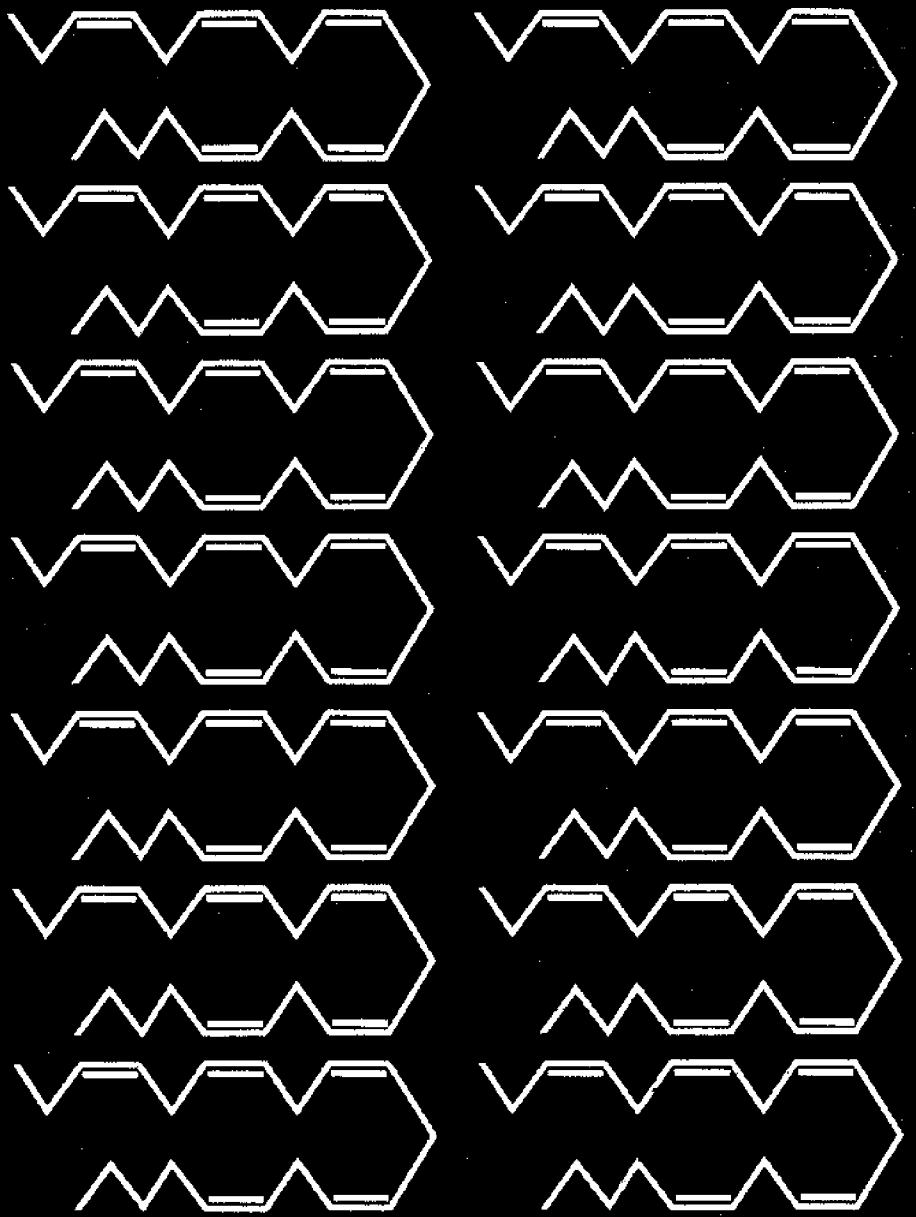
The Fifteenth Annual
MIT Sea Grant Lecture

Keynote address of:
Health Effects of
Omega-3 Fatty Acids:
Fish Oil and
Other Sources

# The Impact of Dietary Fat on Human Health

Dr. Robert S. Lees



Fifteenth Annual MIT Sea Grant
College Program/Lecture

# The Impact of Dietary Fat on Human Health

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**Director for Research,**
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Every year since 1972, the MIT Sea Grant Program has invited a distinguished lecturer to address the public and encourage discussion on subjects related to the use and mangement of marine resources. In the past, presentations on biotechnology in the marine sciences, arctic policy, remotely operated and autonomous underwater vehicles, and the Georges Bank conflict over fish and fuel have been of interest to the public, marine industries, government agencies, and the academic community.

This year's lecture, summarizing the impact of dietary fat on human health and the potential benefits of omega-3 fatty acids from fish oils, was complemented by a day-long seminar, "Health Effects of Omega-3 Fatty Acids: Fish Oil and Other Sources." Seminar participants included physicians, medical researchers, food and drug manufacturers, and government regulators. Seminar presentations reviewed what is and is not known about the health effects of omega-3's; discussed regulatory issues concerning the potential use of fish oils as a drug; presented information on high quality sources of omega-3's; and outlined necessary directions for future research, particulary human vs. animal studies.

A complete collection of the seminar papers will be published in the fall of 1988 by Marcel Dekker of New York and will be distributed by MIT Sea Grant and the publisher. More imme-diately, in response to widespread public interest, the following proceed-ings of the 15th Annual MIT Sea Grant Lecture are being made available in advance as part of the MIT Sea Grant research, advisory, and education report series.

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Dr. Robert S. Lees is currently a professor of Cardiovascular Disease in the Department of Applied Biological Sciences at MIT and director of Medical Research at New England Deaconess Hospital in Boston. He is a recognized expert in the field of coronary heart disease with a distinguished record of research and clinical practice in the role of fat metabolism in health and disease. The author of more than 120 reports and articles on human metabolism, Dr. Lees has written on such topics as the metabolic relationships among plasma lipoproteins and agents used to treat hyperlipidemia and atherosclerosis.

Dr. Lees graduated magna cum laude from Harvard College in 1955 and received his M.D. from Harvard Medical School in 1959. As well as serving on various committees of professional associations, he is advisory editor of *Zeitschrift fur die Gesamte Experimentelle Medizin* and serves on the editorial board of Stroke.

While Lees finds the accumulating evidence on fish oils exciting he also finds it inconclusive. Extensive clinical studies will be necessary before physicians can truly assess and safely control the effects of omega-3 fatty acids. With Sea Grant support, Lees has begun a clinical study on the health effects of fish oil capsules to better ascertain their effects on human patients.

