Annu. Rev. Nutr. 1990. 10:149–67

EFFECTS OF N–3 FATTY ACIDS ON LIPID METABOLISM

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KEY WORDS: lipoproteins, cardiovascular, cholesterol, liver

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INTRODUCTION

The rediscovery of the unique characteristics of the n-3 fatty acids (or omega-3 fatty acids) and their possible role in preventing coronary heart disease has sparked great interest among nutritionists, clinicians, and the public. This interest is part of a wider reassessment of nutritional means for

combating cardiovascular disorders brought about by the shift in strategy from treating only high-risk individuals to advising entire communities.

The key to any such strategy lies in modifying fat consumption and more particularly the mixture of dietary fatty acids (FA). The replacement of saturated FA can be made satisfactorily by starch or polyunsaturated FA or monounsaturated FA.

In countries where the consumption of polyunsaturated fatty acids, mainly linoleic acid, is high, interest in the n-3 FA has been particularly keen. A change in the high ratio of n-6:n-3 FA is perceived to be desirable. This perception has coincided with the demonstration of the multipotent properties of the longer chain polyenoic n-3 FA found in fish. Whereas the major benefit from eating n-6 FA is the lowering of plasma cholesterol levels, the n-3 FA appear to have a wider range of actions with the potential to reduce cardiovascular risk.

Since the recent interest in these fatty acids was sparked by observations on cardiovascular mortality, most of the initial research focused, not surprisingly, on lipid metabolism. The medical and scientific literature is awash with publications in this area. This review attempts to place more recent findings into the wider context of the nutritional value of these fatty acids.

EPIDEMIOLOGIC CONSIDERATIONS

The consumption of fish and not of fish oil has been associated with low rates of coronary heart disease (CHD). The initial well-known observations of Greenland Eskimos are worth reviewing because they place subsequent experimental findings with fish oils into perspective. Dyerberg (16) reported an average daily intake among the Eskimos of 7 g of eicosapentaenoic acid (EPA) alone and therefore considerably more total marine n-3 fatty acid than the Danes, whose intake was below 0.1 g/day. The Eskimos experienced only about one fifth the incidence of CHD (17). This reduction was reflected in plasma cholesterol and triglyceride levels, which were approximately 20 and 60% lower, respectively (18).

That this benefit from eating fish may be due to high n-3 FA content was reinforced by studies of the experience of Japanese coastal people. Their intake of fatty fish was about one half that of the Eskimos [~200 vs 400 g daily (35)], and the mortality from CHD and risks for CHD, including blood pressure, were lower than among other Japanese. The amount of n-3 FA eaten by the Japanese, about 4 g daily, is closer to the dosages used in subsequent experimental studies than is the amount eaten by the Eskimos.

Therefore, the finding that even small amounts of nonoily fish, eaten over a lifetime, may be protective was surprising. This finding was observed in studies in the Netherlands (42) and in Chicago (71) but not in the Honolulu

Heart Study (13) nor in one Norwegian Study (79). Thus, the question of the precise role of the n-3 FA remains open, since the intakes of those fatty acids were very small in the Dutch and Chicago populations. Thirty grams of fish, half of it lean, was associated with a reduction in the CHD mortality by about half (42); that amount of fish must contain less than 0.5 g of n-3 FA. As will be seen, such small amounts have not yielded important biologic effects in short-term studies in humans. However, a study of several European populations, who are moderate eaters of fish at most, showed an inverse correlation between clinical CHD and adipose EPA concentrations, an indirect measure of fish consumption (88). The consumption of even small quantities of fish over many years (20 years in the Chicago and Netherlands surveys) may raise tissue EPA levels sufficiently to influence the risk of developing CHD.

The possibility that human atherosclerosis is preventible with dietary fish oil is being explored in patients who have undergone coronary artery angioplasty for atherosclerosis. Restenosis is strongly related to hyperlipidemia. One study has reported a significantly lower rate of early reocclusions in patients receiving dietary fish oil (15). However a later, considerably larger study that was double-blind and placebo-controlled failed to observe any benefit from dietary fish oil (60). In fact, the short-term restenosis rate was higher in the fish oil–treated patients. The pathogenesis of atherosclerosis is complex and does not resemble the pathology seen in the stenosing lesions of previously dilated coronary arteries (18a). Different models of human atherosclerosis need to be tested to determine whether fish oil prevents atherosclerosis. In animal studies, researchers have observed protection for large arteries such as the femoral and carotid (14; see below).

