Trans Fatty acids: Is margarine really better than butter?

Margo A. Denke, M.D.

Internal Medicine
Grand Rounds
March 23, 1995

Letterman's "Top Ten New Margarine Slogans"

#10--I can't believe it's not healthy.
#9 --Little pats of poison.
#8 --You can't spell margarine without angina. It's a deadly killer. Run. Run for your lives. It'll explode and kill ya'.
#7 --Pure chemical satisfaction.
#6 --Thought you were healthy? Well, guess again, Pepe.
#5 --For external use only.
#4 --Which are you gonna believe, boring laboratory studies or cool TV commercials?
#3 --Give us a week and we'll shut off your heart.
#2 --Elves ate it; why don't you?
And the #1 new margarine slogan--Mmm-mmm, dead.

In 1993, a report published in **Lancet** took the American public by surprise (1). US women who ate four or more teaspoons of margarine a day had more heart attacks than women who rarely ate margarine. The results of the publication received wide media coverage, including a piece with David Letterman's top ten (see cover). Consumers who had switched from butter to margarine as part of a "heart healthy" diet (2) became angry and confused. How could the deliberate changes they had made to reduce their risk for coronary heart disease put them at increased risk? This medicine grand rounds will review the evidence that ingestion of *trans* fatty acids is associated with an increased risk for coronary disease. To begin this review, we must understand how and why hydrogenated fats were introduced into the food supply.

background: the development of hydrogenated fats.

The type of edible fats harvested and prepared to be sold as a fat (so called "visible fat") have been influenced by changes in the relative cost of rendering fat from different sources, in the need for fats with specific qualities, and growing interest in the healthfulness of the American diet (3,4). In the early 1900's, the major fat available for commercial use was lard. Lard was easily and inexpensively rendered from pork fat. Lard had a good shelf life and excellent shortening properties. However, without the benefits of refrigeration, lard would become semi-solid at warm temperatures. Lard was also rich in saturated fat, and growing health concerns of the dangers of excess dietary saturated fat stimulated efforts to find an alternative source for fat.

Pressing seeds and vegetables could produce a liquid fat, but this oil lacked the stability and shortening properties of lard. The hydrogenation process was developed to modify liquid vegetable oils so these fats could be suitable substitutes for lard. The first hydrogenation products were a blend of totally hydrogenated cottonseed oil and refined cottonseed oil. This created a product that had the consistency of lard but was less likely to liquefy at warmer temperatures. The totally hydrogenated vegetable oil produced a waxy aftertaste. In addition, the product provided little health benefits, since cottonseed oil is as high in saturated fatty acids as lard (Table 1).

Table 1

FATTY ACID COMPOSITION OF COMMON FATS AND OILS RANKED BY SATURATED FATTY ACID CONTENT[1]

	S	M	P
Coconut	87	6	2
Palm Kernal	81	11	2
Butter Oil	62	29	4
Cocoa Butter	60	33	3
Beef Tallow	50	42	4
Palm	49	37	9
Lard	39	45	11
Menhaden Oil	34	32	30
Chicken fat	30	50	21
Cottonseed	26	18	52
Herring Oil	19	60	16
Peanut	17	46	32
Soybean	14	23	58
Olive	14	74	8
Corn	13	24	59
Safflower	9	12	75
Canola	7	56	33
Safflower-Hi Oleic	6	75	14

S = Total saturated fatty acids; the definition currently used by FDA.

M = Monounsaturated fatty acids

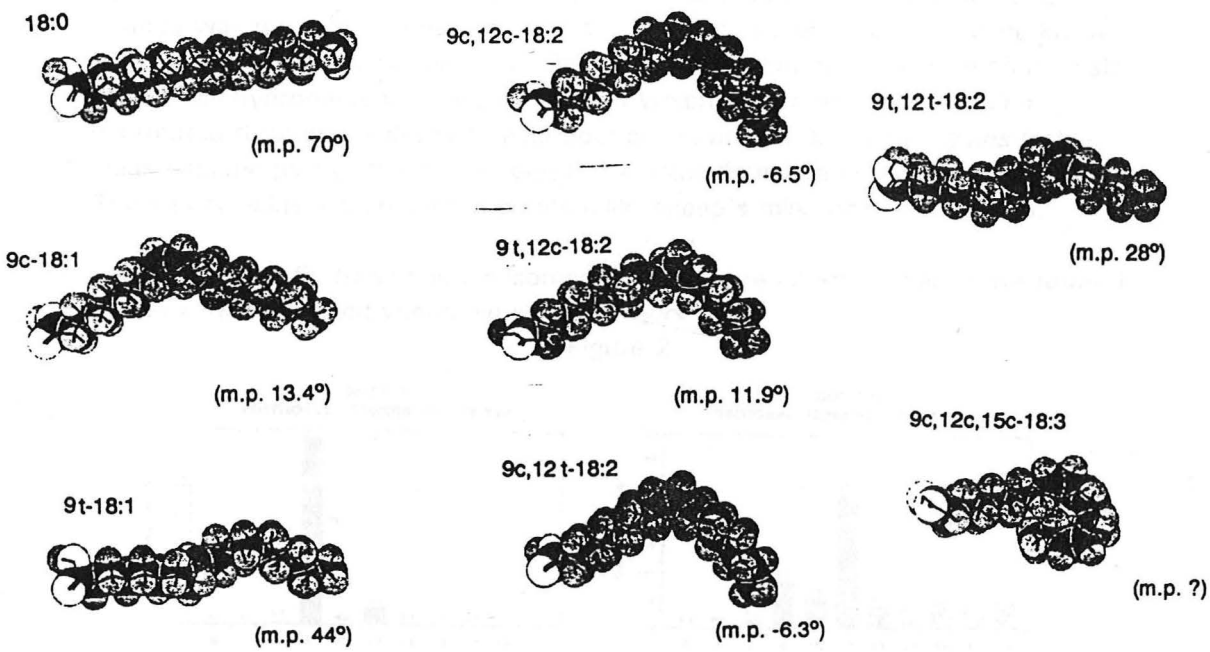
P = Polyunsaturated fatty acids

[1]*Source:* US Department of Agriculture: Composition of foods, "Fats and oils." Agriculture Handbook No. 8-4. Washington, DC, US Government Printing Office, 1979.

The technique of partial hydrogenation was developed in the 1930's and complemented the development of a high-yield, solvent-extraction method to render fat from vegetables and seeds. In the process of partial hydrogenation, using pressure, temperature, and a metal catalyst, hydrogen gas is bubbled through liquid vegetable oil. Under these conditions, the double bonds on monounsaturated and polyunsaturated fatty acids in the liquid oil are subject to modification. Three different modifications can occur: 1) A double bond can be changed to a single bond, e.g., changing a 2-polyunsaturated fatty acid into a monounsaturated fatty acid or a monounsaturated fatty acid into a saturated fatty acid, 2) the location of the double bond can be moved up or down the fatty acid chain, and/or 3) the configuration of the double bond can be changed to either *cis* or *trans* (5). Highly polyunsaturated fatty acids are most susceptible to the process of hydrogenation because they contain more double bonds than other fatty acids.

Changing the number, location, and type of double bonds in fatty acids changed the characteristics of the fat. Reducing the number of highly polyunsaturated fatty acids reduced the fat's susceptibility to oxidation, improving the shelf-life of the still liquid fat. Creating *trans*-monounsaturated fatty acids produced a fat with greater hardness because *trans* fatty acids have a melting point inbetween *cis*-monounsaturated fatty acids and saturated fatty acids (Figure 1).

FIGURE 1



Hence, partial hydrogenation provided the ability to take a vegetable oil and alter, in a highly controlled fashion, its stability, plasticity, mouth feel, melting point so the fat could be tailor made for a specific use. Partial hydrogenation minimally increased the saturated fat content of the oil; since the majority of vegetable oils have far less than half of the saturated fatty acid content of lard, partially hydrogenated vegetable oils were considered more healthful than lard. (Table 1).

The partial hydrogenation process was widely commercialized beginning in the 1940's and resulted in a marked change in the source of visible fat used for food manufacture, home cooking oil, and as spreadable fat in the United States (6). Before the introduction of hydrogenation, two-thirds of the per capita consumption of visible fat was from animal origin and about one-third was from vegetable origin; in the mid-1960's one-third was from animal origin and two-thirds was from vegetable origin. This change in primary sources of visible fat resulted in a significant increase in polyunsaturated fat and a decrease in the intake of saturated fat in the United States.

Besides reducing the saturated fat content of the American diet, changes in hydrogenated fat consumption increased the *trans* content of the diet. This change was not considered innately artificial, since *trans* fatty acids were known to be present in some animal fats. Specifically, bacteria that colonize an animals' rumen will hydrogenate dietary fatty acids which in turn are absorbed and distributed throughout the fatty acid pool of the animal (6). These *trans* fatty acids became part of the human diet if the animal's milk and meat are ingested. *Trans* fatty acids are present in cow's milk, sheep's milk, beef and mutton.

The specific *trans* and *cis* isomers in butter are different than those found in partially hydrogenated vegetable oil (7). (Figure 2)

Figure 2

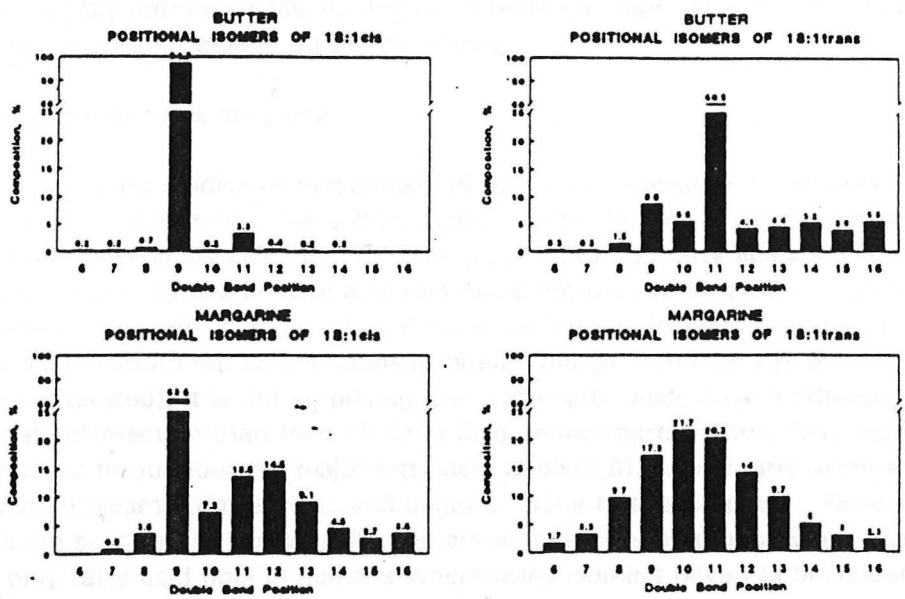


Figure 2 Distribution of positional isomers of the *cis*- and *trans*-octadecenoate fraction of butter and margarine. [Data for butter from Parodi (1976b); data for margarine from Sampugna et al. (1982).]

