Current Studies on Relation of Fat to Health[1]

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ABSTRACT

An increase in the linoleic to oleic acid ratio by an increase in the percentage of the polyunsaturated ω6 family of fatty acids in culinary fats and a decrease in the consumption of cholesterol-rich food were believed necessary as a prerequisite to early intervention in coronary heart disease. A decrease in total fat consumption also has been recommended. However, a decrease in the percentage of fat in the diet may not be nutritionally sound, as it may only increase the percentage of carbohydrates consumed and, thus, the synthesis of the ω9 family of fatty acids from the surfeit calories. It may be more judicious to decrease the total number of calories through less consumption of a well balanced diet. Furthermore, as the *trans*-fatty acids, which are formed during hydrogenation, are not discriminated against completely by acyl-glycerol-3-phosphoryl-choline transferase or acyl coenzyme A cholesteryl transferase, it would be, from a biological viewpoint, advantageous to eliminate *trans*-fatty acids from both stick and tub type margarines.

INTRODUCTION

It is evident that fats and oils supply an economical source of calories to the American diet. However, it is also evident that the level of fat intake, the mixed fatty acid composition of the fat, and the character of the sterols in the fat or oil may all have a nutritional impact. Laboratories located in every one of the five continents are engaged in determining the extent of this impact upon heart disease and stroke, the leading cause of death in every country that keeps mortality records.

ROLE OF LIPIDS IN DISEASES OF LONGEVITY

To understand the relationship of cancer, heart disease, and stroke to mortality, it is important to recognize that a shift in death rate/100,000 of population has occurred since 1900 (1). The diseases caused by bacteria or viruses have been controlled by antibiotics, so that cancer, heart disease, and stroke are responsible for 81.6% of all deaths in the U.S. A task force of the National Cancer Institute has just completed an intensive study on how best to conquer cancer and has asked for additional funding (2).

Cancer represents a complex disease process. At a recent symposium on "Molecular Biology and Pathology," it was stated that the cancer cell is capable of a higher rate of glycolysis than a normal cell. However, Potter (3) stated, "Pick any enzyme whose activity is modulated in the normal cell of origin, and there is a good chance that it will respond in a peculiar fashion in the neoplasm."

The fat and oil industry has a stake in the future direction of cancer research, as neither the mechanism that converts a normal to a malignant cell nor the mechanism that might be useful in controlling the key enzyme presently is known. It is known that oxidized sterols (4) and complex cyclic sterol-like hydrocarbons, such as methyl cholanthrene and dibenzanthracene, are carcinogenic, i.e. capable of converting a normal to a malignant cell (5). However, it is not known whether the methyl silicone, propylene glycol monostearate, or the mono- and diglycerides in shortenings aid in the absorption of oxidized sterols. This is an area of research that deserves further effort.

Pearce and Dayton (6) indicated that an experimental group of human subjects, fed diets which contained polyunsaturated fats, developed more tumors than subjects fed saturated fats. This is an interesting observation in light of the results of Nichaman, et al., (7) that dietary linoleate stimulated the conversion of linoleic acid to carbon dioxide and that linoleic acid is more readily metabolized in rats fed corn oil than in those fed coconut oil (8). It would be worth further study to determine whether polyunsaturated fatty acids (PUFA) influence the rate of glycolysis or the assembly of cell membranes in malignant cells.

In a recent review, Reiser (9) has critically discussed the complex interrelation between plant sterols and polyunsaturated and saturated fats and concluded that saturated fats do not increase serum cholesterol levels significantly. As an elevated serum cholesterol level has been considered an important risk factor in the development of coronary heart disease, the conclusions of Reiser warrant serious consideration. However, at least two parameters that were not taken up in this review also may be relevant to the development of atherosclerosis. They are: (A) the tissue level of polyunsaturated fatty acids on a minimum linoleic acid intake and (B) the possible role of *trans*-fatty acid upon the biophysical properties of cell membranes.