EFFECTS ON PLASMA LIPOPROTEIN LIPIDS: HUMAN STUDIES

Introduction

Substantially lower plasma lipid concentrations in Greenland Eskimos than in Danes led to the investigation of fish oils for the treatment of hyperlipidemia. Results are less clear than might have been expected. Levels of plasma triglycerides and of the lipoproteins that transport triglycerides, especially very low density (VLDL) and intermediate density (IDL) lipoproteins, have been lowered very satisfactorily. Questions remain about the relative efficacy of the two major n-3 FA, EPA and docosahexaenoic acid (DHA), the advantages of partly purified oils such as the esters of EPA, and the responsiveness of chylomicron remnant disorders to fish oils.

Even greater uncertainty surrounds the effect on plasma cholesterol levels, especially on the concentrations of low density lipoproteins (LDL) and high

density lipoproteins (HDL). Findings appear to conflict; the concentrations of both LDL and HDL are variably reported to rise, fall, or not change. Some of the discrepancies relate to dosage, the sources of fish and hence to the nature of the n-3 FA, the type of hyperlipidemia treated, and the matching of the control and test diets.

Triglyceride-rich Lipoproteins

The concentrations of endogenously derived triglyceride-rich lipoproteins, VLDL and IDL, have been almost uniformly reported as lowered (20, 26, 54, 59, 64, 72). Fish oils have been effective in normal subjects and in patients with common phenotypes of hyperlipidemia in which VLDL levels are raised. The minimal effective dose of n-3 FA appears to be slightly more than 1 g per day (23); at intakes of more than 2 g per day, the fall in VLDL averages 25% in normal subjects (23). The average reduction is greater in hypertriglyceridemic subjects; it is approximately 50% in those with types 4 or 5 phenotype (23, 59) and approximately 40% for those with type 2B (20, 23, 59). The degree of triglyceride lowering is also related to dosage but approaches maximal reduction at between 5 and 10 g n-3 FA. Much higher doses have also been used, up to 40 g n-3 FA (59), with no apparent further efficacy. Nevertheless, some patients have had up to 90% reduction in plasma triglyceride concentration. A recent study of longer duration has reported failure to maintain triglyceride lowering with approximately 3 g n-3 FA (67), but this point should become clearer as more patients are treated.

So far, the evidence shows that n-3 FA lower the concentrations of endogenous triglyceride-rich lipoproteins. What of chylomicrons and chylomicron remnants? In more severe forms of hypertriglyceridemia, such as type 5 hyperlipoproteinemia, in which both VLDL and chylomicrons (or remnants) are present, excess n-3 FA can be highly effective (59). Whether this result reflects enhanced removal of chylomicrons is uncertain. Catabolized VLDL and chylomicrons compete for similar removal mechanisms (52); diminished chylomicron removal may therefore occur whenever VLDL overproduction increases the need for VLDL removal as in type 5 hyperlipoproteinemia.

Dietary fish oil does not influence the level of two key enzymes of triglyceride catabolism, lipoprotein lipase and hepatic triglyceride lipase (24, 82). Therefore, the improved clearance of chylomicrons also observed in normal subjects after a fatty meal cannot be due to increased activity of lipolytic enzymes. Two studies have shown that the postprandial lipemia after dietary fat intake is lessened if the background diet has been enriched with fish oil (24, 82). Single meals rich in saturated FA or in fish oil were metabolized similarly, but the degree and duration of the lipemia were significantly reduced if fish oil were part of the longer term background diet. This effect is unlikely to have been due to poorer absorption of the fish oil

(24). In addition, the alimentary particles formed from the dietary fish oil were more susceptible to lipolysis in vitro (82). Fish oils, however, are highly unlikely to have a role in the therapy of primary hyperchylomicronemic states caused by defective or absent lipoprotein lipase or hepatic triglyceride lipase.

The nature of the predominant n-3 FA (EPA or DHA) does not seem important in determining plasma triglyceride lowering in humans. Fish rich in EPA appear as effective in humans as fish rich in DHA (23); the similarity may not be true for all species (see below). Fish oils vary considerably in their content of EPA and DHA as well as of long-chain monoenes such as gadoleic (20:1 n-9) and cetoleic (22:1 n-11) acids (39), factors that may influence plasma levels of cholesterol rather than of triglyceride. Virtually nothing is known about the metabolic effects of docosapentaenoic acid (22:5 n-3), which is also present in most fish oils.

