated, mono-unsaturated or polyunsaturated. The main saturated fatty acids in animal and vegetable fats include lauric(C12:0, ie.,12 carbons, no double bonds), myristic(C14:0), palmitic(C16:0) and stearic(C18:0) acids. Oleic acid(C18:1) is the predominant monounsaturated fatty acid in animal and vegetable fats whereas linoleic acid(C18:2) is the main polyunsaturated fatty acid in liquid vegetable oils. That the quantity and type of fatty acids in the diet strongly influences plasma cholesterol and LDL concentrations was first documented in the 1950's by several investigators(64, 65, 66). The most comprehensive series of studies, however, was carried out in the mid 1960's by Keys et al.(37) and Hegsted et al.(38). In these studies, volunteer men from mental hospitals were kept in locked metabolic wards and fed a series of experimental diets (each alternating with a control diet) over a period of several years. Fig. 13 summarizes the general pattern of responses seen in these studies. Plasma cholesterol concentrations averaged about 225 mg/dl on the usual (control) diet containing about 40% of calories from fat (one-third saturated and two-thirds unsaturated). Under circumstances where dietary cholesterol was constant, replacing most of the fat in the control diet with a highly saturated fat such as coconut oil raised plasma cholesterol concentrations by about 50 mg/dl. On the other hand, replacing most of the fat in the control diet with a polyunsaturated vegetable oil such as safflower oil lowered plasma cholesterol levels by about 50 mg/dl. Similar reductions, although not always as dramatic, were seen when olive oil or carbohydrate were substituted for fat in the control diet. As shown in panel B, when added to a low fat diet, saturated fats markedly raise plasma cholesterol levels whereas polyunsaturated and monounsaturated fats modestly lower plasma cholesterol. Thus, mean plasma cholesterol levels in a group of individuals can be changed by more than 100 mg/dl by altering the amount and/or the type of fatty acids in the diet. Since the average American diet falls approximately midway between these two extremes, reductions of 40-50 mg/dl are at least possible in most individuals by altering the type and/or the amount of fatty acids in the diet. As discussed above, even greater reductions in plasma cholesterol are possible if the cholesterol content of the diet is also decreased.

Although saturated fats generally raise plasma cholesterol and LDL levels, the magnitude of this effect varies widely depending on the fatty acid composition

Figure 13. Adapted from references 37-38.



of the fat. Many saturated fats actually contain substantial amounts of unsaturated fatty acids. For example, most animal fats contain about 50% saturated and 50% unsaturated fatty acids. Important exceptions include butter fat which is more highly saturated and marine lipids which are less saturated. In addition, the saturated fatty acids in common saturated fats vary in chain length from 4 to 18 carbons and the chain length of a fatty acid markedly influences its effects on plasma cholesterol concentrations. The relative cholesterol elevating activity of saturated fatty acids of various chain lengths is summarized in Fig.14. Saturated fatty acids with 10 or fewer carbons have essentially no effect on plasma cholesterol concentrations(67). The practical significance of this is limited since most commonly used saturated fats contain relatively small amounts, if any, of these fatty acids. These fatty acids are absorbed directly into the portal vein(rather than incorporated into chylomicrons as are longer chain fatty acids), but whether this accounts for their lack of effect is not known. Stearic acid(C18:0) also has essentially no effect on plasma cholesterol levels(38, 68). Indeed, Bonanome and Grundy recently showed that stearic acid was equivalent to oleic acid in its effect on plasma lipoproteins and suggested that the reason may be rapid conversion of stearic acid to oleic acid in the body(69). In this country, the highest amounts of stearic acid are found in cocoa butter (~34%) and beef fat (~18%). Consequently,

Figure 14. Hypercholesterolemic effects of individual saturated fatty acids. Adapted from references 37, 38, 67,69.

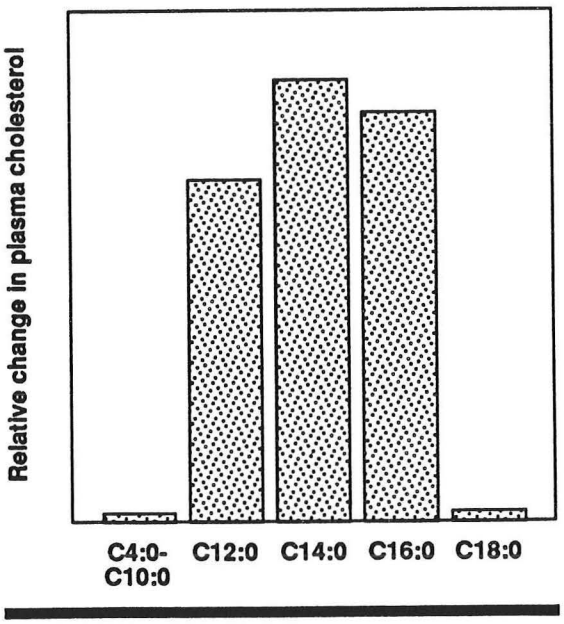
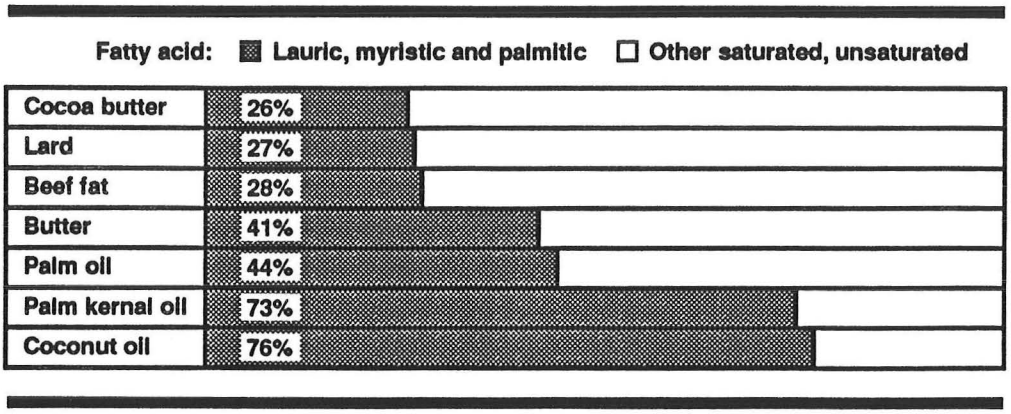


Figure 15. Amount of cholesterol elevating saturated fatty acids in several common saturated fats.



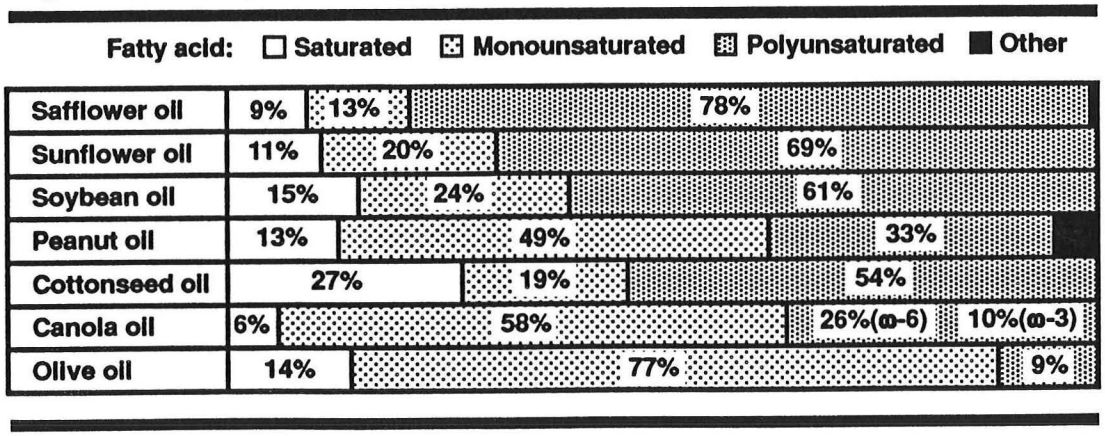
beef and chocolate are somewhat less hypercholesterolemic than might be expected based on their total saturated fatty acid content(70). Thus, the cholesterol elevating effect of saturated fats is due entirely to their content of lauric(C12:0), myristic(C14:0) and palmitic acids(C16:0). Fig. 15 shows the amount of these cholesterol elevating fatty acids in several common animal and vegetable fats.