NUTRITIONAL AND PHYSIOLOGICAL PROPERTIES OF PUFA

That PUFA occur in families or series in which the terminal structures are similar is now widely recognized. Original studies in this area, as well as further investigations of the metabolic conversions of several families of PUFA, have been reported by Klenk (10-12). These fatty acids include the mono-, di-, and polyenes of the ω6, ω7, and ω9 families and of chain lengths C_{20}-C_{22}. With respect to the ω6 acids, rats fed diets rich in linoleate (corn oil), or barely adequate with respect to this acid (tallow or butterfat), resulted in almost equal levels of ω6 long chain unsaturated fatty acids in the cholesteryl esters and phospholipids (Table I) in the isolated tissues (13). Therefore, the sum total of the ω6 family of fatty acids in a given tissue and lipid class does not necessarily bear a direct relationship to the quantitative level of linoleic acid in the diet. However, dietary linoleate is known to lead to increased levels of the members of the ω6 family, especially

TABLE I

Dietary Fat and Total Fatty Acid Composition Greater than C_{18} in Cholesteryl Esters and Phospholipids in Heart Tissue (13)[a]

	Cholesteryl esters		Phospholipids	
Dietary fat	ω9	ω9	ω9	ω9
Corn oil	6.9	8.7	—	23.6
Milk fat	9.4	38.5	—	32.0
Beef tallow	4.3	4.9	4.9	31.6
Hydrogenated fat	19.8	7.5	3.3	17.8
Fat free	18.1	16.5	22.4	12.8

[a]Results are summation of total ω9 and ω6 fatty acids, respectively, and are expressed as percentage of total fatty acids.

[1]One of five papers presented at the symposium, "Status of Fat in Food and Nutrition," AOCS Fall Meeting, Chicago, September 1973.

TABLE II

Acyl-Coenzyme A Cholesteryl Ester Acyltransferase Activity in Rat Liver Microsomes (45)

Diet	Enzyme activity[a]
Stock	25.0
Corn oil	29.0
Fat-free	8.0
Hydrogenated fat	10.0

[a]Expressed in millimicromoles cholesterol esters synthesized/mg protein/hr.

arachidonic acid (20:4ω6), in cholesteryl esters and phospholipids (14,15). The absolute ω6 concentrations in heart tissue were generally higher in the phospholipids than in the cholesteryl esters and were largely accounted for by high levels of 20:4ω6. Tissue fatty acid specificity was also evident; for instance, relatively high amounts of 22:4ω6 were seen only in the adrenal cholesteryl esters. This observation has been reported by other workers (16,17). Furthermore, Walker has shown that corn oil supplementation of a fat-free diet resulted in increased levels of the ω6 acids with time of supplementation in the total lipids of rat liver, heart, and plasma (18,19). There was a simultaneous decrease in the level of the ω9 series of fatty acids.

TRANS-FATTY ACIDS AND THE BIOPHYSICAL PROPERTIES OF CELL MEMBRANES

An extensive literature has been developed in regard to cell membrane configuration, and various types of models have been suggested to represent the manner in which lipids are incorporated or sandwiched into bilayers with protein (20-24) at the cellular level. All of them agree that the phospholipids and cholesterol in cell membrane seem to line up in tightly bound columns with hydrophobic and hydrophilic orientation and that the fatty acid structure could influence the properties of cell membranes. For example, Chapman, et al., (20) found that the replacement of a *cis*- by a *trans*- acid in the 2 position of phosphatidylethanolamine or phosphatidylcholine caused monomolecular films to be appreciably more condensed in character in vitro. The association of cholesterol with *trans*-phospholipids also was believed to differ from the association of cholesterol with the isomeric *cis*-phospholipids. Mulder and Van Deenen (21) have shown that there is a free interchange, in vitro, of fatty acids between the lipids in the plasma and the lipids in the red blood cell. Demel and Eibl and coworkers (22,23) also have shown that even a difference in chain length and the degree of unsaturation of the incorporated fatty acids influenced the interfacial characteristics of phospholipids significantly. The classical studies of Burr and Burr (24) and Holman (25) provide examples for the importance of the ω6 series of fatty acids to the rate of water transpiration through skin tissue.