Dietary fish oils also modify the hypertriglyceridemia that is normally inducible by carbohydrate (25). This modification might be expected from the known effects of these two nutrients on triglyceride metabolism: carbohydrates stimulate and fish oils inhibit VLDL production.

While naturally occurring fish oils clearly lower VLDL triglyceride levels uniformly, doubts have been raised about the equal efficacy of esters of individual n-3 FA. Conversely, whole fish oils are rich in saturated fatty acids that may be undesirable. Much larger amounts of fish oil than of individual n-3 FA must be taken.

The absorption of the ethyl or methyl esters of EPA, however, are inferior to that of EPA in the glycerides of the fish oils (19; see below). That EPA esters have been found therapeutically effective in dosages roughly equal to those in the fish oil is therefore interesting (28, 36).

Cholesterol-rich Lipoproteins

In contrast to the effects on triglyceride levels, plasma cholesterol levels may fall, rise, or remain unchanged. Harris (23) recently summarized much of the reported data. The average changes in plasma levels of total cholesterol and in LDL cholesterol in normolipidemic subjects are small and of little clinical importance. Yet within the body of evidence, there is considerable variability; some groups show significant falls (33, 73) and others rises (66) in LDL cholesterol levels.

Much the same applies to patients with pure hypercholesterolemia due to excess LDL; with a few exceptions, LDL cholesterol levels are reported as unchanged (23). Only in hypertriglyceridemic subjects and only when a substantial proportion of the plasma cholesterol is carried in VLDL and IDL is the plasma total cholesterol concentration frequently lowered by dietary fish oil (59). Equally, these are the subjects, i.e. patients with combined hyperlipoproteinemia and primary hypertriglyceridemia, in whom the LDL cho-

lesterol often rises (20). This phenomenon is not unique to fish oils; LDL cholesterol levels often rise (albeit from low levels) in patients with hypertriglyceridemia who are treated with fibrates.

The influence of the nature of the background lipoprotein phenotype on the response to dietary fish oil was shown clearly in a recent study in which the composition of the LDL particle was a reliable index of the response (69). In hypertriglyceridemia, LDL are frequently polymorphic; numbers of smaller, denser, cholesterol-poor LDL exceed those of the more normal, larger, cholesterol-rich LDL. Dietary fish oils raised LDL cholesterol levels when the predominant LDL was of the small, dense variety. This observation is important, since most previous studies concluded that LDL composition was not influenced by fish oil.

Although the possible mechanisms for this LDL-raising effect will be examined below, several points can be made now. First, people treated with dietary salmon oil, which was used frequently in earlier studies, apparently did show falls in total and LDL cholesterol levels (26, 33). The same may be true for dietary tuna oil (10), which raises the possibility that DHA-rich fish oil may lower LDL levels while EPA-rich fish oil does not. Second, several studies that have reported fish oil–induced reductions in LDL cholesterol levels failed to control for differences in saturated fatty acid intake. Clearly, if fish oils substitute for saturated fats, the LDL cholesterol level should fall. However, when dietary saturated fatty acids were equal in control and test diets, then LDL levels changed little in normotriglyceridemic individuals, except when salmon or tuna oil was used. The dual influence of the type of lipid disorder and the saturated fatty acid content of the control diet has been dissociated in several well-designed studies (e.g. 20, 37). Substituting either fish oil or a polyunsaturated vegetable oil for a saturated fat diet was about equally effective in lowering LDL cholesterol levels in normolipidemic subjects, but fish oil alone raised LDL levels in combined hyperlipoproteinemia (20).

Despite the variable responses in the LDL concentration, a fish oil diet apparently prevents the rise in LDL level that is normally induced by dietary cholesterol. In one study (53) large intakes of egg yolk failed to raise levels of LDL cholesterol or LDL apo-B. In a novel confirmation of this result, egg yolks were compared from hens fed fish oil or vegetable oil; only eggs from hens on normal diets raised the plasma cholesterol level (56). The possible reasons for this discrepancy will be discussed later but include suppression of the resecretion of cholesterol-enriched lipoproteins from the liver and reduced cholesterol absorption.