# Introduction

## "I come to bury Caesar, not to praise him"

Human beings, for many centuries, have implied almost mystical properties to their dietary fats. The ancient Romans not only ate their beloved olive oil, they anointed themselves with it. Modern Americans not only eat fish in hopes that its oil will keep them healthy, they swallow fish oil capsules as a drug. In this discussion, I will attempt to review the salient facts concerning dietary fat and its effects on human health. My goal is to provide an overview of the major categories of disease with which dietary fats have been associated.

To my mind, the modern era of dietary fat research began with an animal, rather than a human, experiment. Parenthetically, I will quote relatively little animal data here because laboratory animal experiments are often not extrapolable to the situation in the free-living human being. The experiment I have in mind, however, is highly extrapolable. In the early 1920s, Simon Henry Gage and Pierre Fish, at Cornell University, fed a sheep some vegetable oil colored with a fat-soluble dye called cochineal. Then they dissected the sheep and found that the animal's intestinal lymph, or chyle, contained tiny droplets of fat that contained the dye, and that these droplets passed into the blood via the thoracic lymph duct. Finally, the sheep's fat, after several hours, turned pink. In a preliminary publication (Gage, 1920) in the Cornell Veterinarian, Gage named these small particles "chylomicrons," the name we use today. In a later full publication (Gage and Fish, 1924), the investigators postulated that the function of chylomicrons was to transport dietary fat into the lymph, and from there to the blood stream and sites of storage or utilization, the function they are still thought to fulfill. This classic experiment in fat metabolism is, to my knowledge, the first metabolic experiment to use a tracer. The studies of Gage and Fish, which graphically showed the passage of dietary fat through the lymph and blood to the depot fat, set the stage for the next half-century of studies on the metabolism of dietary fat and its effects on human health.

In the spirit of Gage and Fish, we must turn to the composition and the metabolism of dietary fat in order to understand its effects on health. What we eat (Table 1) contains three major lipid classes: triglycerides, phospholipids, and sterols, plus a number of minor components of varying, sometimes major, importance.



Table 1. Some Components of Human Dietary Fats

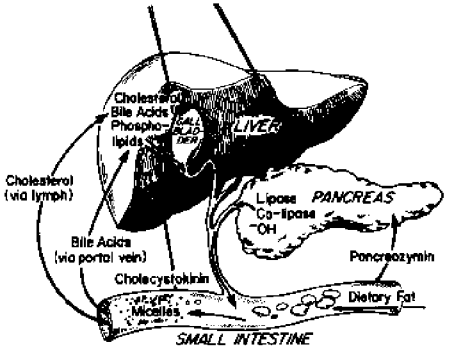
| Component | Subclasses | Natural/Otherwise | Percent of Total |
|---|---|---|---|
| Triglycerides | Saturated | Natural/Synthetic | |
| | Monounsaturated | Natural/Synthetic | 65-95+ |
| | Polyunsaturated | Natural | |
| Phospholipids | Lecithins | Natural/Synthetic | 1-30 |
| | Other Phosphatides | Natural | |
| Sterols | Cholesterol | Natural | |
| | Other Animal Sterols | Natural | 0-5 |
| | Plant Sterols | Natural | |
| Vitamins and Provitamins | Retinol | Natural/Synthetic | |
| | Caroteins | Natural | <1% |
| | Vitamin D | Synthetic | |
| | Tocopherols | Natural/Synthetic | |
| Hydrocarbons | Squalene | Natural | <1% |
| | Alkanes, Alenes | Environmental Contaminants | |
| | Polycyclics | | |
| Antioxidants | BHA/BHT | Added to prolong | |
| | Propyl Gallate | shelf-life | <1 |
| | Tocopherols | | |
| Environmental Toxins | DDT | Insecticide | |
| | PCBs | Insulating Fluid | <<1% |
| | Mycotoxins | Food Spoilage | |
| | Other toxins | | |
| Other Additives | Silicones | Non-stick Agents | <<1% |

This list which is by no means comprehensive, gives us some idea of both the variety and variability of human dietary fat intake. Commercial vegetable oils, for instance, may contain almost pure triglycerides, the sterols and phospholipids having been removed to ensure clarity. Egg yolk, by contrast, is about 65% triglycerides, 25% phospholipids, and 5% cholesterol. When discussing human disease, one must distinguish among the effects of substrate, natural minor components of metabolic importance, and toxic minor components. Let us turn at this point to normal human fat metabolism.

Fat fulfills multiple functions in the human body including: major metabolic fuel to meet caloric needs, major structural component of the cell wall, and essential precursor of several hormones critical to normal existence. A complete review of human fat metabolism is beyond the scope of this lecture and this seminar, and the interested reader is encouraged to seek out other excellent sources of this information (Lewis, 1976; Havel, 1980; Assmann, 1982). However, the major pathways of fat metabolism will be discussed here, as they are essential for understanding the topics that follow.

Of the three major lipid classes in dietary fat, sterols have the simplest metabolic pathway in most respects, and triglycerides the most complex. Nevertheless, we will begin with the latter, as it is necessary to understand triglyceride metabolism in order to place that of the other lipids into proper perspective. The majority of dietary fat is triglycerides, and they pass through the stomach unaltered by its acid pH and the gastric proteases. In the small intestine (Figure 1), the pH is alkaline, and the pancreatic juice that is secreted into the small intestine contains a powerful lipase (Semeriva and Desnuelle, 1979), which with its polypeptide colipase (Borgstrom, 1979), has the ability to hydrolyze triglycerides to monoglycerides and two moles per mole of free fatty acids.

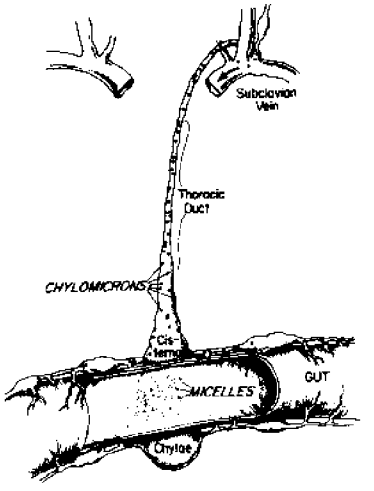
Figure 1. Fat Absorption and the Entero-hepatic Circulation




Pancreatic lipase is virtually inactive on triglycerides in their usual bulk state, however. The enzyme's activity depends on the emulsification of dietary fat into micelles—small spheres of triglycerides along with any other nonpolar lipids present in the dietary fat, such as sterol esters and fat-soluble vitamins— with a surface coat of phospholipids, free cholesterol and bile acids. Most of the first two and all of the third of these polar lipids of the micellar surface come from the bile, which is secreted by the liver with intermediate storage in the gallbladder and bile ducts. Bile is secreted into the small intestine when fat enters it from the stomach. This process (Figure 1) is triggered by the hormone cholecystokinin that is secreted by the duodenal mucosa when fat comes into contact with it.



