BRIEF REVIEWS

The Role of Dietary Polyunsaturated Fat in Lowering Blood Cholesterol in Man

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IT HAS BEEN nearly a quarter of a century since Keys and coworkers (see Refs. 1-3 and the pertinent references therein) demonstrated a statistically significant relationship between the death rate from coronary heart disease (CHD), plasma cholesterol levels, and the proportion of calories derived from total dietary fat. In these pioneer studies, it was shown that a Cape Town population which consumed foods rich in fat had a higher serum cholesterol level than a Bantu tribe which consumed less fat. Following these observations, there were a number of experimental studies in the mid-1950's in patients[4-7] and animals[8-11] which showed that the feeding of saturated fat led to an elevation of plasma cholesterol, whereas polyunsaturated fats produced a decrease. The strong correlation between elevated plasma cholesterol levels and CHD led to several clinical trials which attempted to relate the consumption of the type of dietary fat and the course of the disease.[12-15] Although the overall results of these trials were inconclusive, many of the participants had prior CHD and were less likely to be helped by dietary modification.[16] Nonetheless, based on epidemiological evidence and animal experiments, the American Heart Association,[17] the American Health Foundation,[18] the Food and Nutrition Board of the National Research Council and the American Medical Association,[19] and the Royal College of Physicians of London and the British Cardiac Society[20] all have recommended changes in the diet which include a reduction of the total consumption of fat and cholesterol and the substitution of polyunsaturated for saturated fat. It is possible that one of the reasons for

the 15% decrease in the heart attack death rate in the United States in the last 7 years[21] may be in part related to a decreased intake of total dietary fat and to an increased proportion of polyunsaturated fat of that consumed. Because of the widespread consumption of dietary polyunsaturated fat, there has been considerable interest in the mechanism by which these fats lower plasma cholesterol levels. Various mechanisms have been proposed, but to date no single explanation has received general acceptance. Keys et al.[22] and Hegsted et al.[23] have derived equations relating changes in serum cholesterol with various diets as follows:

Keys et al.:[22]
$$\Delta \text{ cholesterol} = 2.74\Delta S - 1.31\Delta P$$
Hegsted et al.:[23]
$$\Delta \text{ cholesterol} = 2.16\Delta S - 1.65\Delta P + 6.66C - 0.53$$

In these equations, S and P are the changes in the percent of dietary calories derived from saturated and polyunsaturated fatty acids, respectively; C is the dietary cholesterol content in mg%. Based on these equations, it may be concluded that saturated fatty acids have, per weight basis, approximately twice the effect on the change in serum cholesterol as do the polyunsaturated fats which act in the opposite direction. These quantitative equations suggest why a number of earlier studies have failed to show that polyunsaturated fat feeding lowered serum cholesterol.[24-27] For example, Connor et al.[24] did not observe a change in serum cholesterol following increases in dietary polyunsaturated fat. However, the iodine values which are a measure of the degree of unsaturation only changed from 64 to 100. In a later study, Connor et al.[28] found that when the iodine values of the fat were increased from 32 to 127, there was a decline in serum cholesterol of 21%. Thus, as suggested by Ahrens et al.[7] some 20 years ago, it would appear that a certain percentage of polyunsaturated fat is required in the diet before serum cholesterol is lowered.

Another point which should be emphasized concerning the equations of Keys et al.[22] and Hegsted et al.[23] and the mechanisms involved is the question of whether the decrease in serum cholesterol with increased polyunsatu-

rated fat feeding actually is due to the polyunsaturated fat itself or to the decrease in saturated fat. The equations show that both types of fat affect the change in serum cholesterol.