The so called tropical oils are saturated vegetable oils that together account for about 7% of the fat consumed in this country. These oils include coconut oil, palm kernal oil, palm oil and cocoa butter. Of these, coconut oil and palm kernal oil are among the most hypercholesterolemic of all saturated fats (on a per gram basis) due to their high content of cholesterol elevating saturated fatty acids(Fig.15). Palm oil and cocoa butter are somewhat less hypercholesterolemic due to substantial amounts of oleic and/or stearic acids.

Hydrogenated vegetable oils(primarily hydrogenated soy bean oil) account for nearly 15% of the fat consumed in this country(71). Hydrogenation is used to convert liquid oils to semisolid fats, which facilitates their formulation into margarines and shortenings. Linoleic acid(C18:2) is the main polyunsaturated fatty acid in vegetable oils and exists in the cis configuration, ie., the hydrogen atoms are on the same side of the double bonds causing a kink in the fatty acid. During hydrogenation, fatty acid isomers are formed in which some of the double bonds have been rearranged from the cis to the trans configuration and in which the double bonds have migrated to new positions along the fatty acid chain. Typically, 10-15% of cis linoleic acid is converted into trans oleic acid whose physical properties(melting point, etc.) more closely resemble a saturated than an unsaturated fatty acid. The widespread usage of partially hydrogenated vegetable oils in the U.S. has prompted a great deal of interest in the nutritional, biochemical and toxicological effects of trans fatty acids. Overall, it has been difficult to demonstrate any adverse consequences of consuming hydrogenated vegetable oils. In general, partially hydrogenated vegetable oils have little effect on plasma cholesterol levels(36) and in this respect appear to be a good substitute for saturated fats such as butter, coconut oil and palm kernal oil, which have marked hypercholesterolemic effects.

A great deal of discussion still surrounds the role that unsaturated vegetable oils should play in a cholesterol lowering diet and whether any of the many preparations available offer any advantages over the others. Fig.16 shows the fatty acid composition of several common unsaturated vegetable oils. Canola

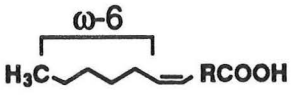
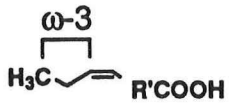
Figure 16. Fatty acid composition of common unsaturated vegetable oils.



oil and olive oil contain predominantly oleic acid (monounsaturated) whereas the other oils contain mainly linoleic acid (polyunsaturated). The large series of experiments by Keys et al. and Hegsted et al. discussed above suggested that polyunsaturated fats may lower plasma cholesterol levels more effectively than monounsaturated fats when used to replace saturated fat in the diet. More recent studies directly comparing the effects of these two types of fats indicate that at ordinary intakes, polyunsaturated and monounsaturated fatty acids have essentially identical effects on plasma lipoprotein levels(72). At very high intakes, polyunsaturated fatty acids, but not monounsaturated fatty acids, appear to reduce HDL levels(73). Thus, the highly polyunsaturated vegetable oils appear to offer no advantage over the monounsaturated oils. Since liquid vegetable oils tend to lower plasma cholesterol levels, these oils need not be restricted from the standpoint of their effects on plasma cholesterol(74-78). On the other hand, these fats contribute nine calories per gram and provide essentially no other nutritional benefits. Thus, the major emphasis should be on a shift from foods rich in saturated fat and cholesterol to whole grains, fruits and vegetables. These later foods contain no cholesterol and only small amounts of fat, which are predominantly unsaturated. Furthermore, these foods are rich in fiber which may exert a favorable influence on plasma lipoprotein levels. High-carbohydrate, low-fat diets have been associated with reductions in HDL levels in some(74, 76, 77, 79), but not all(75, 78, 80, 81), studies. This may be a transient phenomena as it seems to be less common in the longer term studies. In addition, the significance of diet-induced changes in HDL levels is not known since diet induced changes are due to changes in the production of the major apoprotein of HDL whereas differences in HDL levels among people on the same diet reflect differences in the catabolism of this protein(82).

The apparent low incidence of CHD in Greenland Eskimos has stimulated interest in the potential beneficial effects of their unusual diet, which consists largely of seal, whale and fish(83, 84). Although this diet is rich in cholesterol and fat, the fat is unique in that it contains large amounts of the long-chain ω-3 polyunsaturated fatty acids eicosapentenoic acid(C20:5) and docosahexaenoic acid(C22:6)(Fig. 17). These fatty acids affect all cell membranes and modify prostaglandin and leukotriene formation(85). As a consequence, they have a variety of biologic activities that include effects on platelet function, inflammation and blood lipid levels. When used to replace saturated fat in the diet, fish oil

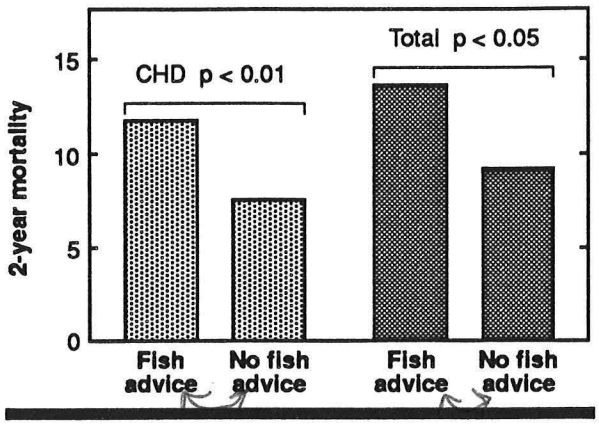
Figure 17. Structure and dietary source of ω-6 and ω-3 polyunsaturated fatty acids.

Fatty acid	Structure	Dietary source
Linoleic acid (18:2, ω-6) Arachidonic acid (20:4, ω-6)		Vegetable oils
Eicosapentaenoic acid (20:5, ω-3) Docosahexaenoic acid (22:6, ω-3)		Marine oils

lowers plasma cholesterol levels as effectively as polyunsaturated vegetable oil despite the fact that fish oil contains some cholesterol. Indeed, purified preparations of ω-3 polyunsaturated fatty acids are significantly more active than ω-6 polyunsaturated fatty acids(64, 86). Importantly, neither fish oil nor polyunsaturated vegetable oils(in reasonable amounts) significantly reduce plasma cholesterol levels when simply taken as a supplement to the usual Western diet and in some hypertriglyceridemic patients, fish oil supplements may actually raise plasma LDL levels(87). In contrast to polyunsaturated vegetable oil, fish oil markedly reduces plasma triglyceride levels. The effect of fish oil on platelet function is likely the result of alterations in prostaglandin metabolism(85). Ordinarily, dietary linoleic acid is converted to arachidonic acid which is the precursor of the "2" series prostaglandins. Platelets generate thromboxane A_2, which causes platelet aggregation and vascular smooth muscle constriction whereas blood vessel endothelium produces prostacyclin(PGI_2), which has the opposite effects. Eicosapentaenoic acid inhibits the cell membrane formation of arachidonic acid and substitutes for it giving rise to the "3" series prostaglandins and reducing "2" series prostaglandins. This leads to a moderate hemostatic defect since PGI_3 is almost as potent as PGI_2 whereas thromboxane A_3 is hardly, if at all, biologically active. Indeed, when fed in sufficient amounts(3.6 gram/day or more of ω-3 fatty acid for at least two weeks) fish oil prolongs the bleeding time.