Figure 2. Chylomicron Synthesis and Transport

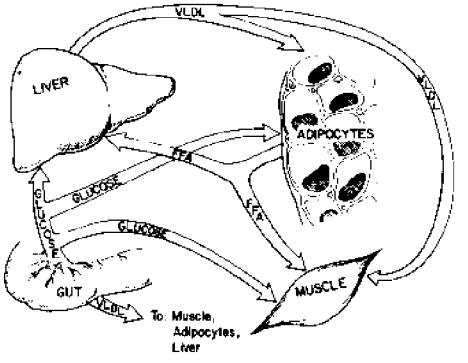



The monoglycerides and free fatty acids produced by lipase activity are absorbed by diffusion into the gut mucosal cells. There they are resynthesized into triglycerides, with the admixture of 10-20% of endogenous fatty acids (i.e. fatty acids from the body stores, not from the digested fat), and assembled into chylomicrons. The chylomicrons, as described by Gage and Fish, pass into the intestinal lymphatics; from there into the large lymphatic chamber in the abdomen, called the cisterna chylae; and finally via the thoracic (lymphatic) duct into the venous blood (Figure 2). The chylomicrons then pass through the heart in the venous blood and are distributed to the entire body via the arterial and finally, the capillary circulation. There, something akin to the mirror image of chylomicron production occurs. As the chylomicrons move along the capillary surface, their triglycerides are lipolyzed by the concerted action of two important enzymes—lipoprotein lipase (LPL) and lecithin:cholesterol acyl transferase (LCAT). LPL and a related enzyme, hepatic lipase, hydrolyze the triglycerides to glycerol and free fatty acids. The free fatty acids diffuse through the capillaries into the underlying parenchymal cells where they are either burned as metabolic fuel or resynthesized to triglycerides and stored. The glycerol is returned via the blood to the liver where it enters the hepatic glycerol pool and is resynthesized into triglycerides, or converted to carbohydrate, or undergoes one of a multiple of other metabolic reactions.

Since as much as 100 grams of dietary fat may be absorbed and transported through the blood each day, the process of chylomicron formation and transport must be swift and efficient. The half-life of chylomicrons in plasma, for example, is about 10 minutes in normal human beings. Thus, the triglyceride fatty acids from dietary fat are rapidly and efficiently transported to peripheral tissues where they may be stored, as in the adipose tissue, or burned for energy as occurs in muscle. Multiple metabolic studies (Frederickson and Gordon, 1958; Fritz, 1961; Cahill, 1966) have shown that up to 50% the "fed" state and up to 90% of total fasting caloric needs of human beings are met by the metabolism of fatty acids.

Figure 3. Glucose and Fatty Acid Metabolism

What then happens in the fasting state? How are metabolic needs met when dietary fat is not directly supplying chylomicron fatty acids? The human body has an alternative system for supplying fatty acids for metabolism (Figure 3). Depot fat has a rich sympathetic innervation and is vascularized with an extensive capillary network. Norepinephrine, delivered at the sympathetic nerve endings, and circulating epinephrine and norepinephrine, delivered via the capillary blood, activate a hormone-sensitive lipase in adipose tissue, which rapidly lyses triglycerides into glycerol and free fatty acids (FFA). These molecules move rapidly down a concentration gradient across the capillary endothelium into the blood. The FFA bind immediately to the plasma albumin, which has multiple high-affinity FFA binding sites (Goodman, 1958; Spector, 1975). They are then transported via the blood to muscle, where they are extracted and burned for energy. Any unburned FFA are removed efficiently by the liver, as is the glycerol, resynthesized into triglycerides, and resecreted as triglyceride-rich very-low-density lipoproteins (VLDL) (Figure 3).




Where blood glucose is also low, the glycerol returned to the liver is converted to glucose, and the fatty acids to "ketone bodies" (the oxygenated fatty acids beta-hydroxy butyric and acetoacetic), which are alternative fuels for the brain in place of glucose. The neural and hormonal sympathetic control of fatty acid mobilization is so sensitive and rapid, that significant rises in FFA concentration can be detected within a few seconds of a stimulus that disturbs a person—a loud noise, for instance, or a perceived physical threat. If the mobilized FFA are not burned for energy, they are re-esterified by the liver and returned to the adipose stores as VLDL. The cycle of endogenous fatty acid availability is not only finely tuned, it is rapid, much like that for chylomicrons. The half-life of FFA in the blood is about two minutes, and that of VLDL about an hour, in normal subjects. As much as 100 grams or more of fat may pass through this cycle each day.



Cholesterol metabolism, by contrast, is relatively indolent. In most human beings on Western diet, cholesterol intake is between one-half and one gram daily. It is consumed as a component of animal fat, almost always accompanied by a much larger amount of triglycerides. There is no cholesterol in vegetable fat—the critical biochemical distinction between plants and animals is that *only* animals can synthesize cholesterol. Dietary cholesterol, as noted above, is incorporated into micelles in the small intestine. It is almost all free cholesterol, so no hydrolytic step is necessary. From that point, its fate is very different from that of triglycerides. First, it is diluted by a much larger amount of biliary cholesterol. Then, rather than being almost completely absorbed as are triglycerides, it is only partially absorbed. The percent absorption varies widely, depending both on how much cholesterol is in a given meal and on what other lipids are included in the meal (Quintao, 1979). In general, about 50-75% of dietary cholesterol is absorbed at low intakes, and this falls progressively to 25% or less at high dietary intakes (Borgstrom, 1969; Quintao, 1971).

Once it reaches the gut mucosal cell, the cholesterol again behaves very differently from triglycerides. It enters the blood stream only very slowly, over a period of 24 hours or more after a given meal, and only in part as chylomicron cholesterol. Much of the dietary cholesterol is probably secreted into the portal venous blood as intestinally synthesized VLDL. At the same time, endogenous cholesterol synthesis goes on in virtually every cell in the body, although half or more of the total occurs in a single organ, the liver. Total endogenous synthesis is about a gram a day and in human beings, unlike many animals, changes relatively little as dietary intake changes. This important and underappreciated fact means that the more cholesterol a person eats, the more the body accumulates, despite its poor absorption (Borgstrom, 1969; Quintao, 1971).