High-density Lipoproteins

The variability observed in the LDL cholesterol response to dietary fish oils also extends to HDL cholesterol (23). Although much of the variability in

LDL responses can be attributed to the background lipoprotein phenotype, the experimental design, or the EPA/DHA ratio, the variability in HDL responses appears to be partly a function of dosage. High intakes of fish oil lower HDL cholesterol levels (33, 54, 59), whereas more moderate intakes either raise or do not affect HDL levels (72, 75, 78). On average, the HDL cholesterol concentration tends to rise rather than fall.

The nature of HDL particles is influenced more than that of LDL. We have observed no change in LDL particle size, but HDL particles show a significant size redistribution from smaller, denser particles to larger particles (1). That is, the proportion of HDL-2 to HDL-3 rose significantly, as reflected by an increase in the apoprotein A1:A2 ratio (particles with A1 alone occur within HDL-2, whereas A1, A2 particles characterize HDL-3).

Some of the possible mechanisms responsible for the changes induced in HDL will be discussed below. Production and removal rates are altered, but fish oils probably affect a wide range of regulatory mechanisms.

FISH OIL FATTY ACIDS AND TRIGLYCERIDE METABOLISM

Because plasma triglyceride levels are characteristically lowered by dietary marine n-3 FA, the greatest research effort has been in triglyceride metabolism. The two major n-3 FA, EPA and DHA, are incorporated into circulating glycerides in approximate proportions to that in the dietary oil (7). This finding suggests that both are absorbed equally and that the mechanisms responsible for the initial catabolism of chylomicrons and the subsequent repackaging in the liver into VLDL are influenced by both fatty acids similarly. As discussed below, this is not entirely true. Nevertheless there is not the discrimination seen with cholesterol esters, which become enriched with EPA but not with DHA (1, 30). EPA and DHA also show specificity for different phospholipids (47).

Absorption of Marine N-3 Fatty Acids

An unresolved inconsistency remains between studies of people eating single meals of different oils and the direct measurements of fatty acid absorption in rats. In humans, the postprandial rise in plasma triglyceride level after a single meal of fish oil is similar to that after a similar amount of other edible fats (24). Yet in lymph-cannulated rats, fish oil concentrate has been claimed to be less well absorbed than other oils, although the absorption of free EPA is as efficient as that of oleic acid (8). When the oil is emulsified with bile acid, absorption in the rat is similar for fish oil and olive oil. Emulsification with phospholipid also improves absorption in humans (27). The importance of the physical environment in the gut is demonstrated by the enhanced absorption

of the ethyl ester of EPA when taken with a meal containing an adequate amount of triglycerides (44).

Under different circumstances, EPA ester may be poorly absorbed (19). This poor absorption has implications for the therapeutic uses of these oils, especially with the increasing use of esters of specific fatty acids.

The initial hydrolysis of these oils by pancreatic lipase may not be as efficient as for other fats (6). Since lipolysis and absorption of triglyceride take place over many hours in vivo, minor differences in short-term in vitro incubations may have little practical significance. The distribution of EPA and DHA in the triglyceride molecule may also be important. Pancreatic lipase splits the outside FA at the 1 and 3 positions more readily than that at the middle or 2 position. Released EPA and DHA are then absorbed efficiently. In fish triglyceride, however, most of the n-3 FA is in the 2 position (51). The absorbed monoglyceride with the fatty acid at the 2 position preserves this fatty acid position during reesterification in the enterocyte and resecretion in chylomicrons. The subsequent catabolism of n-3 FA in this position may differ from that in the 1 and 3 positions, which are absorbed as free fatty acids. It is therefore relevant to their utilization that EPA and DHA are preferentially positioned at the 2 position in fish oils but not in the oil of marine mammals (51).

These issues may become important for consumption of smaller amounts of dietary oils as in fish; when large amounts are consumed, minor differences in the efficiency of absorption are difficult to discern. Furthermore, supplements apparently are best taken with meals to optimize emulsification in the gut (44). Nevertheless, no clear conclusion can be made at this time about the optimal forms of EPA and DHA (esters or glycerides) in supplements.

An excellent review of the digestion and absorption of fats and oils has been published recently (51).