A major question regarding fish oil is whether any of its many biological effects is important clinically. In cross-sectional surveys, there generally is an inverse relationship between fish consumption and CHD. Within populations, some prospective trials have shown an inverse relationship between fish consumption and CHD mortality(88, 89) whereas others have not(90, 91). In a recent randomized controlled trial in men who had recovered from myocardial infarctions(Fig. 18), advice to increase fish intake led to a 4-fold increase in the intake of ω-3 polyunsaturated fatty acids that was associated with significant reductions in total(27%) and CHD(36%) mortality at the end of 2 years(92). In animals, fish oil has been shown to retard the development of CHD when added as

Figure 18. Effect of advice to eat fish on 2-year mortality in men who had recovered from myocardial infarction (reference 92).



a supplement to an atherogenic diet(93). In a dog vein-graft model, intimal proliferation was inhibited by fish oil compared to aspirin-dipyridamole(94). Recently, several trials have examined the effect of fish oil supplements on the rate of early restenosis following angioplasty. Dehmer et al. from this institution showed that fish oil significantly reduced the incidence of early vessel restenosis following angioplasty(95) although two subsequent studies have failed to confirm these findings(96, 97). The positive results of Dehmer et all may be related to the fact that they began with a population at relatively high risk for restenosis, that the fish oil supplement was started 7 days before angioplasty and that the incidence of stenosis was determined by performing followup arteriography in all patients. Taken together, these studies suggest that modest fish intake (two to three times per week) may favorably influence atherosclerosis and CHD. Whether supplementing the diet with fish oil capsules will have a similar effect is not known but is not recommended at the present time.

Although dietary fatty acids have been known to alter plasma cholesterol and lipoprotein levels for several decades, their mechanism of action is not well understood. As discussed above, the concentration of LDL in plasma is determined by the rate at which LDL is produced relative to the rate at which LDL is removed from plasma. In humans, replacement of saturated fatty acids with polyunsaturated fatty acids or with carbohydrate appears to favorably influence both of these processes, ie., removal of LDL from plasma is increased and LDL production is decreased(98, 99, 100). The same pattern is seen in animal studies where the increase in LDL clearance can be shown to be due to an increase in receptor dependent LDL uptake by the liver(54, 101). This results in a fall in LDL concentrations and a transient increase in the amount of LDL-cholesterol that is delivered to the liver, which is apparently eliminated in the bile as biliary cholesterol or bile acid(102-105). As plasma LDL levels fall, however, a new steady-state is achieved in which an increased number of hepatic LDL receptors results in the uptake of LDL cholesterol at the same rate as before but now at a lower plasma LDL concentration. In the long term, the type of fat in the diet appears to have relatively little effect on cholesterol absorption, total body cholesterol synthesis, bile acid synthesis or biliary cholesterol output(106).

Although liquid and partially hydrogenated vegetable oils can be used in cooking and baking to replace more hypercholesterolemic fats, these oils still provide nine calories per gram of fat and as such pose a problem for those trying to lose weight. Thus, there is a great deal of interest in developing nonabsorbable fats. Sucrose polyester(Olestra) is a nonhydrolyzable fat developed by the Proctor and Gamble Company that will probably be the first product of its kind approved by the FDA. Sucrose polyester is a mixture of hexa-, hepta- and octa-esters that

are formed by esterification of sucrose with long chain fatty acids. It has the appearance and physical properties of conventional fats but differs in that it cannot be hydrolyzed by pancreatic enzymes(107) and as a consequence is not absorbed(108). When substituted for dietary triglycerides or when added to the normal diet, sucrose polyester has been shown to reduce plasma LDL concentrations in humans(109-114). This effect appears to be secondary to a reduction in the absorption of dietary and biliary cholesterol resulting from the partition of cholesterol in the intestinal lumen between the micellar and sucrose polyester oil phases; cholesterol retained in the unabsorbable oil phase of sucrose polyester is excreted. Sucrose polyester does not interfere with the hydrolysis or absorption of dietary triglycerides. The consistency of sucrose polyester can be varied from liquid to semisolid depending on the fatty acids that are esterified to sucrose. Liquid formulations tend to have a mild cathartic effect; however, substitution of up to 50% or more of the fat in the diet with sucrose polyester seems to be well tolerated. Whether sucrose polyester will be useful in long-term weight reduction is not known. However, if substituted for conventional fats, it should have a favorable effect on plasma lipoprotein levels.

Role of elevated LDL concentrations in the initiation and progression of atherosclerosis

The arterial intima is the cell layer principally involved in athersclerosis. The earliest recognized gross lesion of athersclerosis is the fatty streak which is first observed at about ten years of age in the coronary arteries. Fatty streaks are characterized by an accumulation of cells loaded with cholesteryl esters(foam cells) just beneath the endothelium. Although the precise cellular and biochemical mechanisms are not understood, it is now clear that most foam cells arise from circulating monocytes that have taken up residence beneath the vascular endothelium and that the plasma is the source of most of the cholesteryl ester present in atherosclerotic lesions(115-120). With time, fatty streaks may progress to fibrous plaques, which are characterized by an accumulation of cholesteryl esters (both intra and extracellular), smooth muscle cells and connective tissue. These lesions may become further complicated as a result of cell necrosis, calcification and hemorrhage. The response to injury hypothesis, which is one of the oldest and most widely accepted theories on the pathogenesis of atherosclerosis, is based on the similarity between atherosclerosis and the response of arteries to experimental injury. In this theory, injury to endothelial cells exposes the subendothelial space to platelets and plasma lipoproteins. As a consequence, lipid accumulates and platelet derived growth factors lead to the proliferation of smooth muscle cells and connective tissue. More recently, from the work of Drs. Brown and Goldstein, it has become apparent that high LDL concentrations (resulting from a single gene defect) uniformly results in premature athersclerosis suggesting that infiltration of plasma LDL from the bloodstream into the vessel wall may be sufficient in and of itself to initiate the process of athersclerosis.

LDL has been shown to accumulate in atherosclerotic lesions both in animals and in humans(116, 118, 119). The amount of LDL in the subendothelial space is determined by the rate of LDL influx relative to the rates of LDL degradation and LDL efflux from the arterial wall. LDL is transported across endothelial cells by vesicular transport and the flux of LDL across the

endothelium into the intima is directly proportional to the concentration of LDL in plasma(121) In addition, the flux of LDL into the intima is generally higher in areas prone to develop fatty streaks than in areas more resistant to fatty streak development. Thus, when animals are placed on a high cholesterol diet, the rate of LDL entry into the subendothelial space increases in direct proportion to the increase in circulating LDL levels. Although rates of LDL degradation and LDL efflux from the arterial wall also increase under these circumstances they do not keep pace with influx and LDL begins to accumulate(122). Thus, the first detectable alteration in the arterial intima in an area destined to become a fatty streak is an increase in the content of LDL. Subsequently, blood monocytes adhere to the overlying endothelium, penetrate into the subendothelial space and become engorged with lipid to become foam cells.

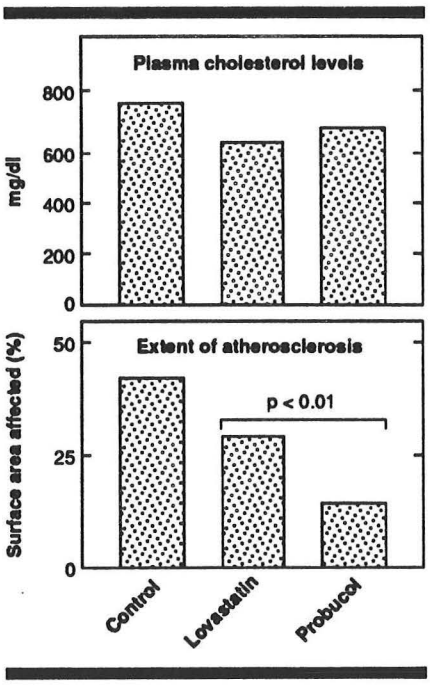
Another series of studies has examined the mechanisms by which monocytes and monocyte-derived tissue macrophages are converted to foam cells(123, 124). Although monocyte/macrophages take up LDL through the classic LDL receptor pathway, these cells express a relatively limited number of receptors for native LDL and the receptors they do have are under feed-back regulation by cellular cholesterol. Thus, monocyte/macrophages in culture cannot be converted to foam cells even when incubated with high concentrations of native LDL(123). Subsequently, Drs. Brown and Goldstein and coworkers described a second receptor on monocyte/macrophages that did not recognize native LDL but rather recognized LDL that had been modified chemically by acetylation(125). This receptor, termed the scavenger or acetyl LDL receptor, is not subject to feedback regulation by cellular cholesterol and has been found only on monocyte/macrophages, Kupffer's cells and liver sinusoidal endothelial cells. Thus, when offered to monocyte/macrophages in vitro, chemically modified LDL, but not native LDL, leads to the accumulation of large amounts of cholesteryl esters and eventually to foam cell formation. Subsequently, other chemical modifications, such as treatment with the lipid peroxidation product, malonaldehyde, were found to convert native LDL into particles recognized by the scavenger receptor(126). These modifications have in common the alteration of lysine residues of apoprotein B which results in an increase in the net negative charge of LDL.