Cholesterol can leave the human body by only a few restricted pathways. The most important is via the gut. Much of the endogenously synthesized cholesterol in the liver is secreted into the bile either as such, or as bile acids, an oxidative breakdown product of cholesterol. The bile eventually ends up in the intestine, where much of the biliary cholesterol and most of the bile acids are reabsorbed and eventually returned to the liver (Carey, 1982; Hofmann, 1983, 1984). This enterohepatic circulation of cholesterol and bile acids is an important control mechanism for body sterol metabolism and is the site at which certain cholesterol-lowering drugs act, for instance. Some of the biliary cholesterol, some of the bile acids, and some of the dietary cholesterol—that which is not absorbed or reabsorbed—is excreted in the stools.

Ideally, the total excreted would equal the sum of that taken in in the diet plus that synthesized, since the only other means of cholesterol loss, or catabolism, are via the small amount of skin that is desquamated each day, and the few milligrams of cholesterol that are converted into steroid hormones by the adrenals and the gonads. In reality, most human beings are in very slightly positive cholesterol balance, and slowly deposit cholesterol over time in their adipose and connective tissues (Crouse, 1972) and, unfortunately for many, in the walls of their arteries.

The fate of dietary phospholipids is relatively simple. They are hydrolyzed almost quantitatively in the small intestine by pancreatic phospholipases, and their multiple constituents absorbed and mixed in the gut, metabolic pools of fatty acid, phosphoric acid, choline, and so on. Phospholipids can be synthesized and broken down by every nucleated cell in the body, so there is little need for net phospholipid transport. In terms of fat absorption, they have a major role as a primary constituent of the bile being one of the two biliary detergents (the other being bile acids) that emulsify the dietary triglycerides and cholesterol into micelles, and facilitate hydrolysis and absorption of the former, and partial absorption of the latter.

As can be noted from Table 1, there are many other lipids, at least potentially, in human dietary fat. Several are important to consideration of the role of dietary fat in etiology of disease and will be mentioned briefly here. The reader is encouraged to consult the cited references for the details of their chemistry and metabolism. These other lipids include plant sterols (Tilvis and Miettinen, 1986), animal sterols other than cholesterol (Lin, 1984), fat-soluble vitamins (Machlin, 1984), antioxidants (Coppen, 1983; Loliger, 1983), and environmental toxins— both natural toxins, such as mycotoxins (Wogan and Busby, 1980), and man-made toxins, such as polychlorinated biphenyls (PCBs), insecticides like DDT, and herbicides like dioxins (McEwen and Stephenson, 1979; Poland and Knutson, 1982).

Plant and animal sterols are specifically important in the understanding of lipid-lipid interactions in the maintenance of plasma cholesterol concentrations and deserve review here. Plant sterols are found in all vegetable oils in their natural state, but are usually removed from the oils for the commercial marketplace. Their limited solubility in triglycerides often causes turbidity and precipitation on the shelf, and thus, decreases the eye appeal of vegetable oil to the purchaser.

These plant sterols, which generally differ from cholesterol in that they have side-chain aliphatic substitutents (Figure 4), are more poorly absorbed than cholesterol, except in certain rare inherited states (Bhattacharyya, 1974;



Figure 4. Some Common Plant Sterols

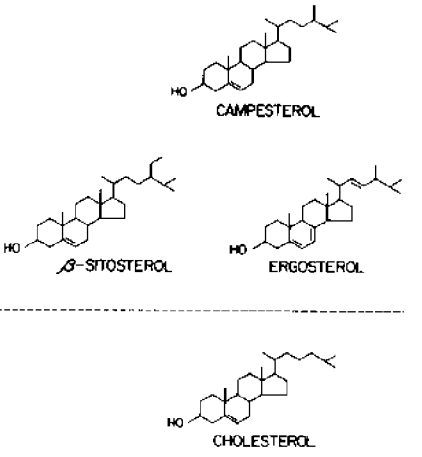
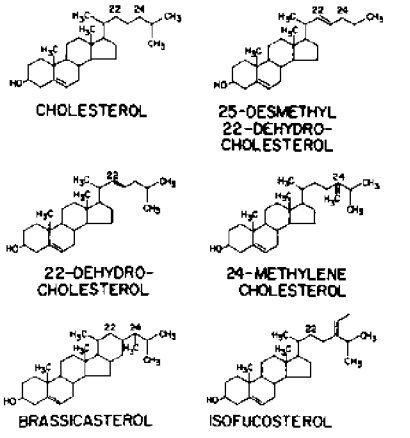



Figure 5. Mollusc Sterols




Salen, 1985a, 1985b), and inhibit cholesterol absorption, presumably by blocking cholesterol-binding sites on the gut mucosal cells. Thus, their frequent removal from food fats is probably not beneficial from a public health point of view.

Sterols like campesterol and beta-sitosterol are present in low concentration in normal human blood plasma (Gould, 1969; Salen, 1970; Lees and Lees, 1976; Gregg, 1986) and are occasionally found in tumors, presumably secondarily. Except in rare subjects, who lack the normal absorption barrier for them, (Bhattacharyya, 1974; Gregg, 1986), they do not cause disease. Other plant sterols such as ergosterol (Figure 4) are vitamin D precursors (Machlin, 1984).

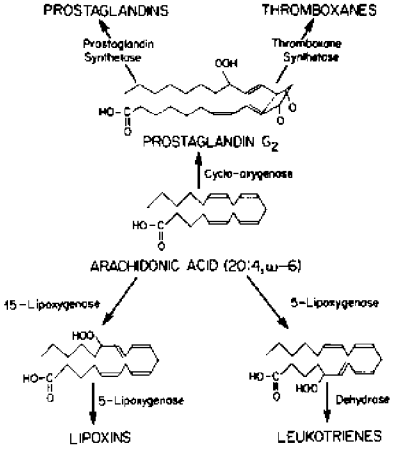
Not very many years ago it was thought that clam and oyster sterols, like those of shrimp and lobster, were almost pure cholesterol. Recent research, mostly from the laboratory of Connor and his colleagues in Oregon (Connor and Lin, 1981), has shown that there is a marked difference between the sterols of the molluscs, such as oysters and clams, and those of crustaceans, such as shrimp, crayfish and lobster. The latter are largely cholesterol, while mollusc sterols are only about 50% cholesterol and include several other compounds (Figure 5)— some of which, although clearly animal sterols, share with the plant sterols the property of poor absorbability. Others are well absorbed, however, and like cholesterol appear both in the bile and the blood (Connor and Lin,

1981, 1982). In limited clinical studies (Connor and Lin, 1982), crustacean ingestion was associated with a modest rise in plasma cholesterol while mollusc ingestion raised plasma cholesterol in a hypercholesterolemic patient, but not in six normal men.