In butter, due to the specificity of ruminant bacterial enzymes, 11*t*-18:1 is the predominate *trans* isomer; 9t-16:1 is a shorter *trans* isomer present and 9 *c*-18:1 and 11*c*-18:1 are the only *cis* isomers found. In partially hydrogenated vegetable oils, the location of the *trans* bond forms a Gaussian distribution that centers around 10*t*- and 11*t*-18:1 with *cis* isomers occurring between the 7th and 16th carbon. Far fewer t16:1 isomers are present in vegetable oil because of its lower 16:1 and 16:2 content.

The percent of fatty acids that are *trans* fatty acids in fats of animal origin fats is relatively fixed (dairy fat is 3-4%; beef tallow 2-5%)(Table 2). The total *trans* fatty acid content of hydrogenated vegetable fats is quite variable, depending on the type of oil (i.e., requirements to remove fatty acids sensitive to oxidation), the specifics of the tailored hydrogenation process (e.g., desired hardness, plasticity, and shortening qualities)(8,9), and on the relative proportion of hydrogenated oil contained in the product (**Table 2**). For example, the *trans* fatty acid content of soybean stick margarine is 19-49% compared to soybean tub margarine at 11-28% and soybean cooking oil at 1-13%. The *trans* fatty acid content of foods that contain hydrogenated fats is even more variable. Specifically, whereas the percent *trans* in soybean stick margarine varies two-fold, the amount of *trans* in e.g., sugar cookies, may vary four-fold because of the source of the ingredient fat, its degree of hydrogenation, and the amount of hydrogenated fat contained in each serving.

Metabolism of trans fatty acids.

Based on studies of metabolism of deuterium-labeled and carbon-13 labeled 18:1 isomers in humans, *trans* fatty acids are metabolized in a similar manner to non-*trans* fatty acids (10,11). This suggests that *trans* fatty acids are recognized by the major enzymes in fatty acid metabolic pathways. Since the rates of oxidation, acylation, elongation, and desaturation are different for every class of fatty acids (saturated, *cis*-monounsaturated, Omega 3 and Omega 6 polyunsaturated), it is not surprising that *trans* fatty acids have a different affinity and rate of reaction than their *cis*-fatty acid counterparts. *Trans* fatty acids are distributed throughout the major fatty acid pools(3,5). *Trans* fatty acids are present in plasma triglycerides and adipose tissue fatty acid pools. *Trans* fatty acids are secreted in human milk, and are incorporated into phospholipid pools. The only fatty acid pool in humans where *trans* isomers have not been identified is the longer chain fatty acids (arachidonic acid and eicosanoids)(12).

In monkeys fed *trans* fatty acids for 6 months, *trans* fatty acids appeared in adipose tissue and phospholipid membranes fatty acids (13). If monkeys fed *trans* for six months were returned to chow diet and sampling was repeated six months later, no *trans* fatty acids were detected in these tissues. This suggests that *trans* fatty acids easily exchange between fatty acid pools and *trans* fatty acids can be completely catabolized.

TABLE 2
TRANS FATTY ACID CONTENT OF SELECTED MAJOR FOOD PRODUCT CATEGORIES

Product Category	gm *trans* per serving	average % *trans*18.1	Average % *trans* 18.2	Range % total *trans*
Animal Fat				
butter	0.40	2.9	0.3	2-7
Vegetable fat: Soybean				
Shortening, commercial	4.87	33.6	3.8	34-42
Shortening, household	2.54	14.5	4.1	3-30
Margarine: Stick	3.4	24.1	2.1	19-49
Margarine: tub	1.95	14.4	1.9	11-28
Cooking oil	1.67	8.0	2.8	1-13
Salad oil	0.48	0.9	0.7	0-5
Meats, lean, raw				
Beef	0.21	3.2	0.2	2-5
Pork	0.01	0.2	0	0.1-0.3
Chicken	0.04	0.9	0.3	0.7-1.4
Fast Foods				
Milk shake	0.20	2.0	0.3	2-4
Hamburger	0.53	3.6	0.3	3-5
French fries	1.83	18.7	1.4	3-34
Bakery				
Cookies, sugar	1.36	15.0	1.8	4-36
Cake, coffee	1.68	9.6	1.0	10-13
bread, commercial	0.16	6.8	1.22	0-32
Snacks				
Potato chips	1.38	10.0	1.7	0-40

Adapted from *trans* Fatty Acid Content of Selected Foods, J Am Oil Chem Soc (In preparation) and HNIS/USDA contract data for *trans* Acid Content of Foods.

Animal Models on the Effects of *Trans* Fatty Acids on Atherosclerosis

Animal models of diet-induced atherosclerosis have been developed to assess the extent of how changes in diet can alter atherogenesis (14). In most models, fatty acid feeding alone does not induce atherosclerosis unless dietary cholesterol is added to the chow. Some animal models are also influenced by the polyunsaturated fatty acid content of the diet. Whether these factors (dietary cholesterol, dietary linoleic acid) are equally important in human atherogenesis is unclear. There are reasons to suspect that human atherosclerosis may be preferentially responsive to different dietary factors than animal arthersclerosis. For example, in animal models, dietary cholesterol is a more potent cholesterol-raising nutrient than saturated fatty acids. In humans, saturated fatty acids are more potent cholesterol-raising nutrients than dietary cholesterol. Despite these differences, animal model systems of diet-included atherosclerosis provide valuable insight into a diet-CHD relationship.

The effects of *trans* fatty acids on atherosclerosis has been evaluated to a limited extent in animal models. Dr. Robert Nicolosi at the University of Lowell, Massachusetts (15), has recently reviewed this area and his review is presented in Table 3.

Dr. Nicolosi found a consistent effect of saturated fat feeding, in the presence of dietary cholesterol, on diet-induced atherogenesis. In cholesterol-fed rabbits, palm oil or coconut oil feeding produces more atherosclerosis than corn oil (16,17). More detailed fatty acid manipulations suggested that the higher linoleic acid and lower saturated fatty acid content of corn oil was responsible for this effect (18,19). Besides the rabbit model, saturated fatty acids have been shown to induce atherosclerosis in cholesterol-fed rhesus monkeys (20) and in cynomolgus monkeys (21). In a prolonged feeding study of African green monkeys, saturated fat feeding raised serum cholesterol levels, increased atherosclerotic lesion area, and resulted in more complex lesions which contained extracellular cholesterol crystals (22,23) than safflower oil feeding.

The consistent findings that saturated fatty acid feeding increases cholesterol levels and increases atherogenesis are in contrast to the inconsistent findings that *trans* fatty acids alter ather*ogenes*is. In rabbits, *trans* 18:1 feeding yielded significantly higher cholesterol levels than *cis* 18:1 feeding (24,25,26). However, no significant differences between the extent of atherosclerosis in the

Table 3
Effects of fatty acids on atherosclerosis in Animal Models

Study	Animal Model	Results
Saturated fatty acids		
Kritchevsky (13)	cholesterol-fed rabbits (n=9).	Serum cholesterol (mmol/L [mg/dL]) / Atheromata methyl-18:0 — 56.04 [2167] — 1.78 methyl 18:1 — 60.43 [2337] — 1.11 methyl-18:2n-6 — 66.67 [2578] — 0.94
Kritchevsky and Tepper (11)	cholesterol-fed rabbits (n=12 or 13 per group)	Serum cholesterol (mmol/L [mg/dL]) / Atheromata (Arch, Thoracic) coconut oil — 73.11 [2827] — 3.1, 2.5 Corn oil — 49.34 [1908] — 1.6, 1.3
Kritchevsky and Tepper (12)	cholesterol-fed rabbits (n=4-10 per group)	Serum cholesterol (mmol/L [mg/dL]) / Relative atherosclerosis coconut oil — 36.22 [1401] — 1.04 Cocoa butter — 44.12 [1706] — 0.84 Palm oil — 36.39 [1407] — 1.09 Corn oil — 43.39 [1678] — 0.69
Kritchevsky and Tepper (14)	cholesterol-fed rabbits (n=4-43 per group)	Serum cholesterol (mmol/L [mg/dL]) / Atherosclerosis (Arch, Thoracic) 12:0 — 54.40 [2104] — 1.98, 1.15 14:0 — 52.47 [2029] — 1.82, 1.24 16:0 — 52.55 [2032] — 2.07, 1.30 18:0 — 50.17 [1940] — 1.74, 1.08 Corn oil — 61.52 [2379] — 1.65, 1.10
Vesselinovitch (15)	cholesterol-fed rhesus monkeys (n=6).	Aortic atherosclerosis (% area, x±SD) / Coronary lesions (Frequency, Severity) / Lumen narrowing of coronary arteries (%, x±SD) butter — 82±3 — 53, 18 — 34±7 corn oil — 63±6 — 22, 8 — 11±4

Study	Animal Model	Results			
Anderson et al (16)	cholesterol-fed cynomolgus monkeys (n=6)		Plasma cholesterol (mmol/L[mg/dL])	Thoracic aorta thickness (μm)	Coronary arteries Intimal thickness (μm)
		butter,olive oil,corn oil,peanut oil			
		10%, 7%, 3%, 0%	7 [270]	77±15	15± 8
		8%, 5%, 2%, 5%	5 [190]	69± 9	4± 3
		6%, 3%, 2%, 10%	8 [310]	55± 8	17±11
		0%, 0%, 0%, 20%	4 [150]	44± 7	3± 2
Wolfe et al (17) and Parkes et al (18)	African Green monkeys. (n=16-25 per group)		Plasma cholesterol (mmol/L [mg/dL])	Incidence	Intimal area (mm^2 x 103)
		lard	8.30 [321]	57%	45
		safflower oil	7.75 [261]	20%	15
		menhaden oil	5.97 [231]	--	10
Trans fatty acids					
Weigensberg et al (19), McMillan et al (20), and Weigensberg and McMillan (21)	cholesterol-fed rabbits (n=20-36 per group)		Serum cholesterol (mmol/L [mg/dL])		Aortic lesions (%)
		9t-18:1	99.04±6.26 [3830±242]		37±5
		18:1	62.40±5.82 [2413±225]		32±5
		corn oil	21.67±3.93 [838±152]		10±3
		9t-18:1-treated olive oil	63.25±6.36 [2446±246]		30±4
		olive oil	37.16±4.16 [1437±161]		23±5
		9t-18:1-treated 18:2n-6	39.51±4.19 [1528±162]		33±8
		18:2n-6	36.54±5.22 [1413±202]		21±6
		fat free	25.21±3.44 [975±133]		28±7