Since none of these chemical modifications are likely to occur in vivo attention has been focused on modifications of LDL that could potentially occur in the arterial wall in vivo ("biologic modification") and account for foam cell formation. In this regard, cell-induced oxidation of LDL has been best studied. When LDL is incubated with cultured endothelial cells it undergoes a series of physical and chemical changes that result in its recognition and rapid uptake by scavenger receptors on cultured macrophages(124). Similar oxidative modification of LDL can also be produced by incubating LDL with cultured smooth muscle cells or monocyte/macrophages. Thus, the three major cell types in the artery wall can convert LDL in vitro to a form recognized by the scavenger receptor. During cell-induced oxidation of LDL, the initiating step is the peroxidation of polyunsaturated fatty acids in the 2-position of phospholipids, a step that appears to involve phospholipase A_2, which is carried in the LDL particle itself, and cellular lipoxygenases(127, 128). The chemical alterations that take place during oxidative modification of LDL include 1) complete depletion of vitamin E carried in the LDL particle followed by extensive conversion of lecithin to lysolecithin, 2) generation of lipid peroxidation products some of which

covalently bind to lysine groups on apoprotein B and thereby increase the net negative charge of LDL and 3) fragmentation of apoprotein B(124, 129, 130). It should be noted that cell-induced oxidative modification of LDL has only been demonstrated in in vitro studies and requires the complete absence of antioxidants and the presence of at least low concentrations of copper or iron in the medium. The addition of plasma strongly inhibits cell-induced oxidation suggesting that if this process occurs in vivo, it must take place extravascularly in microenvironments where antioxidants might become depleted.

Two lines of evidence suggest that oxidative modification of LDL does occur in vivo and plays a pathogenetic role in atherosclerosis. First, LDL can be detected in atherosclerotic lesions of animals and humans that has many of the characteristics of LDL that has been oxidatively modified in vitro(131-133). In addition, when extracted from atherosclerotic lesions, this LDL is rapidly taken up via scavenger receptors on monocyte/macrophages but is not recognized by the LDL receptor. Second, the antioxidant probucol has been shown to selectively inhibit LDL degradation in macrophage-rich fatty streaks and to retard the progression of atherosclerosis in rabbits that have high LDL levels due to a genetic lack of functional LDL receptors(134, 135). Probucol is a strong antioxidant that is transported in the LDL particle itself and effectively blocks the oxidative modification of LDL in vitro. Since probucol is also a lipid lowering agent, the effect of probucol was compared to a dose of lovastatin that produced a similar reduction in plasma cholesterol levels. In addition to oxidative modification, LDL in the arterial intima may become modified through interaction with various components of the matrix (collagen, elastin and proteoglycans) so as to be rapidly taken up by macrophages(118, 136).

Figure 19. Effect of Probucol on atherosclerosis in hypercholesteroemic rabbits (reference 134).



A very early event in experimental atherosclerosis is the adherence of circulating monocytes to arterial endothelial cells followed by migration into the subendothelial space at sites destined to become fatty streaks. Oxidized LDL may be involved in the recruitment and retention of macrophages since, at least in vitro, it is a potent chemoattractant for monocytes and reduces the mobility of resident macrophages. In addition, monocyte/macrophages from hypercholesterolemic animals are much more adhesive to normal endothelial cells than are monocyte/macrophages from normocholesterolemic animals(137). Finally, hypercholesterolemia may induce a receptor (adherence molecule) on endothelial cells that specifically binds circulating monocytes (Dr. Gimbrone, University lecture series, 1990).

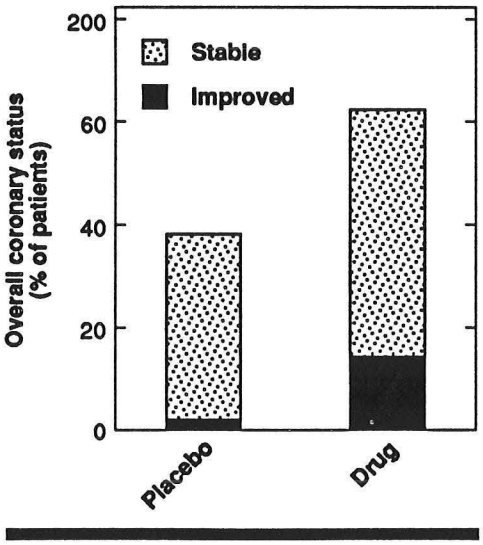
Together, these studies suggest that a high plasma LDL concentration may, in and of itself, lead to the formation of fatty streaks and atherosclerosis. High circulating LDL concentrations lead to the retention of LDL in the arterial

intima. (Susceptible sites of the arterial tree appear to be more permeable to LDL than nonsusceptible sites.) In the intima, LDL is modified so as to be rapidly taken up by monocyte/macrophages via the scavenger receptor or by phagocytosis leading to the formation of foam cells. Modification of LDL probably occurs in part through oxidation and in part through interaction with matrix components. Subsequently, macrophage-smooth muscle cell interactions, platelet-macrophage interactions and platelet-endothelial cell interactions lead to the development of more advanced proliferative lesions(138).

Regression of atherosclerosis

Since atherscleroses is present to some degree in the coronary arteries of most adult males and of many females over the age of fifty, any hope of benefit from dietary therapy must be based, to a large degree, on the possibility that existing lesions can be made to regress or at least be stabilized. Several studies have been undertaken to examine the hypothesis that lowering plasma cholesterol concentrations reduces the rate of progression of coronary atherscleroses as measured angiographically. In only one of these was plasma cholesterol levels lowered by diet alone(139). In this uncontrolled trial, 39 men with angina and a least one vessel with 50% stenosis were placed on a low saturated fat low cholesterol diet for two years at which time they underwent repeat coronary arteriography. Dietary therapy achieved a mean reduction in plasma cholesterol concentrations of 10%. Overall, 21 patients showed no lesion growth and 18 showed progression of disease and there was a significant correlation between lesion growth and the total cholesterol/HDL cholesterol ratio. Since there was no control group, even this later correlation is hard to interpret. Much greater reductions in plasma cholesterol levels for longer periods of time have been achieved using drug therapy. In the NHLBI Type II Coronary Intervention Trial, 116 patients were randomized to cholestyramine (which produced a mean reduction in plasma LDL cholesterol of 26%) or to placebo(140). After 5 years, 25% of the cholestyramine group and 35% of the placebo group showed definite evidence of progression, a difference that did not achieve statistical significance. Even greater changes in plasma lipoprotein levels(43% reduction in LDL-cholesterol, 37% increase in HDL cholesterol) were achieved in the Cholesterol-Lowering Atherosclerosis Study(CLAS), a randomized, placebo-controlled trial testing combined colestipol and niacin therapy in 162 men with previous coronary bypass surgery(141). After two years, drug therapy resulted in significant reductions in the average number of lesions per patient that progressed, the percentage of subjects with new atheroma formation in native coronary arteries, and the percentage of subjects with new lesions in bypass grafts; perceptible improvement in overall coronary status occurred in 16% (compared to 2% of controls). This trial shows that large changes in plasma

Figure 20. The Cholesterol-Lowering Atherosclerosis Study (reference 141).



lipoprotein levels can produce stabilization and in some cases improvement in coronary atherosclerosis. However, it is unlikely that the changes in plasma lipoprotein levels achieved in this study could be produced by diet therapy alone.