In 1970, Grundy and Ahrens[29] reviewed the various mechanisms that might explain the hypocholesterolemic effects of polyunsaturated fats. The action of polyunsaturated fat could result from (1) an increase in fecal excretion of neutral steroids and/or bile acids, (2) a reduction in cholesterol absorption in the small intestine, (3) a decrease in endogenous cholesterol synthesis, and (4) a redistribution of circulating cholesterol between the plasma and tissue pools. The literature contains evidence both for and against each of these theories. Spritz and Mishkel[30] have also suggested the possibility that polyunsaturated fat may influence lipoprotein structure. In this review, we have assessed the evidence for the various mechanisms of cholesterol lowering in man by polyunsaturated fats and have attempted to integrate information available from previous studies with current knowledge of lipoprotein structure and metabolism. It is not our purpose to propose a single mechanism that will apply to all subjects. Rather, it is our aim to review the existing data for the reader so that he may understand the reasons for conflicting viewpoints and may have a framework to which additional facts may be added as further knowledge evolves.

Dietary Fat, Neutral Steroid, and Bile Acid Excretion

It has been known for a long time that the synthesis, secretion, and turnover of bile acids is influenced by diet. Ahrens and his colleagues[31, 32] gave the field a great impetus in the mid-60's with the development of chemical methods to measure fecal steroids directly. These methodologies allowed many investigators to measure quantitatively the effects of dietary fat on bile acid and neutral steroid excretion. Polyunsaturated fat has been reported to cause both an increase[33-35] and a decrease[36, 37] in bile acid excretion; other investigators have reported no change.[29, 38] Avigan et al.[38] administered lipoproteins which had been labeled in plasma with [^{14}C]cholesterol to six subjects on a formula diet containing a high content (60%) of total calories as fat and found no effect of the type of fat on bile acids and neutral steroid excretion. The fat was either saturated (coconut oil) or polyunsaturated (corn oil or safflower oil). Although the daily measured fecal bile acid excretion in five of the six subjects ranged from 0.24 to 1.27 g/day (mean 0.79) on the saturated fat diet and 0.10 to 0.88 g/day (mean 0.54) on the polyunsaturated fat diet, the differences were not statistically significant. The daily excretion of neutral steroids in the same five subjects averaged 0.73 g/day, and the value was the same for both diets. Of the five patients who showed no change in bile acid and neutral steroid excretion, four were hypercholesterolemic and one was normolipemic. The sixth subject, who was hypercholesterolemic, showed no change in bile acid excretion but did show a marked increase in neutral steroid excretion on the polyunsaturated compared to the saturated fat diet; 2.98 g/day vs. 1.27/day, respectively. The factors which

limit the interpretation of these data are that only one of the six patients was normolipemic, and no method was used to determine the adequacy of the 2- or 4-day fecal collections. It should also be pointed out that coconut oil is metabolized differently from other types of saturated fat, since it contains mostly medium-chain triglycerides. These triglycerides are hydrolyzed in the small intestine, and the short-chain fatty acids which are released are transported directly to the liver via the portal vein rather than being incorporated into chylomicrons, as occurs with the longer-chain fatty acids. Hence, it may not be possible to compare the results of the studies involving medium-chain fatty acids to those using longer-chain fatty acids.

Moore and coworkers[39] studied five normal subjects and found an increase in both fecal bile acid and neutral steroid excretion on a polyunsaturated fat diet compared to the saturated one. In this study, the subjects were given solid food with 40% of the calories derived from fat, either safflower oil or butter fat. The average total fecal steroid excretion rate was 19% higher per day during ingestion of safflower oil as compared with butter; the increase was equally divided between the bile acids and neutral steroids. Although the two diets were not equivalent in total cholesterol content (347 vs. 197 mg), it is of interest that the *decrease* in serum cholesterol (28%) on the safflower diet was associated with an *increase* in fecal steroids of a similar magnitude (19%), suggesting a correlation between the two. Limitations in this study include the differences in cholesterol and plant steroids on the two diets and the lack of appropriate methods to verify the adequacy of fecal collections.