Study	Animal Model	Results
Rutenberg et al (22)	atherosclerosis in rabbits (n=8 per group)	Plasma lipids (mmol/L[mg/dL]): Cholesterol / Triglycerides; Atherosclerosis: Arch / Thoracic 6.0% t-18:1: 3.54±0.03 [137±52] / 0.78±0.01 [69±1] / 0.2±0.1 / 0 3.2% t-18:1: 2.25±0.47 [87±18] / 0.78±0.01 [69±1] / 0.2±0.1 / 0.1±0.1 control: 1.86±0.26 [72±10] / 0.84±0.02 [74±2] / 0.2±0.1 / 0
Mortensen (23)	cholesterol-fed rabbits (n=15 per group)	Plasma cholesterol (mmol/L) / Thoracic aorta cholesterol (nmol/mg wet wt) / Atherosclerosis lesion (%) olive oil: 23±1.1 / 11.4±1.0 / 14 margarine: 21±0.4 / 12.6±1.4 / 15 high cholesterol diet: 20±0.1 / 16.6±1.6 / 12
Toda et al (24)	swine (n=8-10 per group)	Incidence / Average thickness (x10mm^2) hydrogenated soybean oil: 21/74 / 2.5±0.2 high oleic soybean oil: 27/63 / 4.2±0.4 lard: 28/84 / 3.6±0.2
Kritchevsky et al (25)	vervet monkeys (n=7-8 per group)	Serum cholesterol (mmol/L[mg/dL]) / Atherosclerosis: Incidence / % of surface 3% t-18:1 fed 12 mo: 3.46±0.31 [134±12] / 6/8 / 2.5±1.3 Reverted to control after 6 mo: 4.34±0.44 [168±17] / 6/8 / 5.1±2.6 6.0% t-18:1 fed 12 mo: 4.22±0.34 [163±13] / 5/8 / 5.3±4.0 Reverted to control after 6 mo: 3.78±0.13 [146±5] / 6/7 / 3.0±1.0 Control: 4.29±0.21 [166±8] / 12/15 / 6.6±2.4

two diet groups was observed (24,25,26). In a study where less cholesterol was added to the feed, despite a relatively higher cholesterol level on the *trans* diet, no differences in the extent of atherosclerosis was seen (27). In a comparison study of olive oil vs margarine vs high cholesterol diet, all feedings gave a similar extent of atherosclerosis (28). In a monkey model, feeding 3%t18:1 for 12 months resulted in no different incidence of atherosclerosis than 6% t18:1 or a control diet (13).

Dr. Nicolosi concluded in his review (15) that the inconsistent nature of the association between *trans* feeding and resultant atherosclerosis suggests that *trans* fatty acids do not promote atherogenesis. The findings also suggest that other factors altered in the dietary design may be important in atherogenesis. A detailed analysis of studies of diet-induced atherogenesis using the swine model can help illustrate these inconsistencies (Table 4).

In the first study by Kumerow et al (29), 120 swine were divided into 10 different feeding groups. After 8 months, the animals were sacrificed and the degree of atherosclerosis was quantitated as a percent coverage of the thoracic and abdominal aorta. Butter feeding resulted in higher cholesterol levels and higher grade thoracic and abdominal atherosclerosis. However, a *trans* fat feeding of 50% trans fatty acids and no essential fatty acids achieved higher cholesterol levels and worse atherosclerosis. The study was interpreted by some that *trans* fat was as bad as butter; however, the *trans* fat used was decidedly uncharacteristic of hydrogenated fat prepared for human consumption. In the same experiment, discarded frying oil (20% *trans*), although achieving higher cholesterol levels than butter feeding, did not achieve more atherosclerosis than the basal diet.

In a study by Rowsell et al (30), 39 swine were divided into 3 feeding groups and fed 3-9 months a diet rich in butter, margarine, or fat-free chow. No differences in serum cholesterol levels were seen; however, the butter feeding resulted in a much higher grade of atherosclerosis than the margarine or basal diet. Atherosclerosis observed on the margarine diet was no different than that of the basal diet, suggesting that margarine feeding did not increase atherogenesis.

In a study by Jackson et al (31), 80 swine were divided into 4 feeding groups and fed 6 months a diet rich in either a) discarded frying oil (hydrogenated fat 13% *trans*) b) discarded frying oil plus cholesterol, or c) beef tallow, and compared this with the basal diet. Whereas the hydrogenated fat feeding

achieved higher cholesterol levels than the beef tallow diet, less atherosclerosis was observed. The addition of dietary cholesterol to the hydrogenated fat further increased the cholesterol levels, and resulted in more atherosclerotic lesions; however the magnitude of atherosclerosis on the hydrogenated fat plus cholesterol was not as great as that observed on the beef tallow diet.

In a recent study by Toda et al (32), 28 swine were divided into 3 feeding groups and fed either a) hydrogenated soybean oil (29% *trans*), b) lard, or c) hi-oleic safflower oil. The hydrogenated oil achieved lower cholesterol levels and less coronary atherosclerosis than either the lard or hi-oleic safflower oil diets.

Table 4

Kumerow et al	120 swine fed 8 months, sacrificed		Cholesterol	Grade of Atherosclerosis (%) Thoracic	Abdominal
		butter	120	9	7
		beef tallow	124	4	3
		corn oil	104	2	5
		isomerized soy + cottonseed (0% *trans*)	125	2	5
		trans fry fat (50% *trans*)(no essential fatty acids)	138	9	10
		used fat + sugar (*trans* 20%)	131	4	8
		cholesterol	93	4	4
		egg yolk	98	5	4
		whole egg	112	3	5
		basal	95	4	5
Rowsell et al	39 swine fed 3 months, sacrificed		Cholesterol	Grade of Atherosclerosis (Aorta, carotid, renal, coronary)	
		butter	87	30	
		margarine	86	11	
		basal diet (no fat)	92	9	
Jackson et al	80 swine fed 6 months, sacrificed diets cholesterol free except when cholesterol added		Cholesterol Mg/dL	% involvement Abdominal aorta	Thoracic aorta
		used hydrogenated soybean oil (13% *trans*)	124	1.32 ± 0.67	0.18 ± 0.03
		Used hydrogenated soybean oil +0.4% cholesterol	149	2.17 ± 0.98	0.42 ± .12
		beef tallow	119	3.22 ± 0.67	1.24 ± 0.09
		basal	99	1.26 ± 0.78	0.44 ± 0.12
Toda et al	28 swine fed 4 months, sacrificed		Cholesterol mg/dL	Coronary artery intinal thickness	
		hydrogenated soybean (29% *trans*)	113	2.5 ± 0.2	
		Hi oleic safflower oil	117	4.2 ± 0.2	
		lard	134	3.5 ± 0.2	

These four studies suggest that, even if hydrogenated fat feeding in swine raises cholesterol levels above a basal diet, the extent of atherosclerosis does not parallel the magnitude of the hypercholesterolemia. In all four studies, *trans* fatty acid feeding, as long as fed with essential fatty acids, produced less atherosclerosis than butter, lard or beef tallow.

Does ingestion of hydrogenated fats, or specifically *trans* fatty acids, increase the incidence of coronary heart disease in humans?

Unlike animal model systems, where factors involved in diet-induced atherosclerosis can be directly tested, in humans, diet relationships must rely in part on inference. Atherosclerosis is a process that takes 20-40 years to manifest (34). Data compiled in nutritional epidemiology studies can evaluate long-term associations but data from metabolic diet studies and clinical intervention trials can only test relatively short-term cause-effect relationships. It was findings from observational studies that raised public concern about the health effects of *trans* fatty acids.

Nutritional epidemiology can contribute valuable information concerning possible diet-disease associations. However, observations do not establish causality (33). Nonetheless, associations uncovered in observational studies have provided hypotheses that can be tested in other research.

Nutritional epidemiology looks for associations between two factors, diet and disease. The study population can be either a cohort or a case-control design. The evaluation can be prospective or retrospective. The evaluation depends on two estimates: an estimate of the prevalence of disease, and an estimate of the intake of the dietary factor.

Estimating disease rates.

"Hard" disease endpoints such as death or heart attack, while definitive, are the end result of a process that took years to develop (34). This time delay makes disease relationships difficult to test in a prospective manner during the life span of the investigator. There are several different ways to circumvent this problem. One is to establish methods to detect occult or early disease (so called "soft" endpoints) such as history of angina like chest pain (35), positive exercise tolerance test (36), or lesions seen on coronary angiography(37). Another is to establish whether the presence of certain factors can be associated with the

disease (38). The concept of "risk factors," factors which are both associated with and precede the development of disease, has revolutionized hypothesis testing for diet-CHD relationships (39). Changes in risk factors by diet can allude to expected changes in disease rates (40). In additions, risk factors can suggest plausible mechanisms by which disease processes become manifest. These mechanisms can be readily tested in animal models of human disease, where in a relatively short period of time (6 months to 8 years, depending on the model), a diet → mechanism → disease relationship can be evaluated using hard endpoints of disease: pathologic analysis of atherosclerotic plaque (14).

Nutritional epidemiologic studies vary in the certainty of their endpoints; studies basing conclusions on "hard" endpoints of disease are more certain than studies basing conclusions on "soft" endpoints such as risk factors that are associated with the disease but explain only part of the risk for disease.

Estimating dietary intake.

There are several ways to estimate dietary intake for populations, and these methods have varying degrees of precision. The most crude method of estimating dietary intake for nations is to use "food balance" data collected by the United Nations Food and Agriculture Organization (FAO)(41). In this method, dietary intake for a specific category of food is calculated by adding the annual amount produced to the annual amount imported, subtracting estimated losses from farm waste, and dividing by a mid-year estimate of the population. The result is a per capita estimate of intake. The FAO tabulates butter and margarine availability, but no data on the availability of hydrogenated fats is routinely collected.

A less crude estimate of population intake can be made by tracking the disappearance rate of commodities packaged for consumer use. Such data is collected on an annual basis by the USDA (42); the advantage of disappearance data over food balance data is the figures address what the consumer purchases. However neither food method estimate is synonymous with consumption. Neither method accounts for food losses from cooking, discarded food, and non-nutritive uses of food commodities. In addition, neither method accounts for individual differences in food consumption.