A large number of regression studies have been performed in a variety of animal models. In these studies, regression is usually studied after atherosclerosis has been induced by a high-fat, high-cholesterol diet. In general these many studies indicate that the occurrence of regression is related both to the severity of the lesions and to the degree to which plasma cholesterol levels can be lowered. Fatty streaks and relatively uncomplicated lesions regress completely even at plasma cholesterol levels in the 200-300 mg/dl range(142, 143). However, complicated fibrocalcific lesions seem to regress very slowly, if at all, even when plasma cholesterol levels are reduced to very low levels. Taken together these studies suggest that modest reductions in plasma cholesterol concentrations begun early in life may slow the progression of athersclerosis to the point that it may never become a clinical problem. However, once complicated lesions have developed, much more drastic reductions in plasma cholesterol concentrations may be necessary in order to achieve regression or even stabilization of disease.

Summary

It is now clear that an elevated concentration of LDL in plasma is sufficient to lead to the initiation and progression of atherosclerosis and that lowering LDL levels can reduce the risk of CHD. Epidemiologic data and population migration studies suggest that the high mean plasma LDL concentrations in this country are due in large part to the high intake of saturated fat and cholesterol. Individual variation in plasma LDL concentrations in this country is due largely to hereditary factors, the most important of which may be genetically determined differences in response or susceptibility to the Western diet. Based on risk of death from CHD, at least one-third of adult Americans have undesirably high plasma LDL concentrations and diet modification is the primary mode of therapy. Although simple conceptually, diet therapy is probably best carried out under the supervision of a dietician. Diet therapy primarily involves a shift from foods rich in saturated fat and/or cholesterol to whole grains, fruits and vegetables. Such diet modification has great potential for reducing plasma LDL levels and the risk of clinical CHD. However, compliance is a major problem, especially in asymptomatic young adults where the potential long-term benefits are probably the greatest.

1. Goldman, L. and E. F. Cook. 1984. The decline in ischemic heart disease mortality rates. Ann. Int. Med. 101: 825-836.

2. Consensus Conference. 1985. Lowering blood cholesterol to prevent heart disease. JAMA. 253: 2080-2086.

3. Expert Panel. 1988. Report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. Arch. Intern. Med. 148: 36-69.

4. Martin, M. J., S. B. Hulley, W. S. Browner, L. H. Kuller and D. Wentworth. 1986. Serum cholesterol, blood pressure, and mortality: Implications from a cohort of 361,662 men. Lancet. ii: 933-936.

5. Sempos, C., R. Fulwood, C. Haines, M. Carroll, R. Anda, D. F. Williamson, P. Remington and J. Cleeman. 1989. The prevalence of high blood cholesterol levels among adults in the United States. JAMA. 262: 45-52.

6. Inter-society commission for heart disease resources. 1984. Optimal resources for primary prevention of atherosclerotic diseases. Circ. 70: 153A.

7. Keys, A., M. A., M. J. Karvonen, C. Aravanis, H. Blackburn, R. Buzina, B. S. Djordjevic, A. S. Dontas, F. Fidanza, M. H. Keys, D. Kromhout, S. Nedeljkovic, S. Punsar, F. Seccareccia and H. Toshima. 1986. The diet and 15-year death rate in the seven countries study. Am. J. Epidemiol. 124: 903-914.

8. Kato, H., J. Tillotson, M. Z. Nichaman, G. G. Rhoads and H. B. Hamilton. 1973. Epidemiologic studies of coronary heart disease and stroke in Japanese men living in Japan, Hawaii and California. Am. J. Epidemiol. 97: 372-385.

9. Chen. J., T. C. Campbell, J. Li and R. Peto. 1990. Diet, Lifestyle and mortality in China: A study of the characteristics of 65 Chinese counties. Oxford Press, Oxford.

10. Andersen, G. E., P. Louis and B. Friis-Hansen. 1979. Hyperlipoproteinemia in newborn infants A study of 1025 families. Acta. Paediatr. Scand. 68: 683-690.

11. Glueck, C. J., F. Heckman, M. Schoenfeld, P. Steiner and W. Pearce. 1971. Neonatal familial type II hyperlipoproteinemia: Cord blood cholesterol in 1800 births. Metab. 20: 597-608.

12. Jian-zhai, L., L. Pei-ying, N. Qing-tian, W. Shu, J. Lei, Z. Shu-hua, G. Han-bang, G. Hua, Z. Zhi-ming and F. Xiang-zhong. 1988. Serum-lipid and lipoprotein patterns of Beijing populations from birth to senescence. Chin. Med. J. 101: 659-664.

13. Strobl, W. and K. Widhalm. 1985. The natural history of serum lipid and lipoproteins during childhood. In Detection of lipid and lipoprotein disorders of childhood. K. Widhalm and H. K. Naito(eds). Liss, A. R., New York.

14. Wilson, P. W. E., W. P. Castelli and W. B. Kannel. 1987. Coronary risk prediction in adults (the Framingham heart study). Am. J. Cardol. 59: 91G-94G.

15. Connor, W. E., M. T. Cerqueira, R. W. Connor, R. B. Wallace, R. Malinow and H. R. Casdorph. 1978. The plasma lipids, lipoproteins, and diet of the Tarahumara indians of Mexico. Am. J. Clin. Nutr. 31: 1131-42.

16. Whyte, M., P. Nestel and A. MacGregor. 1977. Cholesterol metabolism in Papua New Guineans. Eur. J. Clin. Invest. 7: 53-60.

17. Mendez, J., C. Tejada and M. Flores. 1962. Serum lipid levels among rural Guatemalan Indians. Am. J. Clin. Nutr. 10: 403-409.

18. Miller, K., A. Rubenstein and P. O. Astrand. 1968. Lipid values in Kalahari bushmen. Arch. Intern. Med. 121: 414-417.

19. Kesteloot, H., D. X. Huang, X. S. Yang, J. Claes, M. Rosseneu, J. Geboers and J. V. Joossens. 1085. Serum lipids in the People's Republic of China. Arteriosclerosis. 5: 427-433.

20. Xing-sheng, Y., H. Kesteloot and H. Da-xian. 1986. Serum cholesterol in China and the West. Chin. Med. J. 99: 183-186.

21. Han-zhong, Z., H. Qin-qin and C. Hao-zhu. 1986. Study of serum lipids and lipoproteins of healthy subjects in Shanghai. Chin. Med. J. 99: 657-659.

22. Dayton, S., M. L. Pearce, S. Hashimoto, W. J. Dixon and U. Tomiyasu. 1969. A controlled trial of a diet high in unsaturated fat. Circ. 40(suppl II): 1-62.

23. Frantz, Jr., I. D., E. A. Dawson, P. L. Ashman, L. C. Gatewood, G. E. Bartsch, K. Kuba and E. R. Brewer. 1988. Test of effect of lipid lowering by diet on cardiovascular risk. The Minnesota coronary survey. Arteriosclerosis. 9: 129-135.

24. MRC diet study. 1965. Low-fat diet in myocardial infarction. Lancet. II: 501-504.

25. Leren, P. 1970. The Oslo Diet-heart study. Eleven-year report. Circ. 40: 935-942.

26. Rose, G. A., W. B. Thompson and R. T. Williams. 1965. Corn oil in treatment of ischemic heart disease. Br. Med. J. 1: 1531-1533.

27. Woodhill, J. M., A. J. Palmer, B. Leelarthaepin, C. McGilchrist and R. B. Blacket. 1978. Low fat, low cholesterol diet in secondary prevention of coronary heart diesease. Adv. Exper. Med. Biol. 109: 317-330.

28. Turpeinen, O. 1979. Effect of cholesterol-lowering diet on mortality from coronary heart disease and other causes. Circ. 59: 1-7.

29. Hjermann, I., K. Velve Byre, I. Holme and P. Leren. 1981. Effect of diet and smoking intervention on the incidence of coronary heart disease. Lancet. ii: 1303-1310.

30. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. 1984. JAMA. 251: 351-364.

31. The lipid research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. 1984. JAMA. 251: 365-374.

32. Armstrong, M. L., M. B. Megan and E. D. Warner. 1974. Intimal thickening in normocholesterolemic rhesus monkeys fed low supplements of dietary cholesterol. Circ. Res. 34: 447-454.

33. Shekelle, R. B. and J. Stamler. 1989. Dietary cholesterol and ischaemic heart disease. Lancet. i: 1177-1178.

34. Beveridge, J. M. R., W. F. Connell, G. A. Mayer and H. L. Haust. 1960. The response of man to dietary cholesterol. J. Nutr. 71: 61-65.