Hepatic Metabolism of N-3 Fatty Acids

The catabolism of chylomicrons enriched with dietary n-3 FA occurs at least as efficiently as it does in those enriched with other dietary fatty acids. Postprandial lipemia in patients on diets including n-3 FA is, if anything, less in degree and duration after consumption of n-3 FA than after that of less unsaturated fat (24, 82). The point has already been made that this difference probably reflects several factors. When fish oil is also part of the background diet, the reduced pool of endogenous triglyceride-rich lipoproteins allows more capacity for the removal of the chylomicron remnants. Chylomicrons containing n-3 FA provide excellent substrate for lipoprotein lipase (82).

Having reached the liver, the n-3 FA of marine origin exert specific, profound effects. The net outcome is reduced availability of fatty acid for triglyceride formation and reduced triglyceride secretion. In humans, tri-

glyceride production, measured by kinetic analysis of the flow of labeled triglyceride through plasma, is greatly reduced (54, 65). This reduction has been demonstrated to be dose dependent (54, 65); up to 90% of the normal flux of VLDL triglyceride is suppressible. The supply of plasma free fatty acid is adequate; if anything the fractional turnover rate of EPA is higher than that of oleic acid (54). Hence substrate diversion away from triglyceride formation occurs in the liver.

This diversion is due to (*a*) suppression of fatty acid synthesis, (*b*) increased oxidation of fatty acids, (*c*) reduced activity of esterifying enzymes, and (*d*) diversion to phospholipid formation. These findings have been observed consistently in experimental animals, perfused liver systems, and isolated liver cell preparations.

Reduced lipogenesis is substantial (77, 87) and may reflect inhibition of the key enzyme acetyl CoA carboxylase (34) sufficiently to suppress the high rate of triglyceride production as shown in obese, hyperlipidemic rats (85). It nullifies the hypertriglyceridemia otherwise inducible in people eating carbohydrate-rich diets (25). The stimulation of triglyceride production normally brought about by insulin is also abolished (77).

Increased ketogenesis and oxidation of EPA and DHA are partly due to diminished suppressibility of carnitine palmitoyl transferase (5, 87), a key enzyme involved in transporting fatty acids to mitochondrial sites of oxidation.

These findings have been observed in animals that have become adapted to these diets as well as in short-term experiments in which the fatty acids have been added to hepatocytes for only a few hours. In vitro, DHA and EPA suppress triglyceride formation equally (85). Longer term studies suggest, however, that the subsequent secretion of triglyceride from the liver is inhibited more by DHA than by EPA (86). This difference is not reflected in the degree of triglyceride lowering in subjects eating fish that differ in DHA and EPA (10). Other mechanisms include suppression of the hepatic enzymes phosphatidate phosphohydrolase (46), the enzyme catalysing esterification of phosphatidic acid to diglycerides, and acyl-coenzyme A:1,2-diacylglycerol acyltransferase, which mediates the next step (62). These multiple effects may represent changes in cellular membrane fatty acid composition known to affect the regulation of membrane-bound enzymes and cellular functions.

Although the synthesis of apoprotein B, the obligate secretory protein of VLDL, probably also is reduced by fish oils, the evidence is not consistent in animal studies. In humans, apoprotein B flux is as powerfully inhibited as is triglyceride production (54). Confirmatory evidence has been obtained in the human hepatoma Hep G2 cell line (84). However, only triglyceride and not apoprotein B secretion was suppressed in monkeys fed fish oil (83). The

secretion of other constituents of VLDL, notably cholesteryl ester, is also reduced (58).

The eventual outcome is fewer circulating VLDL. Because these particles contain far less triglyceride, they are reduced in size (75). This reduction is of some importance because smaller VLDL are preferentially converted to LDL; the paradoxical rise in LDL may be related partly to this phenomenon.

FISH OIL FATTY ACIDS AND CHOLESTEROL METABOLISM

Relatively little is known about the influence of dietary n-3 FA on cholesterol absorption. In lymph-cannulated rats, absorption is reduced slightly. Cholesterol synthesis is depressed in the intestine as in the liver. In two studies, one in rabbits (21) and the other in a cell line derived from human colonic cancer (CaCo-2) (50), HMG-CoA reductase activity was decreased. However, cholesterol esterification in the intestine, measured as the activity of the enzyme ACAT (acyl-CoA : cholesterol acyltransferase), was also reduced in the CaCo-2 cells but raised in the rabbit. The modification of egg yolk–induced rises in LDL cholesterol in people eating fish oil (53, 56) may represent interference with cholesterol absorption.