To account for variability in individual consumption, individuals must be directly queried (43). This is a far more expensive method than food balance or disappearance. Three methods are commonly employed: a written food diary, 24 hour recall, or food frequency questionnaire. All three methods assume that the

subject is reporting dietary intake accurately; unfortunately inaccuracies in reporting may be substantial (44). Each of the three methods for individual reporting has advantages and disadvantages (45). Food records obtain the most detailed intake (e.g., participant can specify brand name of product, preparation technique and specific portion size). However, records require that the subject receives instructions on how to complete the record; ambiguous entries require timely follow up to clarify actual intake. Because of their detail, food records provide the best self-recorded estimates of dietary intake. A 24 hour recall is where the subject is prompted to recall everything eaten during the last 24 hrs. This recall can be accomplished during a telephone interview and requires no instructions to the participant; the interviewer must receive extensive training to query for portion sizes, sauces and preparation methods. While the 24 hour recall method may be a poor estimate of the average intake for seasonal foods and for holiday, weekend vs weekday eating, the 24 hour recall data provides data on typical meal composition. Several 24 hour recalls can approach the accuracy of a food diary. Food frequency questionnaires is where the subject is asked to check off the frequency of consumption for a specific portion size of a common food consumed during the past year. The questionnaire form can be optically scanned and data can be directly entered into a computer database. Questionnaires can be designed to query food categories containing the nutrient of interest (e.g., calcium); however, the accuracy of this method is subject to question since the participant is asked to estimate the intake in ways other than how foods are eaten. For example, participants are asked to report how frequently they eat corn, broccoli, carrots, and green beans. The participant may only eat corn when eating beef, and broccoli when eating fish; however beef and fish are in different sections of the questionnaire. In addition, the participant may be influenced by public health messages (what I should eat) when filling out the questionnaire rather than just relying on his own food preferences(what I do eat). The food frequency questionnaire requires reading, writing and calculation skills. Food frequency results correlate with food records for macronutrients, with a typical $r \approx 0.5$.

After the data of food intake is obtained, this intake must be translated into nutrient intake. The most comprehensive database is the USDA Handbook 8(46), where key nutrient content of a specific brand, portion size, and method of preparation are listed. While most agree that Handbook 8 data are the best available, the variability inherent in the nutrient content of food must be underscored. For example, the fat content for common cuts of meat depends not only on trim cut, cooking method, grade of meat, but also on characteristics of the individual piece of meat, such as the thickness of trim and intramuscular fat, or

"marbling". The vitamin content of fruits is variable with growing season and the ripening of the fruit. The selenium content and phytosterol content of grains varies with where the crop was grown and the characteristics of the growing season (rain, temperature, length of season, etc.). Even if a software package uses Handbook 8 data for analyzing dietary intake, errors from missing data are common. For example, if the saturated fat content of a particular brand of ice cream is unknown, some software packages enter "0" instead of an average value for that grade of ice cream. The *trans* content of foods is not reported in Handbook 8 because no large database exists that tabulates the *trans* fatty acid content of specific products. Since manufacturers frequently change the specifications of their hydrogenation requirements a usable database on *trans* must have measurement data during the same time period that the intake data was collected.

A perhaps more objective way to evaluate dietary intake in humans would be to measure the content of a marker in the body that correlated with intake (47). The nutrient itself could serve as a marker if it was not synthesized by the body, and its content in an accessible tissue was in steady state with the amount ingested. In this situation, measurement of the nutrient content of tissue would serve as a surrogate for diet intake. Several nutrients, such as *trans fatty* acids and linoleic acid, may fit these criteria. It is well established that the adipose tissue content of linoleic acid is correlated with dietary intake (48). Such an objective measurement might seem superior to estimating intake from self-reports. However, the use of markers may provide no better measure of intake than the use of self reports.

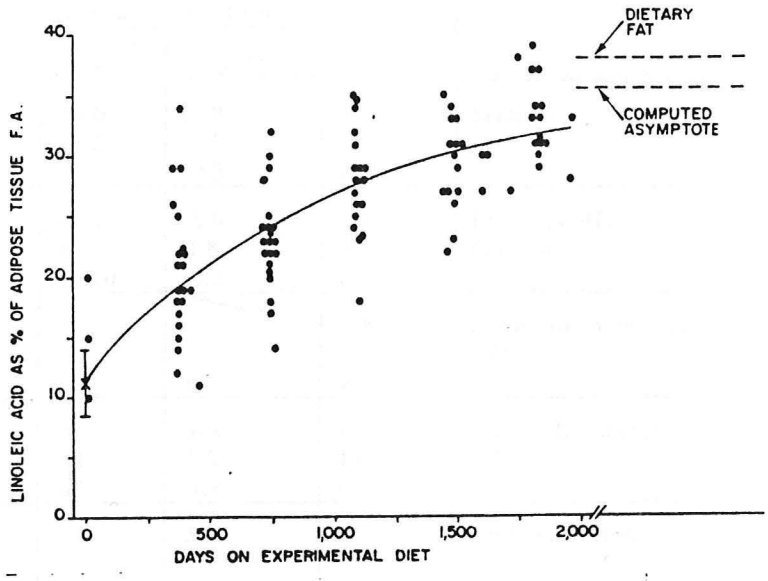
Specifically, the adipose tissue content of linoleic acid as a marker for long term dietary intake was investigated in the VA Domiciliary Study (49). In this study, 846 men who domiciled in a Veteran's Hospital in Los Angeles, were randomized to either a high-saturated, low-polyunsaturated fat diet or a low-saturated, high-polyunsaturated fat diet. As expected, the low saturated fat diet resulted in significant and sustained reduction in serum cholesterol level. The incidence of stroke and CHD events were significantly lower in the diet treatment group. Nineteen subjects had their adipose tissue fatty acids measured both before diet initiation and after one year on the diet (50). The individual variability of these subjects in their incorporation of dietary 18:2 into adipose 18:2 should be noted. Although adherence was correlated when adipose tissue 18:2 content ($r = .20$), initial weight and change in weight were more highly correlated ($r = -0.58$ and $+0.54$, respectively).

TABLE 5

INCREMENT IN ADIPOSE TISSUE LINOLEIC ACID IN FIRST YEAR, RELATED TO OTHER VARIABLES

Subject No.	1 Yr Change in Adipose Tissue 18:2 (ΔL) % of total fatty acid	Adherence in 1st Yr, (A)	Initial Weight, (IW)	Weight Change in 1st Yr, (ΔIW)
		%	lb	lb
1	−3	71	188	−19
2	−1	72	173	+3
3	+5	61	140	−4
4	+5	93	172	−5
5	+5	82	180	−3
6	+6	60	189	−12
7	+7	44	162	+5
8	+9	88	182	+4
9	+10	60	157	+5
10	+10	93	154	+3
11	+12	87	116	0
12	+12	92	177	+11
13	+13	67	140	+8
14	+13	80	103	0
15	+14	80	143	+16
16	+14	85	166	−7
17	+15	80	128	−10
18	+17	58	145	+7
19	+20	86	147	+10
Coefficient of correlation with change in adipose tissue 18:2		+0.20	−0.58	+0.54

FIGURE 3



However, for populations, adipose tissue 18:2 levels can reflect intake. This is easily illustrated by data from 96 adipose tissue biopsies in the VA Domicillary Study. As time on diet increased, the spread of values for 18:2 content of adipose tissue lessened.

All of these methods have been used to estimate the total intake of *trans* fatty acid isomers in the US diet **(Table 6)**(1,51-59). Estimations of *trans* fatty acids in the US diet based on either availability of hydrogenated fats or disappearance data give the highest estimate of 8.1-12.8 g/person/d. Food frequency questionnaire data give the lowest estimate of 2.8 - 4.6 g/person/d. The accuracy of the food frequency method was tested in a separate case-control study of the relationship between dietary intake and breast cancer. In that study, the food frequency questionnaire estimate of trans fatty acid intake was 5.8% of total fat. Adipose tissue aspirates contained a trans isomer content of 4.4% of total fat. The correlation between the food frequency questionnaire estimate of intake vs the content of trans in adipose tissue was r = 0.51. Data based on

Table 6
Estimated per capita consumption of dietary *trans* fatty acids in the United States

	Total *trans* Fatty Acids				
Basis for Estimate	Fat Source	Total Diet Fat (g/day)	Percent of Total Fat Average	Intake, g/day Average	Reference
Disappearance data	Vegetable oil Animal and Dairy Total diet	58.0 41.5 99.5	13.8 5.3 10.3	8.0 2.1 10.2	Senti (1)
Availability data	Vegetable oil Animal and Dairy Total diet	62.9 62.1 125.0	18.6 1.8 10.3	11.7 1.1 12.8	Enig et al (41) (Table 4)
Availability data	Vegetable oil Animal and Dairy Total diet	 nd	 nd	6.8 1.3 8.1	Hunter and Applewhite (40)
Analysis of formulated diets from normal foods, n=5	Vegetable oil Animal and Dairy Total diet	nd nd 83	10.8 1.0 11.8	8.9 0.8 9.7	Craig-Schmidt et al (42)
Analysis of self-selected diets: nursing mothers, n=11	Total diet	54	5.0	2.7	Aitchison et al (43)
Diet records: 8 adolescent females	Total diet	58.8	5.3 ± 0.44	2.8 ±0.26	Van den Reek et al (44)
Duplicate diet analysis: adolescent females, n=8	Total diet	52.3	5.3 ± 0.36	2.6 ±0.22	Van den Reek et al (44)
Food-frequency questionnaire, n= 115F	Total diet	63.5	4.8 ± 1.6	2.8 ±1.6	London et al (45)
Food-frequency questionnaire, n= 748M	Total diet	62.6	5.5	3.4 ±1.2	Troisi et al (46)
Food-frequency data, n= 220M/62F[a]	Total diet	90	4.2	3.8 ±2.0	Ascherio et al (47)
Food-frequency data, n= 187M/52F[b]	Total diet	101	4.6	4.6 ±2.6	Ascherio et al (47)
Adipose data:n=115F [c]	Total diet	63.5	8.8 ± 2.6	5.6 ±1.7	Calc from data in London et al (45)
Adipose data:n=76M[c]	Total diet	105.0	7.2 ± 2.0	7.6 ±2.1	Calc from data in Hudgins et al (48)

[a] Subjects without clinical evidence for coronary heart disease.
[b] Subjects with clinical evidence of myocardial infarction.
[c] For calculation of dietary *trans*, % *trans* in adipose was multiplied by 2.0.
Adipose data used: 4.4 ± 1.1% *trans* (26) and 3.6 ± 1.0% *trans* (total *trans* - 18:2 conjugated diene) (38).

other studies quantitating the *trans* fatty acid content of adipose tissue yield an estimate somewhere inbetween the food frequency questionnaire method and the food disappearance method at 5.6 - 7.6 g/person/d.

Results from Nutritional Epidemilogy
Food frequency questionnaire estimates of *trans* intake are associated with CHD.

In 1980, participants in the Nurse's Health Study filled out a 61-food food frequency questionnaire (1). The 61 foods were selected to allow for a maximum discrimination among intakes of fatty acids in the diet. Participants were asked, given a specific portion size, how often this food was consumed by checking one of nine frequencies ranging from "never" to "six or more times per day." Based on an average content of trans fatty acids for the serving size, multiplied by the frequency of ingestion, a total *trans* fatty acid intake was estimated to be 4.0 ± 1.9 gm or 5.8 ± 1.8% of total dietary fat. Women were also asked to respond yes or no to whether their margarine intake had greatly changed in the previous 10 years; 81% of women answered "no".