Many studies have been designed to correlate the role of dietary fat with the biophysical properties of the lipids in the arterial mesenchyme. One of the most interesting and potentially significant studies involved the use of elaidinized olive oil by McMillan, et al. (26-28). He reported that rabbits "fed elaidinized olive oil showed a little more visible atherosclerosis of the aortic arch, but no more atherosclerosis in the thoracic and abdominal aorta. They had a somewhat higher total cholesterol content per aorta in their aorta than did those rabbits fed natural olive oil and cholesterol." As ca. 60% PUFA in soybean oil are elaidinized during catalytic hydrogenation, such *trans*-isomers of oleic and linoleic acid are present in the shortenings and margarines that provide the major source of the visible fat in the American diet. The high polyunsaturated fatty acid margarines still contain ca. 20% *trans*-fatty acids.

TABLE III

Concentration of Elaidate in Lipid Classes of Heart and Plasma Lipoproteins (Wt% of Elaidate of Total Fatty Acids of Fraction) (46)

Lipids[a]	15 Weeks	20 Weeks
Heart		
Total lipids	10.6 (10.5-10.7)[a]	16.8 (16.2-17.4)
CE	11.2 (10.9-11.5)	4.2 (4.0- 4.4)
TG	10.8 (10.6-11.2)	11.3 (10.9-11.7)
FFA	9.7 (9.4-10.2)	10.5 (10.0-11.0)
PL	15.8 (15.4-16.1)	10.4 (10.3-10.7)
Plasma lipoproteins		
Total lipids	17.9 (17.5-18.3)	17.5 (17.2-17.8)
VLDL:		
CE	7.8 (7.5- 8.1)	16.2 (15.8-16.5)
TG	18.6 (18.2-19.0)	39.5 (38.8-40.0)
FFA	10.2 (9.6-10.8)	21.9 (21.8-22.3)
PL	9.1 (9.0- 9.3)	18.3 (18.1-18.5)
LDL:		
CE	7.1 (7.0- 7.2)	2.1 (2.0- 2.2)
TG	14.0 (13.6-14.4)	17.0 (16.8-17.2)
FFA	14.9 (14.3-15.4)	7.3 (7.1- 7.5)
PL	6.0 (5.9- 6.3)	23.3 (23.0-23.6)
HDL:		
CE	8.8 (8.6- 9.0)	3.9 (3.8- 4.0)
TG	15.3 (14.8-15.6)	10.4 (10.0-10.8)
FFA	2.2 (2.2- 2.2)	23.4 (23.1-23.8)
PL	19.0 (18.5-19.4)	11.7 (11.3-12.0)

[a]Abbreviations: VLDL = very low density lipoprotein, LDL = low density lipoprotein, HDL = high density lipoprotein, CE = cholesterol ester, TG = triglyceride, FFA = free fatty acid, and PL = phospholipid. Range of values in parentheses.

Trans-unsaturated fatty acids have been shown to oxidize as readily to CO_2 as do their *cis*-isomers (29,30). However, anabolism or elongation of the *trans*-fatty acids seems to be selective. The *cis*-9,*trans*-12 octadecadienoic acid has been reported (31) to elongate to an isomer of arachidonic acid believed to be *cis*-5,*cis*-8,*cis*-11,*trans*-14 eicosatetraenoic acid, and *trans,trans*-linoleic acid seemed to elongate to *cis*-5,*cis*-8,*trans*-11,*trans*-14 eicosatetraenoic acid (32). Selinger and Holman (33) found that *trans,trans*-linoleic acid did not increase the level of PUFA normally derived from the *cis,cis*-isomer. The studies of DeThomas, et al., (34) indicated that elongated nonessential fatty acids can esterify the β-position in lecithin. However, a number of studies (35-37) have demonstrated that *trans,trans*-linoleic acid cannot function as an essential fatty acid, presumably because its elongated derivative does not have the functional characteristics of the elongated *cis,cis*-isomer.