35. Connor, W. E., D. B. Stone and R. E. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. J. Clin. Invest. 43: 1691-1696.

36. Erickson, B. A., R. H. Coots, F. H. Mattson and A. M. Klingman. 1964. The effect of partial hydrogenation of dietary fats, of the ratio of polyunsaturated to saturated fatty acids, and of dietary cholesterol upon plasma lipids in man. J. Clin. Invest. 43: 2017-2025.

37. Keys, A., J. T. Anderson and F. Grande. 1965. Serum cholesterol response to changes in the diet II. The effect of cholesterol in the diet. Metabolism. 14: 759-765.

38. Hegsted, D. M., R. B. McGandy, M. L. Meyers and F. J. Stare. 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr. 17: 281-295.

39. Mattson, F. H., B. A. Erickson and A. M. Klingman. 1972. Effect of dietary cholesterol on serum cholesterol in man. J. Clin. Nutr. 25: 589-594.

40. Sedaghat, A., P. Samue., J. R. Crouse and E. H. Ahrens Jr. 1974. Effects of neomycin on absorption, synthesis, and/or flux of cholesterol in man. J. Clin. Invest. 55: 12-21.

41. Crouse, J. R., S. M. Grundy and J. H. Johnson. 1982. Effects of AOMA on cholesterol metabolism in man. Metab. 31: 733-739.

42. Miettinen, T. A. 1979. Effects of neomycin alone and in combination with cholestyramine on serum cholesterol and fecal steroids in hypercholesterolemic subjects. J. Clin. Invest. 64: 1485-1493.

43. Cooper, A. D. 1977. The metabolism of chylomicron remnants by isolated perfused liver. Biochim. Biophys. Acta. 488: 464-474.

44. Sherrill, B. C. and J. M. Dietschy. 1978. Characterization of the sunusoidal transport process responsable for uptake of chylomicrons by the liver. J. Biol. Chem. 253: 1859-1867.

45. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl and K. K. Stanley. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. EMBO J. 7: 4119-4127.

46. Havel, R. J. 1984. The formation of LDL: mechanisms and regulation. J. Lipid Res. 25: 1570-1576.

47. Goldstein, J. L., M. S. Brown, R. G. W. Anderson, D. W. Russell and W. J. Schneider. 1985. Receptor-mediated endocytosis: Concepts emerging from the LDL receptor system. Ann. Rev. Cell Biol. 1: 1-39.

48. Turley, S. D. and J. M. Dietschy. 1988. The metabolism and excretion of cholesterol by the liver. In The Liver: Biology and Pathobiology. D. Schacter, D. A. Shafritz(eds). Raven Press, Ltd., New York.

49. Bilheimer, D. W., J. L. Goldstein, S. M. Grundy, T. E. Starzl and M. S. Brown. 1984. Liver transplantation to provide low-density-lipoprotein receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. N. Engl. J. Med. 311: 1658-1664.

50. McMurry, M. P., W. E. Connor, D. S. Lin, M. T. Cerqueira and S. L. Connor. 1985. The absorption of cholesterol and the sterol balance in the Tarahumara indians of Mexico fed cholesterol-free and high cholesterol diets. Am. J. Clin. Nutr. 41: 1289-1298.

51. Nestel, P. J. and A. Poyser. 1976. Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metab. 25: 1591-1599.

52. Miettinen, T. A. and Y. A. Kesaniemi. 1988. Cholesterol absorption: regulation of cholesterol synthesis and elimination and within-population variations of serum cholesterol levels. Am. J. Clin. Nutr. 49: 629-635.

53. Packard, C. J., K. Carr, L. McKinney and J. Shepherd. 1983. Cholesterol feeding increases low density lipoprotein synthesis. J. Clin. Invest. 72: 45-51.

54. Spady, D. K. and J. M. Dietschy. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. J. Clin. Invest. 81: 300-309.

55. Mistry, P., N. E. Miller, M. Laker, W. R. Hazzard and B. Lewis. 1981. Individual variation in the effects of dietary cholesterol on plasma lipoproteins and cellular cholesterol homeostasis in man. J. Clin. Invest. 67: 493-502.

56. Jacobs, J., D. R., J. T. Anderson, P. Hannan, A. Keys and H. Blackburn. 1983. Variability in individual serum cholesterol response to change in diet. Arteriosclerosis. 3: 349-356.

57. Katan, M. B., A. C. Beynen, H. M. De Vries and A. Nobels. 1986. Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am. J. Epidemiol. 123: 221-234.

58. Perusse, L., J.-P. Despres, A. Tremblay, C. Leblanc, J. Talbot, C. Allard and C. Bouchard. 1989. Genetic and environmental determinants of serum lipids and lipoproteins in French Canadian families. Arteriosclerosis. 9: 308-318.

59. Namboodiri, K. K., P. P. Green, E. B. Kaplan, J. A. Morrison, G. A. Chase, R. C. Elston, A. R. G. Owen, B. M. Rifkind, C. J. Glueck and H. A. Tyroler. 1984. The collaborative lipid research clinics program family study. IV. Familial associations of plasma lipids and lipoproteins. Am. J. Epidemiol. 119: 975-996.

60. Clarkson, T. B., N. J. Alexander and T. M. Morgan. 1988. Atherosclerosis of cynomolgus monkeys hyper- and hyporesponsive to dietary cholesterol. Arteriosclerosis. 8: 488-498.

61. Bhattacharyya, A. K. and D. A. Eggen. 1980. Cholesterol absorption and turnover in rhesus monkeys as measured by two methods. J. Lipid Res. 21: 518-524.

62. Bhattacharyya, A. K. and D. A. Eggen. 1988. Studies on the mechanism of high intestinal absorption of cholesterol and campesterol in high-responding rhesus monkeys. Atherosclerosis. 72: 109-114.

63. Kesaniemi, Y. A. and T. A. Miettinen. 1987. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. Europ. J. Clin. Invest. 17: 391-395.

64. Bronte-Stewart, B., A. Antonis, L. Eales and J. F. Brock. 1956. Effects of feeding different fats on serum-cholesterol level. Lancet. ii: 521-526.

65. Beveridge, J. M. R., W. F. Connell and G. A. Mayer. 1956. Dietary factors affecting the level of plasma cholesterol in humans: the role of fat. Can. J. Biochem. 34: 441-455.

66. Ahrens, Jr., E. H., W. Insulll, R. Blomstrand, J. Hirsch, T. T. Tsaltas and M. L. Peterson. 1957. The influence of dietary fats on serum-lipid levels in man. Lancet. i:

67. Hashim, S. A., A. Arteaga and T. B. van Italie. 1960. Effect of a saturated medium-chain triglyceride on serum lipids in man. Lancet. i: 1105.

68. Keys, A., J. T. Anderson and F. Grande. 1965. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metab. 14: 776-786.

69. Bonanome, A. and S. M. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318: 1244-1248.

70. Reiser, R., J. L. Probstfield, A. Silvers, L. W. Scott, M. L. Shorney, R. D. Wood, B. C. O'Brien, A. M. Gotto and J. Insull W. 1985. Plasma lipid and lipoprotein response of humans to beef fat, coconut oil and safflower oil. Am. J. Clin. Nutr. 42: 190-197.

71. Emken, E. A. 1984. Nutrition and biochemistry of trans and positional fatty acid isomers in hydrogenated oils. Ann. Rev. Nutr. 4: 339-376.

72. Mensink, R. P. and M. B. Katan. 1989. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N. Engl J. Med. 321: 436-431.

73. Mattson, F. H. and S. M. Grundy. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. lipid Res. 26: 194-202.

74. Mensink, R. P., J. M. de Groot, L. T. van den Broeke, A. P. Severijnen-Nobels, P. N. M. Demacker and M. B. Katan. 1989. Effects of monounsaturated fatty acids vs complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. Metab. 38: 172-178.