An old and empirical observation in human fat metabolism, that certain polyunsaturated fatty acids must be in the diet in order for normal growth and sustained health to occur, has been elegantly explained by biochemical findings—these acids are essential precursors of a whole series of highly active substances that have profound effects on smooth muscle cells throughout the body, including those in the vascular, uterine, and bronchial walls, and on blood platelets, to cause or inhibit aggregation. These hormones, called as a class *eicosanoids*, include prostaglandins, thromboxanes, leukotrienes, and lipoxins, and are produced by oxidation of certain polyunsaturated fatty acids (PUFA), which cannot be synthesized by mammals and must be obtained from plant sources (McGiff, 1980; Stenson and Parker, 1984; Samuelsson, 1987). The pathways of oxidative metabolism of PUFA are diagrammed in Figure 6, with arachidonic acid used as an example.

Arachidonic acid is a representative of one of two naturally occurring series of polyunsaturated fatty acids, which are metabolically equivalent, and often interconvertible, but only within a given series. Furthermore, each series produces oxidation products that are metabolically distinct, and these differences may have profound implications for disease.

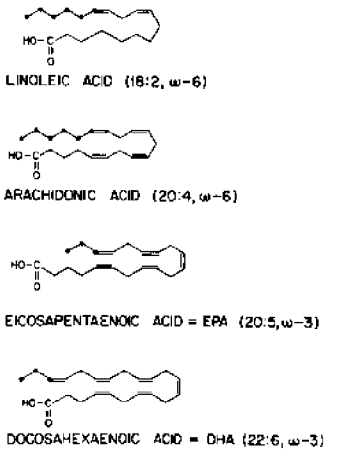
Figure 6. Biologically Active Oxidation Products of Arachidonic Acid




The more common of these fatty acids are synthesized by many terrestrial plants. The first of their two or more double bonds six carbon atoms in from the methyl end of the carbon chain and are therefore called n-6 or w-6 fatty acids (Figure 7). These include linoleic acid and arachidonic acid, which are precursors of the highly active prostaglandins, thromboxanes, leukotrienes, and lipoxins commonly associated with inflammation and thrombosis, as well as with the anti-thrombotic hormone, prostacyclin.



Figure 7. Structure of Some Naturally Occurring Polyunsaturated Fatty Acids




The other fatty acid class is synthesized in small quantities by terrestrial plants, and in larger amounts by the aquatic single-cell plants, which are called phytoplankton (Lin, 1982). Since phytoplankton are the ultimate food source for all marine fish, the same fatty acids are found in high concentration in fish body fat. These acids have the first of their multiple double bonds three carbon atoms in from the methyl end of the side chain, and are therefore called in chemical nomenclature n-3 or w-3 fatty acids. The metabolic oxidation products of these acids are, in general, much less active than their n-6 counterparts. With respect to cardiovascular disease, for instance, thromboxane A3, the n-3 analog of the strong blood platelet aggregant and n-6 derivative thromboxane A2, is almost inactive (Dyerberg, 1978). There is an important exception, however, in that PGI3, the n-3 counterpart of prostacyclin, a strong platelet *disaggregant* synthesized by the vascular endothelium, is almost as potent as the n-6 prostacyclin, PGI2 (Dyerberg, 1978; Dyerberg and Bang, 1979). Thus, eating fish fat in quantity, and increasing the human depot fat content of n-3 relative to n-6 fatty acids, should, and does (Dyerberg, 1978; Dyerberg and Bang, 1979; Glomset, 1985), have a nutritional antithrombotic effect.

Similarly, with respect to inflammatory disease, the n-3 fatty acids are both less effective substrates for the lipoxygenases (Figure 6) which convert them into leukotrienes and lipoxins (Lee, 1984; Lewis and Austen, 1984), and in the case of eicosapentaenoic acid (EPA), inhibits the conversion of arachidonic acid into leukotrienes (Lee, 1984). These data suggest that fish oil ingestion should modify the inflammatory response, and both animal (Prickett, 1981,1984; Robinson, 1986) and recent human (Kremer, 1987) studies suggest that such is the case.

One last general point concerning dietary fat is that the physical chemistry of dietary fat and, therefore, of depot and membrane fat, is most important to its function. Saturated fats, in general, melt at much higher temperatures than do unsaturated fats.

# Dietary Fat Intake and Disease

This is true not only of triglycerides, but of cholesterol esters and phospholipids as well. Many highly saturated fats are crystalline or semisolid at room temperature, for example, cocoa butter, butter, coconut oil. Since most lipids do not function unless in the liquid state, intake or endogenous synthesis of unsaturated fatty acids is essential for the existence of poikilothermic animals (Imagine walking if your own subcutaneous fat had the consistency of a chocolate bar!). At a more subtle level, the physicochemical properties of different fatty acids, as major components of cell membranes or plasma lipoproteins, may have profound effects on cellular and organismal metabolism. We are only beginning to appreciate some of these effects (Spritz and Mishkel, 1969; Mishkel and Spritz, 1969; Spector and Yorek, 1985; Parks and Bullock, 1987). In the next section, we will consider some of the general relationships of dietary fat to human disease, as well as the methods by which these correlations have been elucidated.

This topic is, in a sense, the mirror image of the relationships of dietary fat to human health. We review it here, however, not in physicologic terms, as we did in the previous section, but rather in relation to chemical, toxicological, and pathological parameters.

Dietary fat is an important source of calories for human metabolism and the major substrate that keeps us alive during most of each day. In many ways, fat is an almost perfect food. It is widely available, an efficient source of energy at nine calories per gram (compared with only four for carbohydrate and protein), and can be turned into energy with only minimal metabolic modification. Human beings have existed for centuries on widely varying fat sources, ranging from purely vegetable to fat from terrestrial animals to exclusively marine animal fats. This remarkable human adaptability is often forgotten when we concentrate on the problems that sometimes arise from this generally benign food source.