Considerably more is known about the intrahepatic disposition of cholesterol in animals fed fish oil. Cholesterol synthesis (61, 77) and HMG-CoA reductase activity (21) are inhibited. Less esterified cholesterol accumulates in the livers of cholesterol-fed rats presumably because ACAT activity is reduced (63); free cholesterol may accumulate, however. Although secretion of cholesterol within VLDL is reduced, rats fed fish oil increase the biliary secretion of cholesterol although not of bile acids (3). The rat has a greater capacity for secreting cholesterol into bile than do humans, however; in one study of people eating a large amount of fish oil, the concentration of biliary cholesterol was not affected (12). This finding does not exclude an increase in the rate of secretion, but it suggests that the likelihood of forming gallstones is not increased.

Why, then, does the LDL cholesterol concentration sometimes rise, at least in humans, when all the evidence suggests it should not? Cholesterol synthesis is depressed, biliary cholesterol secretion may rise, and the absorption of cholesterol may even be reduced. This focuses attention on LDL removal and particularly on the LDL (apo B/E) receptor. In a study of LDL apoprotein B turnover in men eating large quantities of salmon oil (richer in DHA than in EPA), LDL concentration and LDL apoprotein B production fell (33). The reduction in formation was attributed to the reduced availability of the precursor VLDL, since direct synthesis of LDL is probably minimal in humans. The fraction of the LDL pool being removed per unit time rose, implying

normal removal mechanisms. The fractional removal rate, however, was not increased by as much as might have been expected on the basis of the much smaller pool; hence this study might be equally interpreted as showing a relative deficiency in LDL clearance. In fact, miniature pigs fed fish oil show a halving in the capacity of receptor-mediated LDL removal (32).

Direct assays of LDL receptor activity in rat liver show at least a third reduction in animals fed fish oil (61). This reduction occurred in conjunction with reduced cholesterol synthesis and is consistent with the coordinate regulation of LDL receptor and HMG-CoA reductase activities. Other studies have reported a reduction in mRNA for the LDL receptor in hamsters fed fish oil (29); in fact, this study showed widespread inhibition of mRNAs for a number of apoproteins critically involved in lipid metabolism (A1, B, and E). Interestingly, however, the binding of HDL to rat liver, possibly through a putative receptor, was raised by fish oil feeding (61). LDL binding to Hep G2 cells is also reduced by preincubating the cells with EPA (84). When, however, a source of dietary fiber such as oat bran or rice bran is included with the fish-oil diet, the down-regulation of the LDL receptor was prevented (76), presumably because some types of dietary fiber stimulate the LDL receptor.

The reductions in HMG-CoA reductase and in LDL receptor activities may reflect the effects of marked changes in membrane fatty acids. One other possibility is a rise in the hepatic free cholesterol concentration resulting from diminished VLDL secretion. The rise in HDL binding to liver cells from fish oil–fed rats (61) suggests that the free cholesterol level might indeed have risen, since uptake by HDL is one mechanism for maintaining cholesterol homeostasis.

As discussed previously, LDL levels may also rise because a high proportion of small VLDL (such as are produced by eating fish oil) become converted to LDL in humans. In fact, in people consuming fish oil, the reduction in VLDL cholesterol is strongly correlated with the rise in LDL cholesterol (1).

Two major systems that control the flux of cholesterol, especially cholesteryl esters, among plasma lipoproteins are the enzyme LCAT (lecithin cholesterol:acyltransferase) and the protein LTP (lipid transfer protein). LCAT esterifies free cholesterol and so regulates the uptake by HDL of free cholesterol generated in cells or during the catabolism of triglyceride-rich lipoproteins. LTP redistributes the cholesteryl esters from HDL to VLDL and LDL, generally in exchange for the other core lipid, triglyceride. This redistribution is a major means of transporting cholesterol through plasma at least in species such as humans that possess LTP activity. Both these systems have been studied recently in people and animals consuming fish oil. LTP activity, measured as the transfer of cholesteryl ester between LDL and HDL,

was reduced by 23% (1); EPA-enriched cholesteryl esters may be a poor substrate for LTP. There appears to be substrate specificity for LTP-mediated cholesteryl ester transfer (49) so that EPA enrichment of HDL cholesteryl ester reduces the transfer from HDL to LDL.