Connor et al.[28] also performed steroid balance studies in normal men but, in contrast to Moore et al.,[39] used formula diets containing no dietary cholesterol. The dietary fat accounted for 40% of the total calories. Gas-liquid chromatography was used to measure fecal steroids directly. Fecal steroid excretion was evaluated during three dietary periods, each lasting 3 weeks. The diets were identical except for the type of fat; cocoa butter was given during the first and third periods and corn oil during the second. The mean plasma cholesterol concentration for the six subjects at the end of each diet period was 222, 177, and 225 mg/dl, respectively. Thus, on the polyunsaturated fat diet, plasma cholesterol declined 21%. During the three dietary periods, total fecal steroids were 709, 915, and 629 mg/day, respectively, corresponding to a 29% increase in fecal steroids on the polyunsaturated diet and a 45% decrease when the saturated diet was reinstituted. The major increase in fecal steroid excretion on the polyunsaturated fat diet was attributed to an increase in bile acids, in contrast to the studies of Moore et al.[39] who found an increase in both bile acids and neutral steroids. Although the studies of Moore et al.[39] and Connor et al.[28] differ in experimental design and method, they both showed an increase in fecal steroid excretion in normal subjects on a polyunsaturated fat diet compared to a saturated one. Connor et al.[28] have also suggested that polyunsaturated fats may cause a loss of cholesterol from tissues, since the increase in fecal steroids was greater than that which could be accounted for by the decline in plasma cholesterol alone.

Nestel and coworkers,[40, 41] using methods similar to those of Connor et al.,[28] recently have shown that fecal steroid excretion in normal subjects increases with polyunsaturated fat feeding during the first 3 weeks while the plasma cholesterol level is falling; however, in the new steady state condition, steroid excretion is similar to that observed with saturated fat in the diet. In their initial study,[40] six healthy normal subjects were given saturated fat (P/S ratio 0.09) obtained from beef meat, dripping, cheese, butter, milk, and cream from cattle fed a conventional diet; the polyunsaturated meat (P/S ratio 0.89) was obtained from cattle which were fed a diet supplemented with "protected" vegetable oil. The vegetable oil was fed to the cattle as formaldehyde-treated safflower oil which prevents it from being converted to saturated fatty acids by microorganisms in the rumen. The major differences in the fatty acid composition of the triglycerides of the two diets given to the normal subjects was the 7-fold greater content of linoleic acid in the polyunsaturated diet compared to the saturated, 22.9 vs. 3.2%, respectively. The content of cholesterol in the two diets was approximately 500 mg/day. After being on the saturated diet for three weeks, five of the six patients had average plasma cholesterol concentrations of 222 mg/dl (range 201–248). After four weeks on the polyunsaturated diet, the five subjects had a mean plasma cholesterol of 202 mg/dl (range 173–234). The plasma cholesterol declined in all five subjects with a mean decrease of 9% (range 6–14%). The sixth subject had a plasma cholesterol value of 158 and did not respond to the polyunsaturated fat diet. Plasma triglycerides were unchanged in three of the six subjects but decreased in the other three by 25%. On the polyunsaturated fat diet, the mean values of triglyceride linoleic acid increased from 6% to 22%, and those of cholesteryl linoleate increased from 38% to 51%. When the subjects were placed on the saturated diet, there was a rapid decrease in plasma linoleic acid content and a corresponding increase in cholesterol. Compared to the saturated diet, the excretion of total fecal steroids in subjects on the polyunsaturated fat diet increased approximately 20% (range 4.6–32.6) in five of the six studied. The total net output of neutral steroids and bile acids in the feces (defined as the total steroid excretion minus dietary cholesterol) amounted to a mean of 268 mg/day on the saturated diet and 416 mg/day on the polyunsaturated diet.