Privett and Blank (38) reported that the *cis,trans*-isomer depressed the conversion of *cis,cis*-linoleic to arachidonic acid (to 33%) as did the *trans,cis*-isomer (to 12%), although not so drastically as the *trans,trans*-isomer (to 1.4%). The presence of 50% *cis,cis*-linoleic acid did not counteract completely the depressing effect of *trans,trans*- (13.5%) or *cis,trans*- (17.5%) linoleic acid on the conversion of linoleic to arachidonic acid. The major share of the arachidonic acid was present in an α-saturated, β-tetraene triglyceride. In animals that had been fed *cis,trans*- or *trans,trans*-linoleic acid, the percentage of arachidonic acid incorporated at the β-position was depressed greatly when compared with that of animals fed *cis,cis*-linoleic acid (14.1, 1.6, and 42.4%, respectively). In those fed *cis,trans*-linoleic acid, an almost equal amount (12.5%) trienoic acid was incorporated at the β-position (39).

In a series of studies in our laboratory (40-42), Sgoutas found that the cholesterol esterases in the microsomal and soluble fractions of rat liver hydrolyzed the ^{14}C-cholesterol esters of *cis*- and *trans*-octadecenoic and octadecadienoic acids at different rates. The substrate concentration curves of the hydrolysis of cholesteryl-oleate, -linoleate, -elaidate,

-linelaidate, -palmitate, and -stearate by the soluble enzyme fractions showed that unsaturated cholesterol esters were hydrolyzed faster than saturated ones, agreeing with previous reports. Sgoutas also showed that the unsaturated esters clearly were divided into two groups: those with *cis*-double bond and those with *trans*-, with the former being hydrolyzed more rapidly than the latter. The substrate concentration curves indicated that the order of their hydrolysis by the soluble protein fraction was: *cis*-unsaturated > *trans*-unsaturated > saturated cholesterol esters. A similar pattern of substrate preference was observed when the crude soluble fraction or the microsomal fraction was used as the enzyme source. Rat liver microsomes also have been shown (43,44) to contain an enzyme system that esterifies cholesterol in the presence of adenosine 5'-triphosphate (ATP) and coenzyme A (CoA). When this enzyme system was obtained from the liver, microsomes of rats that had been fed a hydrogenated fat of 48% *trans*- and 0% essential fatty acid content, a decrease in the ability of these liver microsomes to esterify ^{14}C-cholesterol in vitro (Table II) was observed (45).

At comparable time periods, rats fed hydrogenated fat contained significantly more heart lipids than those fed beef tallow (46). *Trans*-fatty acid (elaidate) concentration in the lipid classes was variable, especially among the lipoprotein classes, and was not a direct function of the duration of feeding (Table III). Plasma very low density lipoprotein lipid classes indicated a doubling of elaidate concentration of each lipid class from 15-20 weeks of fat feeding. Also, at both time intervals, there was a consistent trend in the levels of elaidate in the four lipid classes, the order being triglyceride > free fatty acid > phospholipid > cholesterol ester. These two observations were not seen so clearly in the lipid classes of low density lipoproteins and high density lipoproteins. These results suggest differences in the mechanism of incorporation and turnover of elaidate, not only in different tissues, but also among the major lipoprotein types. Similar results were noted in the plasma lipoproteins obtained from swine fed hydrogenated fat (47) and in human tissue (48). Human adipose tissue was found to contain from 2.4-12.2%; liver, 4.0-14.4%; heart, 4.6-9.3%; and aortic tissue, 2.3-8.8% of *trans*-fatty acids.

The human diet contains a mixture of dietary fats that presumably supply an adequate amount of linoleic acid; yet, human adrenal cholesterol esters do contain some elongated ω9 series of fatty acids which may indicate the presence of less than an optimum level of dietary ω6 fatty acids (Table IV). The percentage of total ω9 polyunsaturated C_{18} and C_{20} fatty acids in the human adrenal (49,50) was in the same approximate range as found in rats fed 20% hydrogenated fat and 2% corn oil, or 8% and 14%, respectively. The adrenal cholesterol esters from rats fed corn oil contained only 3.0% while those fed only hydrogenated fat contained 34% ω9 fatty acids. The total ω6 fatty acids of human adrenal lipids was comparable in amount to that found in rats fed 2% corn oil. Corticosterone is the major corticosteroid of the rat adrenal tissue (51,52), and it has been suggested that essential fatty acid deficiency status leads to a decreased ability of rat adrenals to synthesize corticosteroids (53,54). Recent in vitro studies utilizing the endogenous free cholesterol present in the adrenals have shown that the ability for corticosteroidogenesis was dependent upon the nature of the antecedent dietary fat (55). If adrenal corticosteroid synthesis is modified by the nature of the dietary fat under in vivo conditions, its influence on corticosteroidogenesis could have important implications for some key aspects of carbohydrate, protein, and lipid metabolism (56).