The importance of this reduction in LTP activity may lie in the preferential retention of cholesteryl ester within HDL, which may partly explain the antiatherogenic effect of fish oil in experimental animals. In humans, HDL particles enlarge, owing partly to reduced transfer of core lipid that reflects lowering in LTP activity as well as reduced availability of acceptor VLDL particles. Patients with inherited LTP deficiency also show larger HDL particles and higher HDL2:HDL3 ratios (90).

Diminished transfer of cholesteryl ester from HDL to LDL might be expected to lead to smaller LDL particles. This finding has been observed in monkeys fed fish oil (57). HDL enlargement and reduction in LTP activity have been reported in EPA-fed marmoset monkeys (2). That this response may be antiatherogenic is suggested by the redistribution of plasma cholesterol from LDL to HDL when cholesterol-fed monkeys receive EPA supplements (2) or swine receive fish oil supplements (38). In Rhesus monkeys in which hypercholesterolemia was induced with dietary cholesterol, the nature of VLDL particles was also changed when fish oil was added (74). Not only was the cholesterol content of VLDL reduced, but the VLDL no longer showed capacity to load macrophages with cholesterol.

These multiple effects on the composition of HDL, LDL, and VLDL observed in several species may be responsible for the protection against atherogenesis. Such benefits have been observed in Rhesus monkeys (14) and swine (81) but not consistently in the LDL-receptor deficient, hypercholesterolemic Watanabe rabbit (11). Most important, atherosclerosis was prevented in these animals despite the persistence of hypercholesterolemia.

Plasma LCAT activity has also been reported to be lowered in people eating fish oil (1) but the biologic significance of this reduction is uncertain, since there is no evidence that ratios of cholesteryl ester to free cholesterol are lowered. LCAT activity has also been found to be reduced in people eating large quantities of mackerel (73).

FISH OILS IN LIPID-RELATED DISEASES

Hyperlipidemia occurs as a complication of a number of disorders. Hypertriglyceridemia is particularly common in patients with diabetes mellitus and in those with renal disease. Not unreasonably, fish oil therapy has been attempted in such individuals. As in subjects with primary hypertriglyceridemia, VLDL levels have fallen but LDL cholesterol concentrations

have risen in several reports of diabetic subjects (48, 68, 78). Furthermore, control of glucose levels may have been rendered more difficult (22); glucose secretion is increased from livers of rats fed fish oil (87), presumably owing to increased gluconeogenesis. The precise value of treating such patients with EPA/DHA-rich preparations is therefore uncertain.

The overall reduction in cardiovascular risk with fish oils needs to be considered in terms broader than the effects on lipid levels. Small but not unimportant lowering of blood pressure has been reported in hypertensive (40, 55) and normotensive subjects (37). This finding, however, has not been uniformly observed.

The altered nature of eicosanoids produced from n-3 FA, compared with the more common series derived from n-6 FA, appears to confer benefit. Individuals with extensive atherosclerosis produce large quantities of thromboxane-2 (41), which has several properties conducive to atherosclerosis and symptomatic coronary artery disease. Platelet aggregation, which plays an important role in atherogenesis and in arterial occlusion, is enhanced by thromboxane-2 (80). Indeed, patients experiencing angina pectoris secrete increased amounts of this eicosanoid. Fish oils have generally been reported to reduce platelet aggregation and thromboxane-2 generation (80). Instead, production switches to the much less vasoconstrictive and thrombogenic thromboxane-3 (41). Other benefits have also been claimed but need confirmation; they include reduced blood viscosity (89) and lowered fibrinogen concentrations (31).

N-3 FA likely will be shown to have additional antiatherogenic properties; the initiation of atherosclerosis involves chemotaxis of cholesterol-containing monocytes to the arterial intima and their adhesion to the surface of the artery. Preliminary reports suggest that these processes are inhibited by fish oil (70).

A major fatal complication of coronary heart disease is the development of ventricular fibrillation. This occurrence can be substantially prevented in experimental animals by feeding fish oil for several months prior to the induction of myocardial ischemia (45).