Nestel et al.[41] have repeated these studies in eight subjects on similar diets but for longer periods of time; the subjects were given the polyunsaturated diet (P/S ratio of 0.74) for 36 days and then the saturated diet for 40 days (P/S ratio of 0.06). The dietary cholesterol was 400 and 423 mg/day, respectively. After the polyunsaturated diet was started, the plasma cholesterol values fell from an initial value of 165 mg/dl (on ad libitum diet) to a mean value of 155 mg/dl after the 20th day of the diet. The subjects were then given the saturated diet, and the mean plasma cholesterol increased to 170 mg/dl; the mean difference between the two dietary periods was 15 mg/dl or 9% and was significant at the 0.1% level. The plasma triglycerides on the polyunsaturated fat diet were 17% lower than that on the saturated one. Under steady state

conditions, there was no difference in cholesterol absorption on the two diets. In addition, the mean values for bile acid excretion, cholesterol synthesis, and cholesterol turnover were not statistically different on the two diets. Compared to previous reports,[28, 39, 40] the difference in the findings in this study has been attributed[41] to the nonsteady state conditions in the earlier studies.

To rule out the possibility that the differences in bile acid excretion may have been due to dietary cholesterol levels, Nestel et al.[42] measured fecal steroid excretion on saturated and polyunsaturated fat diets with two different intakes of cholesterol. Five normal men were studied under steady state conditions. The higher cholesterol diet contained from 754 to 849 mg cholesterol/day while the lower cholesterol diet contained 460 to 561. The level of plasma cholesterol was affected by the type of fat but not by the dietary cholesterol. On the saturated diet, the mean plasma cholesterol was 197 and 192 mg/dl, respectively, for high and low dietary cholesterol; for the polyunsaturated diet, the values were 177 and 169 mg/dl, respectively. Cholesterol absorption was not significantly different with the two diets, nor was there any difference in bile acid excretion at the lower level of dietary cholesterol. At the higher level, however, bile acid excretion was greater with the polyunsaturated, compared to the saturated fat diet; the mean values were 381 and 281 mg/day, respectively. The mean net fecal steroid output was nearly identical on the polyunsaturated fat diet for both the high and low cholesterol intake and were 624 and 642 mg/day, respectively. With the higher cholesterol intake, there was a statistically significant increase in net fecal steroid excretion on the high polyunsaturated compared to the saturated fat diet; the value increased from 299 to 624 mg/day. On the low cholesterol intake, these values were 477 and 642 mg/day, respectively. Since the steroid excretion was the same on the polyunsaturated fat diet regardless of cholesterol intake and since the absorption of cholesterol was not significantly different, Nestel et al.[42] have suggested that the major difference in steroid excretion between the saturated and polyunsaturated fat diets is caused by a greater excretion of endogenous cholesterol on the polyunsaturated diet.

Anderson et al.[43] recently have presented a study with similar conclusions. These investigators found no interaction between dietary cholesterol and the degree of saturation with respect to serum cholesterol. The subjects were given either a saturated diet or a polyunsaturated diet with either 3 or 294 mg dietary cholesterol/day. On the low cholesterol diet, there was a significant difference between the serum cholesterol levels with the saturated or unsaturated diets (158 and 122 mg/dl, respectively). When 294 mg cholesterol were added to each diet, the serum cholesterol values rose to 167 and 130 mg/dl, respectively. Thus, the addition of cholesterol to both diets caused an increase, to the same extent, of serum cholesterol. The higher serum cholesterol observed when the subjects ate the saturated diet cannot be due to an increased absorption of dietary cholesterol, since the diet was devoid of cholesterol. A more likely explanation is that polyunsaturated fat causes a greater excretion of endogenous cholesterol.

In addition to using beef as a source of dietary fat, Nestel et al.[44] also carried out studies using saturated or polyunsaturated pork in three normal subjects. The polyunsaturated pork (P/S ratio 1.16) was obtained from swine fed a diet supplemented with safflower seeds and contained 36% linoleic acid, whereas the saturated pork (P/S 0.28) contained only 12%. The mean difference in plasma cholesterol between the two diets was 8%. The net output of fecal steroids (total fecal steroid excretion minus cholesterol intake) was 57% or 290 mg/day higher for the polyunsaturated compared to the saturated diets. The large difference in steroid excretion on the two diets may be related in part to the nonsteady state conditions.