*Intrinsic Properties of Dietary Fat in Relation to Disease*

Many of the relationships between the intrinsic properties of fat and disease have been alluded to in the previous section. These include the saturation of dietary fat, its sterol content, total fat intake, and the stereochemistry of unsaturated fatty acids.

Dietary fat saturation has been the subject of intense study for years, and the interested reader should consult general reviews of the subject (Goodnight, 1982; Mattson and Grundy, 1985; McNamara, 1987). The purposes of this discussion are best served by a very brief summary of current thought. An increase in saturated fat intake as a percent of total fat intake is associated with an increase in total plasma cholesterol, and appears to raise equally low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol (Grundy, 1986). Epidemiologic data link it to an increased incidence of atherosclerotic death (Turpeinen, 1979), and perhaps to certain cancers, although the data on the latter point is, at best, equivocal (Carroll, 1975).

However, some of the world's longest-lived populations, on an average, eat a high saturated fat diet. Furthermore, there may be an inverse correlation between plasma cholesterol concentrations and risk of cancer death (Schatzkin, 1987). An increase in the relative proportion of monounsaturated fats in the diet is associated with moderate plasma cholesterol concentrations, with lower LDL, and with relatively high HDL levels (Grundy, 1986). Such a diet, consumed by much of the population of the Mediterranean basin, is associated with a low cardiovascular death rate, and has recently been championed (Grundy, 1986) as a healthful alternative to the high polyunsaturated fat diet that has been in vogue for some years. The latter diet may or may not lower both LDL and HDL cholesterol (Goodnight, 1982; Gundy, 1986). It increases biliary cholesterol and has been associated with a higher incidence of both gallstones and cancer in one large epidemiologic study (Pearce and Dayton, 1971; Sturdevant, 1973). In animal models, polyunsaturated fats may act as tumor promoters (Carroll and Khor, 1971). However, most of this data, human and animal, was obtained with n-6 fatty acids as the dietary source. Of particular note with respect to this discussion is that n-3 fatty acids may behave very differently. In recent studies, fish oils have been shown to lower plasma triglycerides and VLDL, with a lesser effect on LDL or HDL concentrations. When fed as the sole fat source, fish produce a pathologically low platelet count and a profound bleeding defect (Dyerberg and Bang, 1978, 1979).

Dietary sterol content seems clearly to be associated with risk of atherosclerotic disease. Not only may high cholesterol intake raise plasma cholesterol, it is associated with a higher incidence of atherosclerosis (Quintao, 1971; Turpeinen, 1979), but a high plant sterol intake inhibits cholesterol absorption and lowers plasma cholesterol (Lees, 1977; Tilvis and Miettinen, 1986). Diets high in plant sterols are those high in vegetable fat and low in animal fat. Such diets are often high in fiber and are eaten by populations that have low death rates from both atherosclerosis and gastrointestinal cancer. Very recent studies in Finnish subjects (Kesaniemi, 1987) have shown that plasma apolipoprotein E (apo E) phenotype, a readily studied genetic marker, relates directly to dietary cholesterol absorption, endogenous cholesterol synthesis, and plasma cholesterol concentration. Genetically determined responsiveness to dietary cholesterol intake is almost certainly generally true in humans, since the relationship of apo E phenotype to plasma cholesterol concentration is a world-wide phenomenon (Utermann, 1984; Sing and Davignon, 1985).

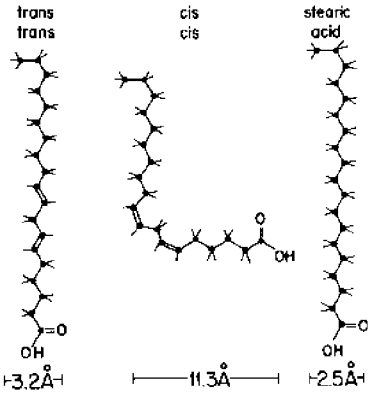
As noted above, the relationship between marine sterol intake and disease is a complex one. Fish and crustacea contain cholesterol, and some fish oils contain a lot of cholesterol, while molluscs contain both cholesterol and a number of other sterols that may behave like cholesterol. The net effect of mollusc sterols on plasma cholesterol appears to be negligible. Diets very high in plant or marine sterols run the risk of raising their levels in plasma (Lees and Lees, 1976) even in normal subjects, and in certain patients, may produce serious illness (Bhattacharyya and Connor, 1974; Salen 1985b; Gregg, 1986).

The relationship between total fat intake and disease is a complex one; in most studies on fat intake, saturated fat, or unsaturated fat, or sterol intake, rather than total fat, has been correlated with a particular disease. It is not clear that a high total fat intake per se is particularly related to any disease.

Finally, the fine structure of unsaturated fatty acids in the diet is important. The prescient studies of Spritz and Mishkel in the 1960s (Spritz and Mishkel, 1969; Mishkel and Spritz, 1969) illustrate this point. Spritz hypothesized that many effects of unsaturated fatty acids were related to their cross-sectional area (Figure 8). Because the normal cis double bond bends the fatty acid chain at roughly a 120-degree angle, the presence of even a single double bond increases the area occupied by that chain; multiple double bonds increase its cross-sectional area even more. Spritz postulated that the cholesterol-lowering effects of polyunsaturated fatty acids were related to their bulk, and that the percent of



Figure 8. Cis & Trans Linoleic Acid




lipid-lowering should be directly related to the ratio of the cross-sectional area of the polyunsaturated fatty acids to that of the saturated dietary fatty acids fed as a control. The investigators then performed human feeding studies to prove their point, and showed that the expected change did occur both for LDL and for HDL (Spritz and Mishkel, 1969). This was perhaps the first study to show that polyunsaturated fat feeding lowered HDL cholesterol as well as LDL cholesterol.

Spritz and his colleagues went much further, however. They realized that all *trans* polyunsaturated fatty acids have virtually the same cross-sectional area as saturated fatty acids (Figure 8), and postulated that triglycerides made from such acids should not lower plasma lipids, *despite their great unsaturation*. Then they put their hypothesis to the test. They had a relatively large amount of *trans, trans* trilinolein custom synthesized for them, and fed it as the sole fat source to a series of human volunteers. The results considerably exceeded their expectations. Not only did the subjects' plasma lipids not decrease, they increased dramatically; and after a few weeks of the *trans* fatty acid diet, the subjects themselves developed, a severe and painful peripheral neuropathy, which slowly subsided when they resumed normal diet (Mishkel and Spritz, 1969).