PLANT N-3 FATTY ACIDS

This review has focussed on the n-3 FA of fish. However, α-linolenic acid, the major n-3 FA of plants, is far more plentiful and might be used to supplement the marine varieties to increase the nutritionally desirable mix of n-3 FA. Although α-linolenic acid is now regarded as an essential fatty acid (4) and the precursor of the longer and more polyunsaturated fatty acids such as EPA and DHA, it has been somewhat disappointing in comparison with the latter. Linolenic acid appears to be strongly discriminated against in mammals; it is rapidly eliminated from tissue pools when fed in excess (51).

Linolenic acid does not lower plasma lipid levels (37, 64) nor does it have the antithrombogenic (64) or antihypertensive properties of its products (37). Some doubt exists about the efficiency of linolenate absorption.

People eating relatively large amounts of α-linolenic acid do show evidence of elongation and desaturation of linolenate. Six weeks of high-dose linseed oil consumption (9 g daily of α-linolenic acid) doubles the concentration of EPA in plasma (37). Although plasma DHA levels did not rise (37), DHA must be generated from α-linolenic acid since DHA is not an essential (though it is biologically crucial) fatty acid. Presumably, DHA has a very high affinity for tissue phospholipid and is either removed from the circulation rapidly or retained in the tissues where it is generated.

IS FISH OR FISH OIL PROTECTIVE?

We return to the original observation that high fish consumption and not fish oil consumption has been associated with reduced coronary disease. Several current studies are comparing the relative benefits of eating fish or fish oil. If quantities of fish that provide a certain amount of n-3 FA induce biologic effects that resemble those after consumption of identical amounts of n-3 FA, there may be justification for recommending fish oil as an alternative to fish. That will require a reorientation in nutritional philosophy. The food industry, which is not overly concerned with the relative merits of foods versus supplements, is already producing n-3 FA–enriched foods that bear no resemblance to fish.

Nevertheless, if the agreed goal is to increase consumption of n-3 FA from various sources, some attainable, quantitative objective must be set. The amounts of fish eaten by Japanese, approximately 100 g daily, would appear to provide 1–2 g n-3 FA, a level that appears adequate from current evidence. This amount would be several-fold higher than is eaten presently by most European and North American populations (39). It would require approximately .75 kg of fatty fish weekly. Cultural constraints make this level unlikely to be achieved. A preferable option may be that of eating fish regularly and receiving small supplements of n-3 FA in processed foods.

CONCLUSIONS

The long-chain polyenoic fatty acids found in fish oils (EPA and DHA) profoundly affect many control systems that regulate lipid metabolism. The net effect is to lower circulating triglyceride-rich lipoproteins. The effects on the cholesterol-rich lipoproteins are inconsistent and at times paradoxical. The triglyceride-lowering effect is readily understood from the multiplicity of induced changes. The required dosage for long-term triglyceride reduction is uncertain, however.

The net effect on the LDL cholesterol concentration is complex. A clear evaluation of the influence of these fatty acids on LDL and HDL metabolism has high priority. Although lowering the level of triglyceride-rich lipoproteins is now increasingly recognized as reducing cardiovascular risk, this reduction must not be negated by adverse changes in LDL and HDL.

Preliminary findings suggest that EPA and DHA have dissimilar effects on lipid metabolism and even greater dissimilarity in their effect on cholesterol metabolism. If confirmed, this dissimilarity would allow a rational strategy for lowering all atherogenic lipoproteins.

Other important questions relate to the wider mechanisms underlying the prevention of atherosclerosis and of cardiovascular mortality, to which the changes in lipids would only partly contribute. These mechanisms include improvements in levels of thrombogenic factors, blood pressure, and precipitating factors for myocardial function.

Some of these effects can be attributed to changes in cell membrane function brought about by changes in fatty acid composition and to the markedly altered metabolism of eicosanoids.

Potential adverse effects of n-3 FA supplementation may arise from the suppression of thrombogenic, immune and inflammatory responses. Although these effects may be useful in treating certain diseases, their impact on healthy people is uncertain.

Finally, that these nutrients have been introduced initially as supplements is fascinating. Active developments in the pharmaceutical and food industries will result in purified, high-dosage forms of EPA and DHA and in their incorporation into a variety of foods. This development will pose a scientific as well as a philosophical challenge to nutritionists. At the very least we must discover rapidly whether eating fish confers greater protection against disease than consuming fish oil. More basic understanding of the actions of fish oils is necessary before fish oils can be recommended widely to the public.

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