The studies described above primarily involved normal subjects with normal plasma lipid values. Grundy[45] has also done cholesterol balance studies with 11 patients with hypertriglyceridemia; nine had a type IV hyperlipoproteinemia phenotype and two had a type V phenotype, according to the Fredrickson typing system. Although the results were variable, in general the polyunsaturated diet caused an increase in the fecal steroid excretion in these patients. The subjects were fed a diet of mixed solid food and formula which contained 40% of calories as fat. During the first period (1 month) of the study, the fat was administered in the form of lard. For the second period of 1 month, safflower oil was the source of the polyunsaturated fat. The daily intake of cholesterol on the two diets was not identical; the intake ranged from 173 to 282 mg/day on the saturated diet and from 63 to 137 mg/day on the polyunsaturated one. All of the patients showed a drop in plasma cholesterol values when the polyunsaturated diet was given, compared to the saturated one; the decrease ranged from 5% to nearly 50%. However, in only seven of the 11 patients was the polyunsaturated diet associated with a significant decrease in plasma triglycerides. The results of fecal steroid excretion were variable. Of the eight patients studied, five had significant increases in total neutral steroid excretion on polyunsaturated fat; the daily increment of neutral steroid excretion ranged from 207 to 521 mg/day. The remaining three patients showed slight increases (62, 63, and 68 mg/day), but the values were not significantly different on the two diets. Excretion of acidic steroids was also variable. Only four of the eight subjects showed a significant rise in bile acid excretion, the increases being 996, 130, 111, and 82 mg/day. Three subjects had only slight increases of 31, 83, and 85, and one subject actually had a decrease of 105 mg/day in bile acid excretion. Although there was a great variation in both neutral steroid and bile acid excretion, all of the subjects showed a total steroid output that was greater than that which could be accounted for by the decline in the plasma cholesterol. Therefore, the increase in fecal steroid excretion may have been derived not only from the plasma compartment but also from tissue pools and/or cholesterol synthesis. Grundy[45] has attempted to determine the source of the excess fecal steroids by measuring the output of biliary cholesterol, bile acids, and phospholipids in six of the 11 patients. On constant feeding of a formula diet, only two of the six subjects showed increases in the output of cholesterol on the polyunsaturated fat diet. In only one patient was there a

significant increase in biliary bile acid output, and no subject showed changes of phospholipid output on the two diets. Thus, it would appear that there is no consistent change in all subjects in biliary cholesterol and bile acids to account for the increased fecal excretion of neutral and acidic steroids on polyunsaturated fat.

In the experiments of Grundy,[45] the patients were on the study diets for from 17 to 38 days. The fecal steroids were measured on 4-day collections throughout the study period; the values reported thus represent the mean of all determinations as the subject approached a steady state condition. Whether or not steady state conditions were ever attained on the different diets is not known.

In summary, it appears that in some subjects, polyunsaturated fat may increase total fecal steroid output under nonsteady state conditions and may account for the lowering of plasma cholesterol. Under steady state conditions, however, the differences in fecal steroid excretion may not be significant. Furthermore, it should be emphasized that there is a great heterogeneity in the response to polyunsaturated fat by both normolipemic and hyperlipidemia subjects. In general, most of the normal subjects studied by Moore et al.,[39] Connor et al.,[28] and Nestel[42] have shown increased total fecal steroid output. The large discrepancy in the data seems to occur with those individuals with familial hypercholesterolemia or hypertriglyceridemia.

Dietary Fats and Cholesterol Absorption and Synthesis

Although an earlier report[46] claimed that there was decreased absorption of cholesterol on polyunsaturated diets compared to saturated, recent studies have shown no difference in either normal subjects[41] or in patients with hypertriglyceridemia.[45] Whether changes in the rate of cholesterol synthesis contribute to the lowering of plasma cholesterol is unknown. There have been no direct measurements of cholesterol synthesis on the two diets. However, indirect measurements in normolipemic[41] or hypercholesterolemic subjects[29] have shown no significant changes in cholesterol synthesis on polyunsaturated fat diets.