TABLE IV

Composition of Adrenal Cholesterol Esters

Obtained from rats fed:	Percent total polyunsaturated C_{18+20} fatty acids ω6	ω9
20% Hydrogenated fat (46)	3.4	34.2
20% Hydrogenated fat + 2% corn oil	9.1	14.2
20% Corn oil	29.5	3.0
Human (49)	24.9	8.3
Human (50)	7.0	4.9

POSSIBLE APPLICATIONS OF LIPID RESEARCH FINDINGS TO HUMAN DIET

It is evident that a dietary source of ω6 fatty acids is essential to proper cell membrane structure and function and that an excessive intake of polyunsaturates may lower serum cholesterol levels. However, there is no evidence that the excess consumption of linoleic acid contributes to the prevention of heart disease (57). The coating of linoleic acid containing dairy feed stuffs with formaldehyde to produce a higher linoleic acid containing butterfat (58) or the incorporation of drugs into poultry feeds to depress cholesterol biosynthesis in a laying hen with the hope of producing egg yolks of lower cholesterol content (59) represents an interesting research approach. However, on a practical commercial basis, it would be far more economical to produce margarines and shortenings of higher linoleic acid content. A stick type margarine easily could contain at least 25% linoleic acid. Furthermore, as *trans*-fatty acids are not discriminated against completely by acyl-glycerol-3-phosphoryl-choline transferase or acyl CoA cholesteryl acyltransferase, it would be, from a biological viewpoint, advantageous to eliminate *trans*-fatty acids from both stick and tub type margarines. *Trans*-fatty acid free margarine base stocks could be fabricated from completely hydrogenated soybean oil and corn or cottonseed oil by a rearrangement process presently in use for the improvement of the shortening value of lard and which already is being used in The Netherlands, Germany, Sweden, and Finland for the production of a *trans*-free high PUFA margarine. The rearrangement process may add to the cost. However, in view of the need for an optimum level of the biologically functional ω6 series of fatty acids, it would seem judicious to eliminate the *trans*-type of unsaturated fatty acids from all dietary fats. It also would seem judicious to get away from the term all purpose shortening, to provide heat-stable frying fats devoid of mono- and diglycerides, to provide baking fats that do not contain methyl silicone, to provide salad oils that do not contain polymerized winterizing agents, and to provide emulsified food substitutes that contain less than 1% propylene glycol monostearate.

A great concern has been expressed in regard to the air we breathe, the water we drink, and the food additives we eat. It is also possible that the kind of fats we eat may be important to the complex and subtle biological process that cause the deposition of lipids into the arteries of all human beings. It is difficult to make diet recommendations in the face of a problem as complex as atherosclerosis. It is evident, however, that the popular habit of consuming high calorie snack items by someone who is already overweight will only add fat and cholesterol to the serum and tissue. However, dietary fats are essential as a sufficient intake of the ω6 series of fatty acids is necessary to prevent the elongation and incorporation into phospholipids and cholesterol esters of the *trans*-fatty acids or of the ω9 series of fatty acids that are synthesized from carbohydrates.

It is also necessary to provide an adequate protein intake, as carrier protein is necessary for the synthesis of lipoprotein. It is unfortunate that the shopper in a modern supermarket can purchase hundreds of food items that will satisfy caloric requirements but not the protein or other

nutrient requirements. It may be possible to satisfy protein requirements by the consumption of unrefined whole grain cereals, legumes, and leafy vegetables. However, in a high calorie diet, such as the American diet, only meat, milk, and eggs can supply a sufficient amount of protein for daily needs. These protein sources admittedly contain either saturated fats or cholesterol. Under normal conditions, the enzyme system that is available for the metabolism of lipids will metabolize the saturated fatty acids and cholesterol that is not needed for tissue maintenance. However, the influence of a dietary source of cholesterol upon human serum cholesterol levels, very low density serum lipoprotein levels, and heart disease needs further clarification. The use of crystalline cholesterol or powdered egg yolk in experimental diets to increase serum cholesterol levels is not analogous to the use of a whole egg which contains egg white protein in addition to egg yolk protein, polyunsaturated fats, and cholesterol.