In 1988, the association between quintiles of *trans* fatty acid intake and 8-yr follow up of the development of coronary disease was analyzed using the risk ratio method. After adjustment for age alone, an increase in relative risk was seen between the first and fifth quintile ($p = 0.002$ for trend), but the 95th percentile confidence intervals of the five quintiles overlapped and a graded effect of intake vs risk was not seen. After the data were adjusted for other risk factors, including the intake of other fatty acids, a significant difference was found between the fifth and first quintile of trans fatty acid intake. Women consuming an average of 5.7gm *trans* fatty acids a day had a risk ratio of 1.57 compared to women consuming an average of 2.4 gm/d (arbitrarily assigned a RR 1.00). (Table 7).

Among the 69,181 women who reported no change in margarine intake in the last 10 years, there were 356 cases of coronary disease. The data were further analyzed with respect to the *trans* intakes from vegetable fats vs the *trans* intake from animal fats. No association was seen between the intake of *trans* from animal fats and CHD (p for trend = 0.23),but a significant relationship was seen between the intake of *trans* from vegetable fats and CHD (p for trend = 0.009). (Table 8)

TABLE 7
ALL HEALTHY NURSES

RISK RATIO FOR QUINTILES OF *TRANS* INTAKE
(95% confidence intervals in parentheses)

Adjustments	2.4 g	3.2 g	3.9 g	4.5 g	5.7 g
Age only*	1.0	1.15 (0.85-1.56)	1.03 (0.74-1.42)	1.16 (0.85-1.59)	1.50 (1.12-2.00)
+CHD risk* factor	1.0	1.12 (0.82-1.52)	0.97 (0.71-1.36)	1.12 (0.82-1.54)	1.35 (1.00-1.82)
++diet*	1.0	1.15 (0.83-1.59)	1.03 (0.72-1.48)	1.22 (0.83-1.78)	1.57 (1.05-2.34)

*all p for trend <0.01

Table 8

ONLY NURSES REPORTING NO CHANGE IN MARGARINE INTAKE

ADJUSTED RISK RATIO FOR QUINTILE OF *TRANS* INTAKE
AFTER ADJUSTMENT FOR STANDARD CHD RISK FACTORS, DIET AND VITAMIN USE
(95 % confidence intervals in parentheses)

Total *trans**	1.0	1.23 (0.50-1.79)	1.11 (0.79-1.68)	1.36 (0.89-2.09)	1.67 (1.05-2.66)
Vegetable *trans**	1.0	1.43 (1.00-2.04)	1.11 (0.74-1.66)	1.39 (0.41-2.13)	1.78 (1.12-2.83)
Animal *trans*	1.0	0.76 (0.51-1.12)	0.69 (0.43-1.10)	0.55 (0.31-0.96)	0.59 (0.30-1.17)

*p for trend <0.01

Concerning specific foods, women who ate four or more teaspoons of margarine a day had a relative risk of 1.66 for having CHD than women who ate margarine less than once per month. Similar, significant relationships were seen with cookies and white bread, two other foods included in the food frequency questionnaire that contained *trans*. The authors concluded that ingestion of partially hydrogenated vegetable oils may contribute to the occurrence of CHD.

Using a similar food frequency questionnaire, data from two case control studies also evaluated the association between *trans* fatty acid intake and CHD. It should be noted that both studies asked participants to complete food frequency questionnaires **after** the diagnosis of CHD was made. Because these analyses are retrospective, their relative strength is less than that of the Nurse's Health Study, since knowing one's diagnosis may alter one's perception of intake.

In a study by Tzonou et al (60), 329 patients with EKG or angiographic evidence for CHD were compared to 570 control patients who were hospitalized for other reasons. All subjects were asked to fill out a 110 item food frequency questionnaire while in the hospital; they were asked to report their typical diet **before** the diagnosis of CHD was made. A specific analysis relating *trans* fatty acid intake was not performed because a database of the *trans* content of Greek foods was not available. Total fat appeared to be associated with a higher risk for CHD, with the fifth quintile having a RR of 2.11 compared to the first quintile (not significantly different, with a 95% confidence interval of 0.87-5.17). Saturated, monounsaturated and polyunsaturated fatty acid intake all appeared to be associated with CHD, but no results achieved statistical significance. Subjects who answered "margarine" as their principal cooking fat had a higher risk for CHD RR = 1.87 (95% confidence interval 0.82-4.28) than those who did not answer margarine. The authors concluded that cooking with margarine was harmful, although this conclusion was not supported by statistically significant associations.

In a study by Ascherio et al (58), a food frequency questionnaire was administered to 521 men and women who had a recent myocardial infarction compared to 197 matched healthy controls. A 116-item food frequency questionnaire was administered approximately 8 weeks after discharge from the hospital. As in the Willet et al study, quintiles of intake of trans fatty acids were calculated and quintiles of intake for the control group were determined. The trans fatty acid intake of the cases was then compared to the control group, and a RR was calculated for each of the quintiles. Comparing the fifth quintile of *trans*

Table 9

RISK RATIO FOR QUINTILES OF *TRANS* INTAKE (g/d)
(95% confidence intervals in parentheses)

	1.69	2.48	3.35	4.52	8.51
Age + Sex Adjusted	1.0	1.0 (0.56,1.79)	0.67 (0.36,1.24)	1.12 (0.63,2.0)	2.44 (1.42,4.19)
Adjusted for CHD risk factors	1.0	0.89 (0.48,1.65)	0.52 (0.26,1.02)	0.83 (0.50,1.75)	2.28 (1.27,4.10)
Adjusted for CHD risk factors + diet	1.0	0.81 (0.42,1.57)	0.40 (0.19,0.83)	0.72 (0.36,1.48)	2.03 (0.98,4.22)

p for trend <0.0001

Table 10

QUINTILES OF *TRANS* INTAKE ACCORDING TO SOURCE

Vegetable *Trans*	0.84	1.56	2.33	3.34	5.04
Adjusted RR	1.0	0.74 (0.38,1.46)	0.43 (0.21,0.90)	0.63 (0.30,1.32)	1.94 (0.93,4.04)

p for trend <0.0001

Animal *Trans*	0.45	0.69	0.98	1.24	1.79
Adjusted RR	1.0	1.17 (0.59,2.34)	1.12 (0.53,2.34)	1.00 (0.45,2.21)	1.02 (0.43,2.41)

p for trend $=0.57$

fatty acid intake with the first quintile, the RR was 2.44 (95th confidence intervals of 1.42 and 4.19) with a p for trend <0.0001. After adjustment for multiple known computed risk factors, the RR 2.03 (95% confidence intervals of 0.98 and 4.22), p for trend <0.0001. (Table 9)

As in the Nurses' Health Study(1), Ascherio performed a subanalysis testing if this association of *trans* isomer intake with CHD was different for vegetable sources of *trans* than animals sources of *trans.* The positive relationship between *trans* intake and risk was highly significant by trend for vegetable sources but not significant by trend for animal sources. The authors concluded that their data supported the hypothesis that hydrogenated vegetable oils contribute to the risk for coronary disease (Table 10)

Adipose tissue estimates of trans fatty acids are not associated with CHD.

Seven case control studies have evaluated if *trans* fatty acids are related to coronary disease by comparing the adipose tissue *trans* fatty acid content of cases with that of controls. None of these studies have found an association between the *trans* content of adipose tissue and the incidence of CHD.

In the first study of this kind, Heckers, Korner, Tuschen and Melcher (61) evaluated if differences in *trans* fatty acid content of myocardium, jejunum, and aorta were present in men who died from coronary disease compared to men who died from other causes. No differences in the *trans* fatty acid content of these tissues were found, suggesting that, if the fatty acid content of these tissues reflected dietary intake, no relationship existed. Heckers et al also analyzed the adipose tissue fatty acid content; although never published, his paper alluded to the lack of an association between the presence of *trans* isomers and the presence of coronary disease.

The analysis of Hecker et al was followed up by several studies in Europe in the 1980's which were specifically designed to reinvestigate the relationship between hydrogenated marine oils and coronary disease. Unlike in the United States, it was common practice in Europe to use marine oils such as Menhaden Oil and Herring Oil in margarines. These oils, although having moderate amounts of polyunsaturated fatty acids, must be heavily hydrogenated for use in margarines creating a wide variety of unusual *trans* fatty acids. Thomas and his group felt that hydrogenated marine oils could be identified by the presence of a 16:1 *trans* isomer. When these investigators analyzed the specific fatty acid content of over

100 brands of hydrogenated fats, t16:1 was present as 0.1-0.2% of the fatty acids in margarines containing hydrogenated marine oils, but was not present in margarines containing hydrogenated vegetable oils (62). Thomas et al found that the presence of t16:1 in adipose tissue was associated with the presence of coronary disease in three different case control studies (63,64,65) (Table 11). The authors concluded that ingestion of hydrogenated fish oils, although providing more polyunsaturated fatty acids than traditional shortening, was associated with an increased risk for coronary disease. It is important to note that Thomas's studies found no relationship between the t18:1 content of adipose tissue and coronary disease, thus finding no association between hydrogenated vegetable oils and coronary disease. In addition, it is important to note that, as expected from the fatty acid content analysis of hydrogenated marine oil margarine, the t16:1 content of adipose tissue was quite small and subject to measurement error.

The issue of the *trans* fatty acid content of adipose tissue and coronary disease has recently been reinvestigated in the EURAMIC study (66). The EURAMIC study is a large, multi-national, case-control study evaluating the relationship between adipose tissue fatty acids and coronary disease. The t16:1 fatty acid level was below detection limits in most of the adipose samples, so a follow-up evaluation of Dr. Thomas's findings could not be done. Instead, the analysis was focussed on the t18:1 content of tissue. The t18:1 level was found to vary dramatically between nations with the lowest levels found among the Spanish nations. Even when evaluating the data without the nations with low *trans* content, no relationship between *trans* fatty acid content of adipose tissue and coronary disease could be seen (Table 11).

These results are further bolstered by another European study (67) comparing adipose tissue from men dying a sudden death from coronary disease vs healthy, living controls. In this study, the unadjusted RR declined with increasing *trans* fatty acid content of adipose tissue (p for trend = 0.03). However, after adjustment for other CHD risk factors, the trend, while in a similar direction, was no longer statistically significant.

In summary, no studies have found a difference in the t18:1 fatty acid content of adipose tissue from cases with CHD to controls. If indeed the adipose tissue content of t18:1 is a valid marker for hydrogenated vegetable oil intake, these studies would suggest that there is no relationship between hydrogenated fat intake and coronary disease.