Needless to say, the human studies were stopped at that point, and definitive data on the cause of the neuropathy was never obtained. Subsequent studies with mono-*trans* fatty acids, which are present in moderate amounts in partially hydrogenated vegetable oils, did not reproduce the toxic effects seen with the *trans, trans* acid (Mattson, 1975). Nevertheless, Spritz's studies dramatically highlighted the importance of the structure and isomerism of dietary fatty acids and set the stage for the more recent findings on the metabolic differences between the n-6 and n-3 isomers.

*Extrinsic Considerations*

Disease may be caused by many factors extrinsic to the major and minor constituent in dietary fat. These extrinsic agents include naturally derived toxins such as mycotoxins, compounds that contaminate dietary fat as a result of fungal growth directly on the fat or on the food from which the fat is extracted. Aflatoxin, a highly potent carcinogen synthesized by the fungus *Asperigillus flavus*, is an almost constant, but usually low-level, contaminant of peanuts and, therefore, of peanut oil (Wogan and Busby, 1980). Keeping the level of this fat-soluble toxin within prescribed limits requires constant monitoring of the supplies of this widely used food fat. Oxidation of polyunsaturated fat, another form of food spoilage, can introduce lipid peroxides, which may be atherogenic and mutagenic (Carroll, 1975). Environmental toxins readily enter through the food chain. Hydrocarbons such as benzpyrene, insecticides like DDT, industrial agents such as PCBs, are found in increasing concentrations as one ascends the food-chain from marine plants to predatory fish like bluefish and striped bass (McEwen and Stephenson, 1979).

A third class of extrinsic food contaminants that may cause disease is additives, in this case, agents to prevent spoilage or rancidity. In the past, the most widely used additives or preservatives, were butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), both phenolic antioxidants (Coppen, 1983). However, both BHA and BHT may be tumor promoters, and perhaps even carcinogens (Shamberger, 1983), and their allowable concentation in foods is now limited. Interestingly, in other studies, BHA and BHT have been shown to inhibit mutagenesis, probably because of their antioxidant properties. Similarly, tocopherols, such as vitamin E, which are natural antioxidant phenols in food fat, inhibit mutagenesis and are sometimes used as food additives; they are perhaps the safest in terms of preventing rather than causing disease (Loliger, 1983).

In summary, while it seems clear that a high-cholesterol, high-saturated fat diet raises plasma lipids and probably thereby increases the risk of atherosclerosis, there is little evidence that the kind of food fat eaten produces other human disease, except through spoilage, environmental toxins, either natural or manmade, and perhaps food additives. The tremendous adaptability of the human organism to its dietary caloric sources allows us to remain healthy over a wide range of diet. Whether certain chronic diseases, such as arteriosclerosis, cancer, or arthritis, can be prevented or ameliorated, is a somewhat different question and will be the topic of the remainder of this discussion.



*Clues to Disease Etiology in Relation to Dietary Fat*

A number of hypotheses have been generated, and some notable conclusions reached, from studies designed either to generate or to test certain clues to the relationship between fat intake and human disease. Almost all fall into two categories: experimental pathology, that is studies with animal models of human disease; and epidemiology, studies of large numbers of people to determine certain associations of dietary habits and disease. Some of the latter have been based on observation alone, with a careful attempt made *not* to affect the population studies, or have been retrospective, in which case different problems arose. Other epidemiologic studies have been interventional: a large number of individuals are fed one diet, a matched group is fed another, and the occurrence of disease in the two groups is compared. In some of these studies, the two groups are switched in the middle of the study, so that each can serve as its own control.

It is well beyond the scope of this overview to review, even in brief the enormous number of animal studies on fat and disease and the almost as large number of human studies. We will note some general advantages and disadvantages of these types of studies and cite a few pertinent examples.

Experimental pathology has many attractive features. An animal model whose metabolism is similar to humans can be chosen, and conditions fixed as far as possible with only a single variable. The animals can be sacrificed and a complete pathological evaluation performed. Perhaps the major problem is that all the diseases under discussion are chronic diseases, often evident late in life, 30 or more years after the beginning of exposure to the diet in question. Since the animals most similar to man are also long-lived, the cost of appropriate studies becomes prohibitive. In most cases, either the intervention is escalated to an unphysiological level, so that the desired end-point is reached in a much shorter time, or a shorter-lived animal species is used, so that chronic disease appears in a convenient time frame. In either case, the extrapolation of these results of naturally occurring disease becomes uncertain and may be quite misleading.

An interesting example is a recent study on the effects of cod liver oil feeding in cholesterol and saturated fat-fed swine (Weiner, et al., 1986). Coronary atherosclerosis, which ordinarily occurs in old age in the pig, was accelerated by feeding a 2.4% cholesterol lard-based diet, and also by balloon abrasion of the left anterior descending coronary artery after three weeks of the test diet. The animals were killed at eight months, and all three coronary arteries examined. There was much less atherosclerotic coronary artery disease in four pigs fed 30 ml cod liver oil daily as a supplement than in seven animals fed the atherogenic diet alone.

On the face of it, it would seen that cod liver oil feeding is anti-atherogenic. However, that conclusion is probably not supported by the evidence. Not only are the numbers of animals small and the diet most unphysiological for either pigs or humans, but the control diet resulted in undetectable levels of n-3 fatty acids in the animals' blood plasma, and its very high cholesterol content minimized the effects of the large amount of cholesterol ingested in the cod liver oil. Thus, as attractive as this experimental study is, it does not necessarily follow that human subjects, on mixed diet, would benefit from fish oil supplements, *nor do the authors suggest it*. Unfortunately, these fine points have not always been brought out in the descriptions of this work in the popular press.

Epidemiological studies similarly have many attractive features. When performed prospectively, observational studies have minimal bias, and intervention studies may allow the investigators to determine the effects of a single parameter on the incidence of disease. It is feasible to design studies of long duration; five years or even 10 is not rare. No extrapolation from animals to humans is needed.