The best that can be done at present for the majority of human subjects is for them to eat a well-balanced diet of cereals, fruits, vegetables and enough meat, milk, and eggs to provide for an adequate protein intake. Heart disease seems to be due to an exceedingly complex metabolic interrelationship. Yet, the rate of incidence may be decreased by eating a well-balanced diet, by providing biologically utilizable polyunsaturated fats, by taking enough exercise to provide for good muscle tone, by getting enough rest, and by avoiding cigarettes. Epidemiological studies indicate that the human subjects in population groups that follow such a regime have less heart disease than those who do not.

CHARGE TO THE EDIBLE FAT AND OIL INDUSTRY

The fat and oil industry should provide the leadership to more adequate funding of not only its own research laboratories but also of governmental and university laboratories. The present practice of spending large sums of money to provide a few percentage increase in sales over a competitor may be nonproductive, as the advertising costs could cancel out the additional money provided by an increase in sales. Furthermore, the added costs due to advertising are paid ultimately by the consumer. The new Food and Drug Administration labeling requirements may solve this problem for any consumer that will take the time to compare cost with the percentage composition on the label. It would serve both the industry and the public if the industry itself combined resources under the auspices of the AOCS and decided to solve common problems, such as the development of hydrogenators that do not use millions of cubic feet of natural gas every day as a source of hydrogen, the objectionable odors from deodorizers, and better means of disposing of bleaching earth. Methods should be developed to hydrogenate soybean oil by other means; objectionable odors could be removed with oxidizers, and bleaching clays can provide valuable by-products. Such an approach would provide for an acceptable public image and also may persuade the U.S. Congress and the Food and Drug Administration that the industry is genuinely interested in its own welfare.

The fat and oil industry should realize that a fundamental change in research funding has taken place within the grant-in-aid program at the National Institutes of Health. The emphasis is now on mission-oriented heart and cancer research, rather than on fundamental science. However, this emphasis should represent an addition of funds rather than a shifting of funds within the various Institutes of the National Institutes of Health. Millions of people still suffer from arthritis, as an example, and funds that are provided by the National Institute for Arthritis and Metabolism may provide as many clues to the cause of heart disease or cancer as funds that are provided by the National Heart and Lung Institute or the National Cancer Institute.

Both the National Research Council of the National Academy of Sciences and the Council of Nutrition of the American Medical Association recently have recommended a decrease in total fat consumption (60). Such a recommendation may not be nutritionally sound, as it may only increase the consumption of carbohydrate and, thus, the synthesis of $\omega 9$ fatty acids from the surfeit calories. It would be more economical to stay at our present percentage level of total fat intake and decrease the number of calories through less consumption of a well balanced diet.

Cancer and heart disease are complex diseases that will require a tremendous research effort for their solution. The fat and oil industry should become more actively involved in this effort for its own sake, as well as for the sake of the consumers of fabricated products of this industry. These products should be reexamined by means of the sophisticated techniques which have become available to lipid laboratories, and every avenue of their possible impact upon cancer and heart disease should be explored thoroughly.

We hope to increase our own efforts in this direction through an additional research facility. This facility, a commercial swine farm which has been made available to us on the basis of a 99 year lease by the Harlan E. Moore Heart Research Foundation, currently is housing 240 swine on 10 different diet patterns. Some of these groups are being fed a source of *trans*-fatty acids and should provide tissue for in vitro studies of the type currently in progress in De Kruyff's laboratory (61). An additional breeding facility will be available in the future. This breeding facility will allow the use of 800 purebred swine/year. The blood or tissue from these animals will be available on a no cost basis to any research group that is interested in such tissue.

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