TABLE 11
STUDIES
LINKING ADIPOSE TRANS FATTY ACIDS WITH CHD

Investigators	Subjects	Findings
Heckers, Körner, Tüschen, and Melcher *Arteriosclerosis* *1977*	15 men who died from atherosclerosis v. 8 men deceased from other causes	No significant differences were seen in the 16.1t and 18:1t content of myocardium, jejunum, aorta between cases and controls. Adipose tissue analysis was apparently performed but data not published.
Thomas, Winter and Scott *J. Epid & Community Health* 1983	136 men who died from atherosclerosis v. 95 men deceased from other causes	adipose tissue from cases had higher levels of total trans than controls. Most of this difference can be attributed to a higher t16:1 content found in cases **% total fat** / **difference case-control** / **P** t16:1: 0.708 / 0.08 ± 0.03 / < 0.005 t18:1: 2.347 / 0.13 ± 0.07 / NS t16:1 + t18:1: 5.327 / 0.23 ± 0.22 / p = 0.02
Thomas, Olpin, Scott and Wilkins *Human Nutrition Food Sciences & Nutrition* 1987	59 cases with EKG evidence of ischemia v. 61 healthy controls	adipose tissue trans fatty acid content were not different between cases and controls **% total fat** / **difference case-control** / **P** t16:1: 0.42 / 0.03 ± 0.03 / NS t18:1: 2.53 / -0.10 ± 0.10 / NS however, the ratio of t16:1 to branched chain acids, consistent with a higher dietary intake of hydrogenated marine oils, was higher in cases than controls (p = 0.06) suggesting that consumption of margarines made from hydrogenated marine oils was associated with CHD.

Investigators	Subjects	Findings
Thomas and Winter *Human Nutrition Food Sciences & Nutrition* *1987*	27 cases who died from CHD v. 27 who died from other causes	cases had higher levels of t16:1 than controls but t18:1 content was no different **% total fat** — **difference case-control** — **P** t16:1 — 0.65 — 0.11 ± 0.05 — p < 0.05 t18:1 — 2.70 — 0.13 ± 0.16 — NS the authors conclude that differences in t16:1 were due to diffrences in consumption of hydrogenated marine oil.
Aro, Kardinaal, Salminen, Kark, Riemersma, Delgado-Rodriguez, Gomez-Aracena, Huttenen, Kohlmeier, Martin, Martin-Moreno, Mazaev, Ringstad, Thamm, van't Veer, Kok *Launcet* 1995	671 men with acute MI v. 717 healthy men	considerable differences in the t18:1 content of adipose tissue between countries was observed (range 0.40 - 2.43%) t16:1 and t20-22 were below detechtion limits in most samples, limiting the analysis to t18:1 isomers t18:1 $\bar{X} \pm SD$ t18:1 Cases — Controls 1.61 ± 0.92% — 1.59 ± 0.86 — NS **all centers: RR of quartiles (n = 1388) of % t18:1** 0.45 — 1.29 — 1.80 — 2.51 Crude RR 1.00 — 0.70 — 1.01 — 1.01 Adj RR 1.00 — 0.68 — 1.05 — 0.97 **Excluding centers with low t18:1 (n = 1095)** 1.12 — 1.55 — 1.98 — 2.65 Crude RR 1.00 — 1.05 — 1.49 — 1.36 Multivarate RR 1.00 — 1.16 — 1.53 — 1.44 Although multivarate RR appears to increase over quartiles, test for trend was NS (p = 0.54).

Investigators	Subjects	Findings
Roberts, Wood, Riemersma, Gallagher, Lampe *Lancet* 1995	66 men, without use of CAD, who died frrom sudden cardiac death v. 286 healthy free-living men	cases had lower total trans and t18:1 than controls $\bar{X}\pm$SEM **Cases** **Controls** **P** t18:1 2.10±0.04 2.27±0.04 <0.05 t18:2 0.58±0.01 0.59±0.02 NS total trans 2.68±0.08 2.86±0.04 <0.05 **quintile 18:1t intake and RR** ≤1.77 1.78-2.09 2.10-2.38 2.39-2.74 ≤2.75 RR‡ 1.00 0.88 0.83 0.56 0.40 multivarate RR 1.00 0.96 1.14 0.61 0.59 ‡P for trend = 0.03 trend no longer significant after adjustment for age, smoking, hypertension, diabetes, and oleic + linoleic content

The relationship between adipose tissue or plasma fatty acid content and lipid risk factors for coronary disease.

An alternative approach to evaluating the relationship between coronary disease and hydrogenated fat intake would be to evaluate, in either a case-control fashion or in an unselected population, the relationship between *trans* fatty acids and the traditional risk factors for coronary disease. Two such studies have been done.

Comparing the adipose tissue content of 76 healthy men with their plasma lipid levels, Hudgins et al (59) found that the total *trans* fatty acid content (average 4.14 ± 0.97%) was unrelated to age, BMI, BP or any lipid/lipoprotein cholesterol levels. Individual *trans* fatty acids were only weakly correlated to CHD risk, and often this correlation was negative. 5t14:1 and 11t18:1 were negatively correlated with total cholesterol, the latter also negatively correlated to LDL cholesterol. The only positive correlation was between 12t18:1 and age ($r = 0.259$). This study would suggest that no relationship between lipids and adipose tissue exists.

Comparing the *trans* fatty acid content of plasma fatty acids with 47 patients with CHD on angiography and 56 individuals previously assayed as controls, Siguel and Lerman (68) found that cases had higher saturated fatty acids and lower polyunsaturated fatty acids than controls. Small but significant differences were found between the total *trans* content of cases (1.38 ± 0.07) compared to controls (1.11 ± 0.05, $p = 0.003$). However, this small difference was attributed to higher 7t16:1 and 6tt18:2 in cases that controls. No significant differences has been found between the 9t18:1 of cases (0.38 ± 0.02; $p = 0.14$) and the 7t18:1 content (cases 0.30 ± 0.02 $p = 0.50$). These results fail to implicate hydrogenated vegetable oil consumption as being associated with CHD.

The Siguel study (68) also evaluated the association between plasma fatty acids and serum lipids. The total *trans* content of plasma was significantly correlated with total triglyceride levels $r = 0.31$, $p < 0.004$, but not correlated with total cholesterol or lipoprotein cholesterol levels. No relationship was found between the t18:1 content of plasma and serum lipids. The 7t16:1 content was significantly and positively correlated with total cholesterol, LDL cholesterol and serum triglyceride levels; in addition, it was negatively associated with HDL cholesterol.

An Animal Model on how dietary fatty acids affect LDL cholesterol levels.

Dr John Dietschy has developed a hamster model to evaluate in a quantitative fashion, how dietary fatty acids can alter LDL metabolism (69). This model has been presented at previous grand rounds. Essentially, the effects of feeding a specific fatty acid on LDL cholesterol levels can be further delineated into the effects of that fatty acid on the production rate of LDL and the clearance rate of LDL. According to the model, *trans* fatty acids appear to be "neutral" in that feeding t18:1 results in similar LDL levels as feeding 8:0, with similar rates of LDL production and clearance (70) (FIGURE 4)

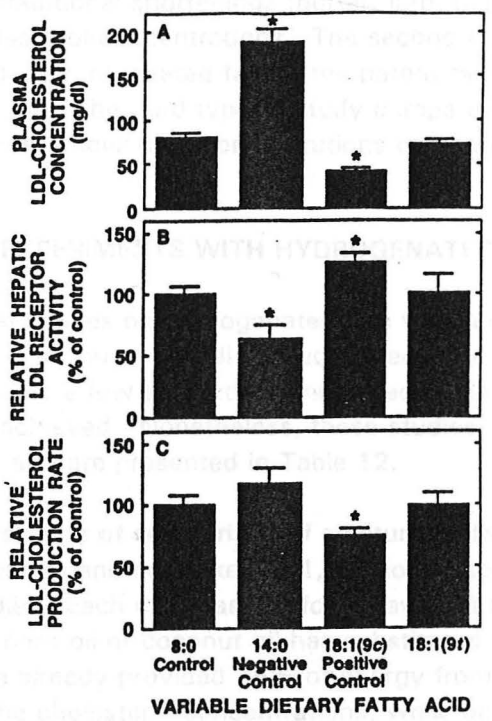


FIGURE 4

The use of the term "neutral" in human feeding studies has historically been applied to factors that achieve cholesterol levels similar to that achieved by either carbohydrate or monounsaturated fatty acid feedings. Since the ultimate goal is to achieve the lowest serum cholesterol levels possible, an alternative approach to avoid defining a "neutral" nutrient would be to consider the effects of a nutrient as compared to the effects of another nutrient.

Human Feeding Studies Comparing the Cholesterol Effects of Various Fats

After data from nutritional epidemiologic studies suggest an association, the cause-effect relationship between a nutrient and CHD risk factors can be further evaluated in human feeding studies. The relationship between *trans* fatty acid ingestion and CHD risk factors has been evaluated in multiple human feeding studies. No relationship between *trans* fatty acid ingestion and blood pressure has been found. However, a consistent relationship between serum lipids and lipoproteins has been found. These studies will be discussed according to the study hypothesis. The first type of study compares whether the substitution of hydrogenated fats for traditional shortenings (butter, lard, palm oil, coconut oil) will change serum cholesterol concentrations. The second type of study compares whether substitution of a hydrogenated fat for the parent fat will change serum cholesterol concentrations. The third type of study compares whether trans fatty acids per se raise or lower cholesterol concentrations compared to other fatty acids.

EARLY EXPERIMENTS WITH HYDROGENATED FATS

Most of the initial studies on hydrogenated fats were conducted before optimal research designs for human feeding studies had been determined. Many were conducted using only a few subjects; some collected lipid data before a steady state had been achieved. Nonetheless, these studies provided pilot data helpful for later studies and are presented in Table 12.