In many ways, the promise of epidemiological studies has been fulfilled. Much of what we consider to be true about dietary fat and human disease is based on prospective, and occasionally retrospective, human studies. The extensive follow-up of the dramatic increase in coronary disease deaths in Jews migrating from the Arab world to Israel, which coincided with, among many other changes, an increase in their caloric intake (Cohen, 1961); the experience of the Finnish and Norwegian populations during the second World War, when a tremendous drop in caloric intake was paralleled by a similar drop in cardiovascular deaths (Turpeinen, 1979); the Framingham Heart Study (Anderson, 1987); and the interpopulation diet-heart disease data nicely summarized by Turpeinen (Turpeinen, 1979) all helped to convince us that dietary fat, as well as total calories, are directly related to the causes and progression of human atherosclerosis. Although the data is much less clear-cut, the prospective dietary intervention study of the late Seymour Dayton and his colleagues (Dayton, 1969; Pearce and Dayton, 1971; Sturdevant, 1973), as well as recent (Schatzkin, 1987) and earlier studies (Armstrong and Doll, 1975; Garcia-Palmieri, 1981; Kagan, 1981), suggest that there might be a relationship among dietary fat intake, serum cholesterol, and certain, but not all, cancers in human populations. However, the epidemiologic approach, too, has many and serious problems. Retrospective studies may have serious built-in biases that often invalidate their results. An obvious one is that those who have died cannot be tested, and survivors may be different in important parameters than they were at an earlier, perhaps critical, time.





Prospective observational studies may suffer from insufficient spontaneous range of variation in the parameter to be studied. For instance, the lack of correlation between dietary cholesterol intake and cardiovascular death in the Framingham study has been attributed to the narrow range of cholesterol intakes in that population, which may not have been wide enough to be meaningful (Connor and Connor, 1972).

Intervention studies are particularly susceptible to ambivalence. A negative result may mean only that the intended intervention did not accomplish its goal. A positive result may be from an *unintended* change which accompanied the *intended* intervention. Diet studies, which involve exchange of saturated fat for unsaturated fat, also exchange animal sterols for plant sterols, as well as the trace constituents of each fat source. Any observed effects of diet could result not from the fat saturation, but rather from one or more of the other ingredients in the fat. Despite all these problems, the epidemiologic approach has remained one of the most useful methods for determining the effects of dietary fat on health.

In the last section of this review, we will look briefly at the three major diseases to which dietary fat has been linked, and attempt to integrate what we have learned from the biochemical, physiological, pathologic, and epidemiologic considerations reviewed above.

*Cardiovascular Disease*

The major cardiovascular disease, atherosclerosis, is responsible for more deaths by far in developed countries than any other disease. As abundantly documented above, dietary fat quality, as well as quantity, has been linked to total plasma cholesterol and LDL and HDL cholesterol, major risk factors for atherosclerosis, as well as directly to the incidence of atherosclerosis complications. Increased intake of saturated fat and cholesterol is clearly associated wih higher plasma lipids and higher risk of atherosclerosis, while intake of vegetable fat as the major source appears to be associated with lower lipids and less atherosclerosis. These epidemiologic conclusions are supported by a considerable body of biochemical and physiologic evidence concerning the effects of fat saturation, and particularly cholesterol intake, on plasma cholesterol concentration.

The last decade has seen the rapid accumulation of information concerning the special effects of marine fat on plasma lipids and atherosclerosis. Epidemiologically, high fish consumption has been associated with low death rates from coronary artery disease (Dyerberg, 1978; Dyerberg and Bang, 1979), although a recent study suggests that eating fish as little as twice a week is associated with a meaningful decrease in coronary disease death (Kromhout, 1985). These studies are frought with the dangers alluded to above, and have stimulated considerable discussion concerning their short-

comings (Bang and Dyerberg, 1985; Curb and Reed, 1985; Greaves, 1985; Vollset, 1985). Biochemical and physiological findings concerning marine fatty acids are perhaps more convincing, but the effects are seen only when n-3 fatty acids are a significant fraction of total fat intake (Phillipson, 1985; Weiner, 1986; Parks and Bullock, 1987). These effects include lowering of plasma triglycerides with little effect on LDL and HDL, inhibition of platelet function with prolongation of bleeding time, and inhibition of granulocyte and monocyte function (Glomset, 1985). Given that monocyte-macrophages are thought to participate actively in atherogenesis (Faggiotto, 1984), inhibition of monocyte function may be an important new mechanism of action of anti-atherosclerotic agents (Glomset, 1985).

*Cancer*

The data on dietary fat and cancer are both less conclusive and less systematized than those concerning atherosclerosis. Epidemiologically, the incidence of breast cancer has been related directly to the intake of animal fat and total fat (Carroll, 1975; Phillips, 1975), and that of gastrointestinal cancer to total fat intake (Carroll and Kohr, 1975; Wynder and Reddy, 1977) and intake of unsaturated fat (Dayton, 1969; Pearce and Dayton, 1971). However, in prospective studies (Phillips, 1983; Willett, 1987), these findings have not been confirmed. Biochemically, unsaturated fats (at least n-6 fatty acids) may be tumor promoters (Carroll, 1975), and bile acids may be as well (Wynder and Reddy, 1977; Hill, 1987). Furthermore, several common environmental contaminants, such as PCBs, are carcinogenic.





*Arthritis*

There is relatively little epidemiological evidence, to this writer's knowlege, concerning dietary fat intake and arthritic diseases. In contrast, a large amount of recent biochemical data suggests that increasing dietary n-3 fatty acids in the diet has an effect on the mechanisms of inflammation, which involve prostaglandins, leukotrienes, and lipoxins (Lewis and Austin, 1984; Samuelsson, 1987). In one experimental animal study, this effect was to exacerbate arthritis (Prickett, 1984). In one very recent clinical study of 33 patients with rheumatoid arthritis, daily administration of 15 grams of fish oil as a diet suppplement was associated within three months with subjective and objective improvement in multiple parameters, including joint tenderness and swelling. Since the study was conducted on a double-blind basis, the results warrant careful consideration.

Although human beings are remarkably adaptable in their ability to remain healthy over a wide range of dietary fat sources and intakes, fat in the diet is clearly associated with the occurrence of, or freedom from, certain diseases. The evidence is particularly strong for coronary heart disease, and less so for cancer. Although little is known about the effects of dietary fat on arthritic and rheumatic diseases, the available evidence suggests that the nature of the dietary fat may be therapeutically important in these syndromes.

In all of these areas, the recent emergence of dietary polyunsaturated fat as a precursor of potent metabolic intermediates has radically changed our concepts of the potential mechanisms of diet-induced effects on health and disease. In particular, the therapeutic effects of dietary fat changes in disease may be signficant, even though health may be maintained over a wide range of intakes. In this regard, the recent findings that certain fish oil fatty acids, the n-3 polyunsaturated acids, are synthesized into eicosanoids with significantly different metabolic effects than those from the n-6 fatty acids, lend a powerful rationale to this concept.

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