75. Baggio, G., A. Pagnan, M. Muraca, S. Martini, A. Opportuno, A. Bonanome, G. Ambrosio, S. Ferrari, P. Guarini, D. Piccolo, E. Manzato, R. Corrocher and G. Crepaldi. 1988. Olive oil-enriched diet: effect on serum lipoprotein levels and biliary cholesterol saturation. Am. J. Clin. Nutr. 47: 960-964.

76. Grundy, S. M. 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N. Engl. J. Med. 314: 745-748.

77. Grundy, S. M., D. Nix, M. F. Whelan and L. Franklin. 1986. Comparison of three cholesterol-lowering diets in normolipidemic men. JAMA. 256: 2351-2355.

78. Ginsberg, H. N., S. L. Barr, A. Gilbert, W. Karmally, R. Deckelbaum, K. Kaplan, R. Ramakrishnan, S. Holleran and R. B. Dell. 1990. Reduction of plasma cholesterol levels in normal men on an American Heart Association Step 1 diet or a Step 1 diet with added monounsaturated fat. N. Engl. J. Med. 322: 574-579.

79. Sacks, F. M., G. H. Handysides, G. E. Marais, B. Rosner and E. H. Kass. 1986. Effects of a low-fat diet on plasma lipoprotein levels. Arch. Intern. Med. 146: 1573-1577.

80. Choudhury, S., P. Jackson, M. B. Katan, C. B. Marenah, C. Cortese, N. E. Miller and B. Lewis. 1984. A multifactorial diet in the management of hyperlipiaemia. Atherosclerosis. 50: 93-103.

81. Thuesen, L., L. B. Henriksen and B. Engby. 1986. One-year experience with a low-fat, low-cholesterol diet in patients with coronary heart disease. Am. J. Clin. Nutr. 44: 212-219.

82. Brinton, E. A., S. Eisenberg and J. L. Breslow. 1990. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J. Clin. Invest. 85: 144-151.

83. Bang, H. O. and J. Dyerberg. 1972. Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. Acta Med. Scand. 192: 85-94.

84. Kromann, N. and A. Green. 1980. Epidemiological studies in the Upernavik District, Greenland. 208: 401-406.

85. Von Schacky, C. 1987. Prophylaxis of atherosclerosis with marine omega-3 fatty acids. Ann. Int. Med. 107: 890-899.

86. Kinsell, L. W., G. D. Michaels, G. Walker and R. E. Visintine. 1961. The effect of a fish-oil fraction on plasma lipids. Diabetes. 10: 316-319.

87. Harris, W. S., C. A. Dujovne, M. Zucker and B. Johnson. 1988. Effects of a low saturated fat, low cholesterol fish oil supplement in hypertriglyceridemic patients. A placebo-controlled trial. Ann. Int. Med. 109: 465-470.

88. Kromhout, D., E. B. Bosschieter and C. D. L. Coulander. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312: 1205-1209.

89. Shekelle, R. B., L. V. Missell, P. Oglesby, A. M. Shryock and J. Stamler. 1985. Fish consumption and mortality from coronary heart disease. N. Engl. J. Med. 313: 820.

90. Vollsett, S. E., I. Heuch and E. Bjelke. 1985. Fish consumption and mortality from coronary heart disease. N. Engl. J. Med. 313: 820-821.

91. Curb, J. D. and D. M. Reed. 1985. Fish consumption and mortality from coronary heart disease. N. Engl. J. Med. 313: 821.

92. Burr, M. L., A. M. Fehily, J. F. Gilbert, S. Rogers, R. M. Holliday, P. M. Sweetman, P. C. Elwood and N. M. Deadman. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction. Lancet. ii: 757-561.

93. Weiner, B. H., I. S. Sckene, P. H. Levine, H. F. Cuenoud, M. Fisher, B. F. Johnson, A. S. Daoud, J. Jarmolych, D. Hosmer, M. H. Johnson, A. Natale, C. Vaudreuil and J. J. Hoogasian. 1986. Inhibition of atherosclerosis by cod-liver oil in a hyperlipidemic swine model. N. Engl. J. Med. 315: 841-846.

94. Landymore, R. W., M. MacAulay, M. Sheridan and C. Cameron. 1986. Comparison of cod-liver oil and aspirin-dipyriamole for the prevention of intimal hyperplasia in autologous vein grafts. Ann. Thorac. Surg. 41: 54-57.

95. Dehmer, G. J., J. J. Popma, E. J. van den Berg, E. J. Eichhorn, J. B. Prewitt, W. B. Campbell, L. Jennings, J. T. Willerson and J. M. Schmitz. 1988. Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with n-3 fatty acids. N. Eng. J. Med. 319: 733-740.

96. Grigg, L. E., T. W. H. Kay, P. A. Valentine, R. Larkins, D. J. Flower, E. G. Manolas, K. O'Dea, A. J. Sinclair, J. L. Hopper and D. Hunt. 1989. Determinants of restenosis and lack of effect of dietary supplementation with eicosapentaenoic acid on the incidence of coronary artery restenosis after angioplasty. J. Am. Coll. Cardiol. 13: 665-672.

97. Reis, G. J., T. M. Boucher, M. E. Sipperly, D. I. Silverman, C. H. McCabe, D. S. Baim, F. M. Sacks, W. Grossman and R. C. Pasternak. 1989. Randomised trial of fish oil for prevention of restenosis after coronary angioplasty. Lancet. ii: 177-181.

98. Shepherd, J., C. J. Packard, S. M. Grundy, D. Yeshurun, J. Gotto A. M. and O. D. Taunton. 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J. Lipid Res. 21: 91-99.

99. Cortese, C., Y. Levy, E. D. Janus, P. R. Turner, S. N. Rao, N. E. Miller and B. Lewis. 1983. Modes of action of lipid-lowering diets in man: Studies of apolipoprotein B kinetics in relation to fat consumption and dietary fatty acid composition. Eur. J. Clin. Invest. 13: 79-85.

100. Nestel, P. J., T. Billington and B. Smith. 1981. Low density and high density lipoprotein kinetics and sterol balance in vegetarians. Metab. 30: 941-945.

101. Ventura, M. A., L. A. Woollett and D. K. Spady. 1989. Fish oil stimulates hepatic LDL transport in the rat. J. Clin. Invest. 84: 528-537.

102. Connor, W. E., D. T. Witiak, D. B. Stone and M. L. Armstrong. 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. J. Clin. Invest. 48: 1363-1375.

103. Moore, R. B., J. T. Anderson, H. L. Taylor, A. Keys and J. Frantz I, D. 1968. Effect of dietary fat on the fecal excretion of cholesterol and its degradation products in man. J. Clin. Invest. 47: 1517-1534.

104. Nestel, P. J., N. Havenstein, Y. Homma, T. W. Scott and L. J. Cook. 1975. Increased sterol excretion with polyunsaturated-fat high-cholesterol diets. Metab. 24: 189-197.

105. Oh, S. Y. and P. A. Monaco. 1985. Effect of dietary cholesterol and degree of fat unsaturation on plasma lipid levels, lipoprotein composition, and fecal steroid excretion in normal young adult men. Am. J. Clin. Nutr. 42: 399-413.

106. Grundy, S. M. and E. H. Ahrens Jr. 1970. The effects of unsaturated dietary fats on absorption, excretion, synthesis, and distribution of cholesterol in man. J. Clin. Invest. 49: 1135-1152.

107. Mattson, F. H. and R. A. Volpenhein. 1972. Hydrolysis of fully esterified alcohols containing from one to eight hydroxyl groups by the lipolytic enzymes of rat pancreatic juice. J. Lipid Res. 13: 325-328.

108. Mattson, F. H. and G. A. Nolen. 1972. Absorbability by rats of compounds containing from one to eight ester groups. J. Nutr. 102: 1171-1176.

109. Fallat, R. W., C. J. Glueck, R. Lutmer and F. H. Mattson. 1976. Short term study of sucrose polyester a nonabsorbable fat-like material as a dietary agent for lowering plasma cholesterol. Am. J. Clin. Nutr. 29: 1204-1215.

110. Crouse, J. R. and S. M. Grundy. 1979. Effects of sucrose polyester on cholesterol metabolism in man. Metab. 28: 994-1000.