Dietary Fats and Redistribution of Plasma Cholesterol

The major proponents of the theory that dietary polyunsaturated fat lowers plasma cholesterol by causing its redistribution have been Ahrens and coworkers.[29, 30, 47] Grundy and Ahrens[29] gave polyunsaturated and saturated fat to 12 patients, 10 of whom had type II, one type IV, and one type V hyperlipoproteinemia. They found no changes in the excretion or synthesis of bile acids and cholesterol and, therefore, suggested that the most likely mechanism for explaining the reduction in plasma cholesterol was that of a redistribution of cholesterol from the plasma compartment to one or more other compartments. A liquid formula was given in which 40% of the calories were fat. There were three levels of dietary cholesterol in the study; six of the type II subjects were on a low (46–83 mg/day) and three were on a high (457–679 mg/day) cholesterol diet. The two hypertriglyceridemic subjects

were maintained on a high cholesterol intake (586–679 mg/day). The fats which were added to the basic diets were butter, corn oil, or safflower oil. Exchanging corn oil for butter produced a significant decrease in plasma cholesterol in all but one subject, the latter being the one with the type IV phenotype. There was considerable variation between subjects in the steroid balance studies. In seven of 11 subjects, there was no change in neutral steroid excretion when dietary polyunsaturated fat was substituted for saturated; one showed a significant increase and three had a decrease. Only two subjects had an increased fecal bile acid excretion on the polyunsaturated diet. The excretion was greater than could be accounted for by the decline of cholesterol in the plasma compartment. In the three subjects in whom there was an increase in fecal steroid excretion (neutral and/or bile acids), the decline in plasma cholesterol was greater than that which could be explained by the increase in total fecal steroids. Grundy and Ahrens[29] postulate that in those individuals in whom the fall in plasma cholesterol was not accompanied by a rise in steroid excretion, there was a transfer of cholesterol from the plasma to tissue (e.g., the liver). However, direct evidence for a transfer of cholesterol to tissues during polyunsaturated fat feeding in man is lacking. On the contrary, Frantz and Carey[48] showed no the cholesterol content of liver biopsies in man after polyunsaturated fat feeding.

Dietary Fat and Lipoprotein Structure and Metabolism

Altered plasma lipoprotein compositions also have been suggested as a possible mechanism to account for the lowering of plasma cholesterol by polyunsaturated fat feeding.[30] Spritz and Mishkel[30] isolated low density lipoproteins (LDL) and high density lipoproteins (HDL) from normal or hyperlipidemic subjects fed either a saturated diet containing coconut or butter fat or a polyunsaturated diet containing corn oil, safflower oil, or trilinolein; the cholesterol content was the same in both diets. In addition to lowering plasma cholesterol, the polyunsaturated diet caused an increase in the linoleic acid content of the plasma phospholipid and a decrease in oleic acid; no attempt was made in this study to determine the fatty acyl groups of the individual lipids in each lipoprotein class. However, there were changes in the lipid-to-protein ratios of LDL; the cholesterol-to-protein and phospholipid-to-protein ratios were decreased by the polyunsaturated diet compared to the saturated diet in all subjects studied. Since unsaturated phospholipids occupy more space, Spritz and Mishkel[30] reasoned that the greater linoleate content of LDL resulted in fewer lipid molecules per LDL particle. These workers[30] did not determine the fatty acyl composition of the lipoprotein lipids.