Regarding the first type of comparison of a saturated fat with a hydrogenated fat, Beveridge and coworkers (71,72) conducted three experiments using a liquid formula diet. Each diet was fed for 8 days. In the first experiment, corn oil, hydrogenated corn oil or coconut oil has substituted for carbohydrate (71). The base formula already provided 25% of energy from butter. Addition of coconut oil increased the cholesterol concentrations, while both corn oil and hydrogenated corn oil lowered concentrations. In a second experiment, the cholesterol lowering effect of 8 different margarines were compared to both corn oil and butter (72); margarine feeding raised cholesterol concentrations only slightly above oil feeding, but to concentrations far less than butter. In a third and final experiment, oil, margarine, or butter was exchanged for carbohydrate in a fat-free formula (72). While the addition of oil produced cholesterol lowering, the 8 different margarines produced a mild cholesterol raising effect compared to the fat-free feeding. The cholesterol raising effect of the margarines was far less than

Table 12
HYDROGENATED OIL VS MORE SATURATED FATS

Study	*Iodine value fat or % energy as trans*	*Study details*	*results*
Beveridge, Connell, Mayer, and Haust 1958	IV 69 hydrog corn	28 ♂, 5 ♀ non randomized parallel design liquid formula diet base formula 35% energy as butter fat test fat added as 25% of energy (Exp III) 8 d	oil ≈ hydrogenated corn oil < coconut oil ΔChol mmol/L -0.52 ≈ -0.59 < +0.23 (ΔChol mg/dL -20 ≈ -23 < +9)
Beveridge and Connell 1958	3 - 11% (trans content various margarines)	77 ♂, 11 ♀ non randomized, parallel design liquid formula initial diet was fat-free test fat provided 45% energy from fat (Exp II) 8 d	oil ≤ 8 different margarine < butter ΔChol mmol/L -0.59 ≤ +0.21 < +0.98 (ΔChol mg/dL -23 ≤ +8 < +38)
Beveridge and Connell 1958	3 - 11% (trans content various margarines)	81 ♂, 13 ♀ parallel design liquid formula diet base diet 22.5% butterfat. test fat provided on additional 22.5% energy fat (Exp I) 8 d	oil ≈ 8 different margarine < butter ΔChol mmol/L -0.21 ≈ -0.03 < +0.44 (ΔChol mg/dL -8 ≈ -1 < +17)
Horlick 1960	IV 95 or 88	4 student volunteers non randomized natural food low fat diet plus 40% energy test fat (Series D) 7 - 21 d	margarine (corn or commercial) < butter ΔChol mmol/L 6.20 < 7.24 (ΔChol mg/dL 240 < 280)
Grasso, Gunning, Imaichi, Michaels, and Kinsell 1962	hydrogenated oil IV 107	patient 1 = diabetic ♂ patient 2 = hypothyroid ♀ randomized liquid formula diet 45% energy from fat 14 d	oil < hydrogenated oil < coconut oil patient 1 Δ Chol mmol/L 4.94 ≈ 5.14 < 7.00 (Δ Chol mg/dL 191 ≈ 199 < 271) patient 2 Δ Chol mmol/L 5.40 < 7.16 < 10.10 (Δ Chol mg/dL 209 < 277 < 319)

OIL VS HYDROGENATED OIL			
Study	***Iodine value fat or % energy as trans***	***Study details***	***results***
Ahrens, Hirsh, Insull, Tsaltas, Bloomstrand, and Peterson 1957	corn oil IV 126 vs hydrog corn IV 80 or hydrog corn IV 58 cotton IV 106 hydrog cotton IV 68	3 men (P+18, 20, 30) non randomized liquid formula 40% energy test fat 56 d	corn oil < corn oil margarine ΔChol mmol/L 3.07 < 3.23 5.17 < 7.05 < 7.70 (ΔChol mg/dL 119 < 125 200 < 273 < 298) Corn > cottonseed < hydrog cottonseed ΔChol mmol/L 4.78 > 7.57 < 5.61 (ΔChol mg/dL 185 > 177 < 217)
Bronte-Stewart, Antonis, Eales, and Brock 1956	nut fat IV 89 hydrog nut fat IV 55	3 men non randomized natural food 100 g test fat added to low fat diet 8 - 11 d	nut fat < hydrogenated fat ΔChol mmol/L 3.10 < 3.88 (ΔChol mg/dL 120 < 150)
DEGREE OF HYDROGENATION STUDIES			
Srinivasarao and Shenolikar 1990	peanut oil IV = 95 37° IV = 65 41° IV = 60 45° IV = 51	4 men randomized natural food diet test fat 15% energy 17 d	peanut oil < 37° oil ≈ 41° oil < 45° oil Δ Chol mmol/L 3.90 < 4.81 ≈ 4.81 < 4.99 (Δ Chol mg/dL 151 < 186 ≈ 186 < 193)

that observed when butter replaced carbohydrates. Beveridge's results paralleled those observed by Horlick (73) in a natural food study of four subjects: margarine feeding at 40% of total energy produced lower cholesterol concentrations than butter feeding. Beveridge's results also were consistent with those of Grasso et al. (74) who found that either partially hydrogenated soy oil or a mixed vegetable oil achieved lower total serum cholesterol concentrations than coconut oil feeding in two patients.

Concerning the second type of comparison of hydrogenated fats with the parent fat, Ahrens et al. (75) and Bronte-Stewart et al. (76) compared the lipid lowering effects of the parent fat with its hydrogenated version. Using a liquid formula, cholesterol-free diet fed for 56 days, Ahrens et al. (75) observed in 2 men that corn oil produced lower cholesterol concentrations than corn oil margarine and in 1 man that cottonseed oil produced lower cholesterol concentrations than hydrogenated cottonseed. Bronte-Stewart et al. (76) found that in three men, an 8 - 11 day feeding of ground-nut fat (likely peanut oil) produced lower cholesterol concentrations than hydrogenated nut fat.

Why hydrogenated fats had apparent cholesterol raising properties compared to their parent oils was unclear. In a study by Srinivasarao and Shenolikar (77), 4 men were fed 3 fats hydrogenated to different melting points for 11 days. The cholesterol raising effects of these fats were then compared to peanut oil. Srinivasarao and Shenolikar found that the higher the melting point of the fat, the higher the total cholesterol concentration. This suggested that the degree of hydrogenation altered the cholesterol lowering effects of the vegetable oil. In a study comparing an unusual triglyceride, trans-trilinolein, with *cis*-trilinolein, Mishkel and Spritz (78) found that trans-trilinolein produced higher cholesterol concentrations than *cis*-trilinolein. Since trans-linolein is rarely found in hydrogenated fats, this preliminary study had limited clinical implications.

Initial studies suggested that while hydrogenated fats increased cholesterol concentrations compared to the parent fat, hydrogenated fat feedings lowered cholesterol concentrations compared to butter. In other words, the effects of hydrogenated fats on serum cholesterol concentrations were intermediate between the parent oil and the more saturated fat. These findings needed to be confirmed in studies employing a more optimum design. Such studies are detailed in tables 13-17 and will be discussed below.

METABOLIC DIET STUDIES

Table 13 presents metabolic studies comparing the cholesterol raising effects of hydrogenated oils and animal fats. McOsker et al. (79) fed 24 men a liquid formula diet for 46 days. Multiple mixtures of oils plus hydrogenated oils (trans content ~6% of total energy) achieved lower cholesterol concentrations than mixtures containing animal fats. Erickson et al. (80) compared several vegetable fat mixtures with different P/S ratios (0.1 to 1.6) and found no differences between fats containing 10% sat and 9% trans, 6% sat and 9% trans, 6% sats and 3% trans, 5% sat and 9% trans, and 4% sat and 0% trans. It should be noted that interpretation of the apparent neutrality of hydrogenated fats in the Erickson et al. study may be subject to β error, since no statistically significant differences were seen despite a 4 - 6% change in percent energy from saturated fatty acids. Comparing margarine with butter, Lerner et al. (81) reported that margarine substituted for butter as a spread and as a fat in food preparation resulted in 12 - 15% decrease in total cholesterol a 15 - 18% decrease in LDL cholesterol and a 5 - 6% decrease in HDL cholesterol concentrations.

TABLE 13

METABOLIC DIET STUDIES
HYDROGENATED OIL VS MORE SATURATED OIL

Study	% energy as trans	Study details	Results
McOsker, Mattson, Sweringen and Kligman 1962	6%	36 men randomized parallel design liquid formula 41% energy as test fat [each subject received 4 test fats] 56 d	partially hydrog winterized soy ≈ partially hydrog cotton + soy ≈ winterized cottonseed ≈ partially hydrog cotton + soy < mixture animal + vegetable fats < butter ΔChol mmol/L 4.08 ≈ 4.08 ≈ 4.08 ≈ 4.21 ≈ 4.31 < 4.52 < 5.27 (ΔChol mg/dL 158 ≈ 158 ≈ 158 ≈ 163 ≈ 167 < 175 < 204)
Erickson, Coots, Mattson and Kligman 1964	4%	36 male prisoners parallel design liquid formula 40% total fat [each subject received 5 test fats] 35 d	no sig difference in fat mixtures despite p/s ratio varying from 0.1 - 1.6 sig difference occurred between formulas containing dietary cholesterol(+) compared with cholesterol free formulas p/s 1.6^{+} ≈ 1.5^{+} < 0.1 ≈ 1.6 ≈0.7 ≈ 1.5 ≈ 0.7 ΔChol mmol/L 5.61 ≈ 5.56 < 5.04 ≈ 4.99 ≈ 4.91 ≈ 4.86 ≈ 4.86 (ΔChol mg/dL 217 ≈ 215 < 195 ≈ 193 ≈ 190 ≈ 188 ≈ 188)
Lerner, Neville, Nalto, Simpson, Raguso and McClish 1983	not measured	26 men randomized to butter vs marg parallel design for margarine 1 vs margarine 2 41% total fat test fat used as spread and in cooking; unknown how much test fat contributed to total energy 35 d	margarine 1 ≈ margarine 2 < butter specific values below obtained by pers. comm. mmol/L (mg/dL CHOL 4.86 ≈ 5.06 < 5.71 188 ≈ 196 < 221 LDL 3.07 ≈ 3.20 < 3.77 119 ≈ 124 < 146 HDL 1.42 ≈ 1.42 < 1.50 55 ≈ 55 < 58)

Concerning the comparison between parent oils and their hydrogenated version (Table 14), Anderson, Grande and Keys (82) fed 27 men either 30 gm of safflower oil or 30 gm of hydrogenated safflower oil substituted for carbohydrate in a high fat base diet. Hydrogenated safflower oil, contributing 3% of energy as trans raised total cholesterol concentrations 0.26 mmol/L (10 mg/dL) compared to safflower oil. Additional experiments feeding 10% of energy as trans raised total cholesterol concentrations 0.54 - 0.65 mmol/L (21-25 mg/dL) suggesting a dose-response relationship. The authors concluded that trans-mono isomers have a cholesterol-raising effect that was slightly less than saturates. They suggested that the Keys' prediction equation relating change in dietary fatty acid intake and serum cholesterol concentrations could be modified by adding a term of +2.1 for change in percent energy from trans. (This equation uses a +2.8 coefficient for changes in percent energy from saturates).

Similar quantitative results were seen in a recent metabolic study by Lichtenstein et al. (83), where lipid and lipoprotein measurements were evaluated. Corn oil margarine feeding (dietary trans 4% of energy) produced a 0.23 mmol/L (9 mg/dL) significant increase in total cholesterol concentrations compared to corn oil; the increase of 0.26 mmol/L (10 mg/dL) in LDL cholesterol concentrations paralleled that of total cholesterol but did not quite achieve statistical significance. No significant effects on HDL cholesterol concentration were seen.