111. Glueck, C. J., F. H. Mattson and R. J. Jandacek. 1979. The lowering of plasma cholestero by sucrose polyester in subjects consuming diets with 800, 300, or less than 50 mg of cholestero per day. Am. J. Clin. Nutr. 32: 1636-1644.

112. Glueck, C. J., R. J. Jandacek, M. T. Ravi Subbiah, L. Gallon, R. Yunker, C. Allen, E. Hogg and P. M. Laskarzewski. 1980. Effect of sucrose polyester on fecal bile acid excretion and composition in normal men. 33: 2177-2181.

113. Jandacek, R. J., F. H. Mattson, S. McNeely, L. Gallon, R. Yunker and C. Glueck. 1980. Effect of sucrose polyester on fecal steroid excretion by 24 normal men. Am. J. Clin. Nutr. 33: 251-259.

114. Mellies, M. J., R. J. Jandacek, J. D. Taulbee, M. B. Tewksbury, G. Lamkin, L. Baehler, P. King, D. Boggs, S. Goldman, A. Gouge, R. Tsang and C. J. Glueck. 1983. A double-blind, placebo-controlled study of sucrose polyester in hypercholesteroemic outpatients. Am. J. Clin. Nutr. 37: 339-346.

115. Gerrity, R. G. 1981. The role of the monocyte in atherogenesis. Am. J. Pathol. 103: 181-200.

116. Smith, E. B. and C. Ashall. 1983. Low-density lipoprotein concentration in interstitial fluid from human atherosclerotic lesions. Biochim. Biophys. Acta. 754: 249-257.

117. Aqel, N. M., R. Y. Ball, H. Waldmann and M. J. Mitchinson. 1984. Monocytic origin of foam cells in human atherosclerotic plaques. Atherosclerosis. 53: 265-271.

118. Hoff, H. F. and M. G. Bond. 1982. Accumulation of lipoproteins containing apo B in the aorta of cholesterol-fed cynomolgus monkeys. Atherosclerosis. 43: 329-339.

119. Hollander, W., J. Paddock and M. Colombo. 1979. Lipoproteins in human atherosclerotic vessels. Exper. Mol. Path. 30: 144-171.

120. Rosenfeld, M. E., T. Tsukada, A. M. Gown and R. Ross. 1986. Fatty streak initiation in Watanabe heritable hyperlipemic and comparably hypercholesterolemic fat-fed rabbits. Arteriosclerosis. 7: 9-23.

121. Schwenke, D. C. and T. E. Carew. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits II. Selective retention of LDL vs. selective increases in LDL permeability in susceptible sites of arteries. Arteriosclerosis. 9: 908-918.

122. Schwenke, D. C. and T. E. Carew. 1989. Initiation of atherosclerotic lesions in cholesterol-fed rabbits I. Focal increases in arterial LDL concentration precede development of fatty streak lesions. Arteriosclerosis. 9: 895-907.

123. Brown, M. S. and G. J. L. 1983. Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. Ann. Rev. Biochem. 52: 223-261.

124. Steinberg, D., S. Parthasarathy, T. E. Carew, J. C. Khoo and J. L. Witztum. 1989. Beyond Cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. 320: 915-924.

125. Goldstein, J. L., Y. K. Ho, S. K. Basu and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. Proc. Natl. Acad. Sci. USA. 76: 333-337.

126. Fogelman, A. M., I. Shechter, J. Seager, M. Hokom, J. S. Child and P. A. Edwards. 1980. Malondialdehyde alteration of low density lipoproteins leads to cholesteryl ester accumulation in human monocyte-macrophages. *Proc. Natl. Acad. Sci. USA.* 77: 2214-2218.

127. Sparrow, C. P., S. Parthasarathy and D. Steinberg. 1988. Enzymatic modification of low density lipoprotein by purified lipoxygenase plus phospholipase A_2 mimics cell-mediated oxidative modification. J. Lipid Res. 29: 745-753.

128. Parthasarathy, S., E. Wieland and D. Steinberg. 1989. A role for endothelial cell lipoxygenase in the oxidative modification of low density lipoprotein. Proc. Natl. Acad. Sci. USA. 86: 1046-1050.

129. Jurgens, G., J. Lang and H. Esterbauer. 1986. Modification of human low-density lipoprotein by the lipid peroxidation product 4-hydroxynonenal. Biochim. Biophys. Acta. 875: 103-114.

130. Esterbauer, H., G. Jurgens, O. Quehenberger and E. Koller. 1987. Autooxidation of human low density lipoprotein: loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J. Lipid Res. 28: 495-509.

131. Goldstein, J. L., H. F. Hoff, Y. K. Ho, S. Basu K. and M. S. Brown. 1981. Stimulation of cholesteryl ester synthesis in macrophages by extracts of atherosclerotic human aortas and complexes of albumin/cholesteryl esters. Arteriosclerosis. 1: 210-226.

132. Yla-Herttuala, S., W. Palinski, M. E. Rosenfeld, S. Parthasarathy, T. Carew, S. Butler, J. L. Witztum and D. Steinberg. 1989. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. J. Clin. Invest. 84: 1086-1095.

133. Palinski, W., M. E. Rosenfeld, S. Yla-Herttuala, G. C. Gurtner, S. S. Socher, S. W. Butler, S. Parthasarathy, T. E. Carew, D. Steinberg and J. L. Witztum. 1989. Low density lipoprotein undergoes oxidative modification *in vivo*. *Proc. Natl. Acad. Sci. USA.* 86: 1372-1376.

134. Carew, T. E., D. C. Schenke and D. Steinberg. 1987. Antiatherogenic effect of probucol unrelated to its hypocholesterolemic effect: Evidence that antioxidants in vivo can selectively inhibit low density lipoprotein degradation in macrophage-rich fatty streaks and slow the progression of atherosclerosis in the Watanabe heritable hyperlipiemic rabbit. Proc. Natl. Acad. Sci. USA. 84: 7725-7729.

135. Kita, T., Y. Nagano, M. Yokode, K. Ishii, N. Kume, A. Olshima, H. Yoshida, C. Kawai and . 1987. Probucol prevents the progression of atherosclerosis in Watanabe heritable hyperlipidemic rabbit, an animal model for familial hypercholesterolemia. Proc. Natl. Acad. Sci. USA. 84: 5928-5931.

136. Wight, T. N. 1988. Cell biology of arterial proteoglycans. Arteriosclerosis. 9: 1-20.

137. Rogers, K. A., R. L. Hoover and J. Castellot J. J. 1986. Dietary cholesterol-induced changes in macrophage characteristics. Am. J. Pathol. 125: 284-291.

138. Faggiotto, A. and R. Ross. 1984. Studies of hypercholesteroemia in the nonhuman primate II. Fatty streak conversion to fibrous plaque. Arteriosclerosis. 4: 341-356.

139. Arntzenius, A. C., D. Kromhout, J. D. Barth, J. H. C. Reiber, A. V. G. Bruschke, B. Buis, C. M. van Gent, N. Kempen-Voogd, S. Strikwerda and E. A. van der Velde. 1985. Diet, lipoproteins, and the progression of coronary atherosclerosis. N. Engl. J. Med. 312: 805-811.

140. Brensike, J. F., R. I. Levy, S. F. Kelsey, E. R. Passamani, J. M. Righardson, I. K. Loh, N. J. Stone, R. F. Aldrich, J. W. Battaglini, D. J. Moriarty, M. R. Fisher, L. Friedman, W. Friedewald, K. M. Detre and S. E. Epstein. 1984. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI type II coronary intervention study. Circ. 69: 313-324.

141. Blankenhorn, D. H., S. A. Nessim, R. L. Johnson, M. E. Sanmarco, S. P. Azen and L. Cashin-Hemphill. 1987. Beneficial effects of combined colestipol-niacin therapy on coronary atherosclerosis and coronary venous bypass grafts. JAMA. 257: 3233-3240.

142. Wagner, W. D., R. W. St. Clair, T. B. Clarkson and J. R. Connor. 1980. A study of atherosclerosis regression in macaca mulatta. Am. J. Pathol. 100: 633-650.

143. Armstrong, M. L., E. D. Warner and W. E. Connor. 1970. Regression of coronary atheromatosis in rhesus monkeys. Circ. Res. 27: 59-67.