Morrisett et al.[49] recently have carried out a dietary study in normal subjects and have shown that the change in the fatty acid composition of plasma also is reflected in the isolated lipoproteins. Four healthy adult males were fed an isocaloric diet consisting of 20% protein, 40% carbohydrate, and 40% fat; the fat was either saturated (P/S ratio of 0.25) or polyunsaturated (P/S ratio of 4.0). On both diets, each individual consumed 400 mg of cholesterol daily. The very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins were isolated after 2 weeks on each diet, and the fatty acyl compositions of each lipid class were determined. Analysis of the triglycerides of each lipoprotein class showed that the largest compositional change occurred in VLDL and LDL; the percentage of palmitate (16:0) in LDL dropped from 39% on the saturated diet to 19% on the polyunsaturated one. The decrease in palmitic acid was accompanied by an increase in the amount of linoleate (18:2) from 12% to 46%. The changes in cholesteryl ester fatty acids were the most pronounced in the VLDL; the 18:1 level dropped from 21% on the saturated diet to 10% on the polyunsaturated diet while the 18:2 content rose from 51% to 63% on the two diets. The fatty acid compositions of the phospholipids differed from those observed in the cholesteryl esters and triglycerides. In the HDL, little or no change was observed in the 18:1 content of the phospholipids. However, there were significant differences in the content of 18:0, particularly in the VLDL phospholipid fatty acids, where the percentage of 18:0 increased from 16% on the polyunsaturated diet to 30% on the saturated one and that of 18:2 decreased from 29% to 11%.

In general, the diet rich in polyunsaturated fat produced lipoproteins which had a preponderance of linoleic acid, fatty acid which has a lower transition temperature and is more "fluid" than palmitic and oleic acids. Morrisett et al.[49] reasoned that the fatty acid differences in the lipoproteins produced in the two diets should be reflected by different thermotropic properties. Using pyrene excimer fluorescence and electron paramagnetic resonance methods, these authors have shown that the lipoproteins from the polyunsaturated diet had thermotropic transitions at lower temperatures than the lipoproteins from the saturated diet. The greatest difference in the transition temperature or fluidity of the lipoproteins was observed in the VLDL and is consistent with the fatty acid composition of the VLDL triglycerides. The importance of the altered lipoprotein composition and fluidity may be reflected in the catabolism of lipoproteins and may account for the hypocholesterolemic effect of polyunsaturated fat feeding.

Blaton et al.[50] have shown that polyunsaturated phospholipids can also change the fatty acid composition of human plasma lipoproteins. In their study, Lipostabil, a commercially available phospholipid which contains 65% linoleic acid, 11% oleic acid, and 13% palmitic acid, was given to 93 patients with type II and IV hyperlipoproteinemia; the subjects were on an ad libitum diet, and each received 1 g of Lipostabil daily. After 2 weeks of intravenous administration of the lipid, there was a mean decrease of 10% in plasma cholesterol. The amount of palmitic and oleic acids in plasma phosphatidylcholine and cholesteryl esters decreased and there was a corresponding increase in linoleic acid. The changes in plasma fatty acid composition were due primarily to alterations in the HDL phospholipids and LDL cholesteryl esters.

Svanberg et al.[51] have carried out a study similar to that described by Blaton et al.[50] However, they did not show a significant decrease in serum cholesterol when Lipostabil was given orally to five subjects, two with type IIb and

three with type IV hyperlipoproteinemia. Lipostabil administration did yield a 17% decrease in serum triglycerides. Although there was no change in the serum cholesterol levels after 9 weeks of Lipostabil, the isolated lipoproteins did show marked compositional changes. There was a 21% decrease in the cholesterol found in the VLDL fraction, no change in LDL cholesterol, but surprisingly a 30% increase in cholesterol in HDL; no attempt was made in this study to fractionate the HDL further. The fatty acid composition of the phospholipids showed a significant increase in linoleic acid between the 3rd and 5th weeks, but by 9 weeks there was no apparent change in 18:2 content compared to the initial values.

Thompson et al.[52] have also shown that Intralipid can effect protein and lipid composition of the plasma lipoprotein. Intralipid is different from the Lipostabil described above in that it contains predominantly polyunsaturated triglycerides and the primary fatty acid in the phospholipid is oleic acid. In their study, Thompson et al.[52] gave Intralipid to four normal individuals receiving isocaloric diets which contained approximately 200 mg cholesterol/day; 20% of the calories were derived from fat which had a P/S ratio of approximately 2. When Intralipid was given by intragastric tube, there was a small decrease in total plasma, VLDL, and LDL cholesterol but an increase in HDL cholesterol. In contrast, Intralipid given intravenously caused a rise in LDL cholesterol and a decrease in VLDL and HDL cholesterol. Although the LDL protein content was significantly higher when the Intralipid was given by the intravenous route, the ratio of LDL cholesterol to protein or LDL phospholipid to protein was identical with the two routes of administration. After intravenous injection of Intralipid, there was a rise in the oleate content of LDL phospholipids and a corresponding decrease in linoleate; after intragastric Intralipid, there was more linoleate than oleate. Thompson et al.[52] concluded from these studies that the infusion of exogenous phospholipid in the form of Intralipid into the circulation was accompanied by phospholipid exchange between the Intralipid and LDL, which resulted in an LDL that is enriched with oleate-containing phospholipids. Although the fatty acid compositons were altered, the cholesterol-to-protein ratios were not, and differ from the results of Spritz and Mishkel.[30]

Thompson et al.[53] have investigated the mechanism for the increase in LDL with intravenously administered Intralipid by carrying out LDL turnover studies. In these studies,[53] there was no evidence for increased LDL synthesis. However, intravenous administration of Intralipid was associated with a decrease in the fractional catabolic rate (FCR) of apoLDL; the FCR decreased from a control value of 0.255–0.218/day. Since the absolute catabolic rates (ACR) were not significantly different by the two routes of Intralipid administration, Thompson et al.[53] have suggested that the increase in LDL protein with intravenous Intralipid was due to an influx into the plasma of preformed LDL.

Chait et al.[54] have also suggested that polyunsaturated fat may decrease the rate of secretion of VLDL into the plasma. Since LDL is derived mainly from VLDL, this could account for the hypocholesterolemic effects of poly-

unsaturated fat. Chait et al.[54] found that [³H]palmitate was preferentially incorporated into VLDL triglycerides compared to [¹⁴C]linoleate when infused into six normal subjects. Consistent with this finding, Yeshurun et al.[55] and Turner et al.[56] have also reported a decrease in LDL protein synthesis in some normals or hyperlipoproteinemic patients by dietary polyunsaturated fat compared to saturated fat. In the study of Turner et al.,[56] there was also an increase in apoLDL fractional catabolic rate in response to polyunsaturated fat.

It is clear from the above discussion that dietary polyunsaturated fat reduces the circulating levels of LDL cholesterol and protein in certain individuals. Whether the decrease is due to decreased LDL synthesis or increased catabolism or a combination of both requires further investigation. Assuming lipoprotein synthesis and/or degradation are influenced by the type of dietary fat, the mechanisms involved are totally unknown. Before the mechanisms are understood completely, it is first necessary to define the biochemical steps involved in lipoprotein metabolism and especially those that are rate-limiting. The effects of dietary fats on the rate-limiting step could then be tested. One possible rate-limiting step may be the conversion of VLDL to LDL. It may well be that the lipolytic enzymes, lipoprotein lipase, and lecithin:cholesterol acyltransferase are the rate-limiting enzymes in catabolism and that they are more active on VLDL or LDL which contain polyunsaturated lipids. Jackson and Gotto[57] have suggested that altered membrane fluidity could also account for increased or decreased LDL catabolism. Since polyunsaturated fat feeding leads not only to changes in lipoprotein composition but also to changes in membrane lipid composition, these authors reasoned that changes in membrane fluidity could alter the translational motion of the LDL receptor described by Brown and Goldstein[58] and affect the rate of uptake of the LDL. It is also possible that the absolute number of LDL receptors may be regulated by membrane fluidity. Although changes in membrane fluidity and lipoprotein metabolism with polyunsaturated fat feeding are only speculative at present, it is clear that an understanding of the mechanisms for cholesterol lowering by dietary polyunsaturated fat must include studies of lipoprotein and membranes. Over the next few years, we can look forward to a substantial increase in information regarding the plasma lipoproteins and the dietary factors which control their structure and metabolism.

Acknowledgments

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