TABLE 14

Metabolic Diet Studies

OIL VS HYDROGENATED OIL

Study	% energy as trans	Study details	Results
Anderson, Grande and Keys 1961	3%	27 men, randomized natural foods 46% energy as fat (60% test) (Experiment K) 21 d	30 gm safflower oil < hydrog safflower ΔChol mmol/L 5.06 < 5.32 (ΔChol mg/dL 196 < 206)
	10%	12 men, randomized 38% energy as fat (74% test) (Experiment N) 21 d	100 gm safflower < hydrog safflower ΔChol mmol/L 4.13 < 4.78 (ΔChol mg/dL 160 < 185)
	10%	14 men, randomized 38% energy as fat (74% test) (Experiment N) 21 d	100 gm corn oil < hydrog corn ΔChol mmol/L 4.21 < 4.75 (ΔChol mg/dL 163 < 184)
Lichtenstein, Ausman, Carrasco, Jenner, Ordovas, and Schaefer 1993	4%	6 men 8 women randomized natural food 40% energy as fat (52% test) 32 d	corn oil < corn oil marg < control **ΔChol mmol/L** CHOL 5.01 < 5.30 < 5.76 LDL 3.23 ≈ 3.48 < 3.96 HDL 1.11 ≈ 1.14 < 1.24 **(ΔChol mg/dL** CHOL 194 < 205 < 223 LDL 125 ≈ 135 < 153 HDL 43 ≈ 44 < 48)

Several studies have simultaneously evaluated how hydrogenated fats compare with their parent fat as well as to a more saturated fat (butter, palm oil or coconut oil; Table 15). In an intensive study of 72 mental hospital patients, de longh et al. (84) evaluated the effects of 9 different fats on serum cholesterol concentrations. Each fat has added to a low-fat base diet and was fed for a total of 4-6 weeks. Safflower oil was tested repeatedly to enhance the dietary comparisons. A total of 33 men completed the entire 2 year study and cholesterol concentrations obtained on these men were subject to analysis of variance testing. A summary of these results are in Table 15; the discussion here will be limited to the comparison of sunflower oil, lightly hydrogenated soybean oil, heavily hydrogenated soybean oil, typical margarine, and butter. Four of the sunflower oil feedings produced significantly lower concentrations than feedings from either lightly hydrogenated soybean oil, heavily hydrogenated soybean oil, margarine or butter. The lightly hydrogenated soybean oil with preserved linoleic content (trans 6% of energy) achieved similar cholesterol concentrations to two of the sunflower oil feedings as well as the margarine feeding (trans 7% of energy). The heavily hydrogenated soybean period (trans 23% of energy) achieved similar cholesterol concentrations seen during the butter feeding period; both were higher than typical margarines or lightly hydrogenated soybean oil. This study suggested that hydrogenated fats generally raise cholesterol concentrations compared to less saturated, unhydrogenated vegetable oils; the degree of cholesterol raising was related to the percent trans. The study also demonstrated that data from a single study cannot be taken as definitive -- variation in mean responsiveness to the six sunflower oil feedings was remarkable enough to achieve statistical significance.

In a parallel design, Antonis and Bersohn (85) conducted a long-term feeding study (154 - 203 days) of Bantu and White prisoners. Hydrogenated sunflower oil added to a normal or low fiber diet produced 1.03 mmol/L (~40 mg/dL) increases in total cholesterol concentrations compared to sunflower oil feeding. Cholesterol concentrations on the hydrogenated oil feeding, however, remained 0.78 mmol/L (30 mg/dL) lower than the butter feeding. No effects on serum triglycerides were seen (86). In another study by Anderson, Grande and Keys (82), 23 men randomized to one of three natural food diets 1) corn oil + olive oil, 2) hydrogenated corn oil (10% energy as trans), or 3) butter found that hydrogenated corn oil produced 0.54 mmol/L (21 mg/dL) higher total cholesterol concentrations than the corn + olive oil mixture; both diets, however produced significantly lower cholesterol concentrations than butter. In the one study of this type that evaluated both lipid and lipoprotein concentrations, Laine et al. (87)

TABLE 15

Metabolic Diet Studies
Oil vs Hydrogenated Oil vs More Saturated Oil

Study	% energy as trans	Study details	Results
de longh, Beerthuis, den Hartog, Dalderup and van der Spek	0 to 23%	33 men non randomized natural food 36 % energy as fat (92% test fat) 28 - 42 d	16 periods were tested in sequence; by ANOVA the cholesterol values achieved fell into five different groups identified below: mmol/L (mg/dL) Sunflower Oil #4 (SF4) 3.85 149 SF # 2 4.03 156 SF # 1 4.06 157 SF # 5 4.16 161 60% hydrogenated fish + 40% SF 4.24 164 SF # 3 4.24 165 SF # 6 4.34 168 lightly hydrogenated soybean 4.34 168 80% heavily hydrogenated soybean oil + 10% soybean + 10% safflower 4.34 168 40% heavily hydrogenated whale oil + 60% SF 4.42 171 40% lightly hydrogenated whale oil + 40 SF + 20% Safflower oil 4.47 173 Typical margarine 4.52 175 lightly hydrogenated whale oil 4.81 186 heavily hydrogenated Soybean 5.06 196 Butter 5.12 198 Normal Diet 5.12 198 heavily hydrogenated whale oil 5.37 208
Antonis and Bersohn 1962	6%‡	8 - 11 subjects/group parallel design natural food 40% energy from fat 25% energy as test fat 154 d (nl fiber; Diet 2) 203 d (reduced; Diet 3)	sunflower oil < hydrog sunflower < butter mmol/L (mg/dL) normal fiber White 4.17 < 5.49 < 6.20 162 < 212 < 240 Bantu Indians 3.72 < 4.81 < 5.84 144 < 186 < 226 Reduced fiber White 4.06 < 5.35 < 6.12 157 < 207 < 237 Bantu Indians 3.85 < 4.94 < 5.87 149 < 191 < 227

Study	% energy as trans	Study details	Results		
Anderson , Grande and Keys 1961	10%	23 men randomized 40% energy as fat (75% test fat) natural food diets (Experiment AD) 21 d	Corn + olive < hydrog corn < butter 4.86 < 5.40 < 6.02		188 < 209 < 233
Laine, Snodgrass, Dawson, Ener, Kuba and Frantz 1982	3%	13 men 11 women crossover from palm to others (randomized; Group 2 and 4) natural food 35% energy from fat (52% test fat) 20 d	corn < lightly hydrog soy < palm CHOL LDL HDL	3.93 < 4.16 < 4.60 2.20 < 2.53 < 2.79 1.47 ≈ 1.47 ≈ 1.47	152 < 161 < 178 85 < 98 < 108 57 ≈ 57 ≈ 57

‡ trans content not listed. Hydrogenation reduced the linoleic content 63.7% to 1.6% and stearic increased 4.1 to 17.4%, oleic 24.7% to 73.1%. Assuming 50% of hydrogenated linoleic formed trans ≈ 6% energy trans.

found a similar quantitative relationship between trans intake and both total and LDL cholesterol concentrations: Lightly hydrogenated soy (3% of energy as trans) achieved higher total and LDL cholesterol concentrations of 0.28 mmol/L (11 mg/dL) and 0.24 mmol/L (13 mg/dL), respectively, compared to corn oil. Palm oil produced even higher concentrations compared to trans with further increases of 0.44 and 0.26 mmol/L (17 and 10 mg/dL), respectively. Laine et al. (87) found no differences in HDL cholesterol concentrations on the three diets.

In summary, 10 of 11 study comparisons have shown that feeding a hydrogenated fat produced higher total cholesterol concentrations compared to feeding the parent fat; however, 11 of 11 study comparisons have shown that this increase was far less than the increase observed when butter, palm oil, or coconut oil were substituted for the parent fat. There are some notable limitations to these conclusions. Some studies did not control for other dietary factors, such as changes in dietary cholesterol, that could confound a conclusion concerning the cholesterol raising potential of trans fatty acids per se. For example, butter contains dietary cholesterol and direct comparisons of margarine vs butter cannot determine whether the results can be attributed to changes in the fatty acids, changes in dietary cholesterol or changes in both factors. Other studies specified the content of trans in the test fat but did not specify the content of trans in the entire diet, and some did not distinguish whether trans referred to all trans isomers, only trans monoene isomers, or to a specific trans monoene (e.g., elaidic acid).

STUDIES COMPARING FATTY ACIDS

An alternative approach to evaluating the effects of hydrogenated fats on serum lipids would be hold every dietary variable constant except the fatty acids of the fats. Since fatty acids have been found to be the strongest predictor of serum lipids in humans, this approach is most consistent scientifically with the hypothesis that dietary fatty acids are the main factor that alter serum cholesterol concentrations in humans.

Several human studies have compared fatty acid feeding on serum cholesterol levels (Table 16). The percent energy derived from specific fatty acids in each study is listed in Table 16a.

In the first studies of this kind, Vergroesen (88), using a parallel design of liquid formula diets, compared three fats: *cis*-mono (11% c16:0, 73% *cis* c18:1) trans-mono (11% c16:0, 37% *cis* c18:1, 35% trans c18:1) and palmitic (43%

TABLE 16
Metabolic Diet Studies
Where Design Employed a Head to Head Comparison
of Fatty Acids

Study	% energy as trans	Study details	Results rating of study groups Cholesterol concentrations in mmol/L
Vergroesen and Gottenbos 1975	14%	12 men and women crossover liquid formula 40 % energy from fat (Experiment A) 28 d	no cholesterol high cis mono ≈ high trans mono high palmitic mmol/L 4.94 ≈ 4.65 ≈ 4.91 (mg/dL 191 ≈ 180 ≈ 190) + cholesterol mmol/L 4.83 ≈ 5.63 ≈ 4.99 (mg/dL 187 ≈ 218 ≈ 193)
Vergroesen and Gottenbos 1975	13%	53 men (~ 9/group) parallel design liquid formula 38% energy from fat (Experiment B) 28 d	oleic < elaidic < lauric + myristic high poly (34% of fat) mmol/L 4.83 ≈ 4.93 ≈ 5.10 (mg/dL 187 ≈ 191 ≈ 198) low poly (10% of fat) mmol/L 4.81 ≈ 5.01 ≈ 5.43 (mg/dL 186 ≈ 194 ≈ 210)
Mattson , Hollenbach and Kligman 1975	15%	33 men parallel design liquid formula 35% energy from fat 28 d	cis ≈ cis; cis ≈ trans mmol/L 4.75 ≈ 4.88 4.81 ≈ 4.89 (mg/dL 184 ≈ 189 186 ≈ 189)
Mensink and Katan 1990	11%	34 men, 25 women randomized natural food 40% energy from fat 21 d	oleic < trans ≈ saturated mmol/L (mg/dL CHOL 4.46 < 4.72 < 5.00 173 < 183 < 194 LDL 2.67 < 3.04 ≈ 3.14 103 < 118 ≈ 122 HDL 1.42 > 1.25 < 1.42 55 > 48 < 55) Mensink 1992 reported Lp(a) concentrations rose from 69 mg/L (both oleic and sat) to 85 (trans).
Zock and Katan 1992	8%	26 men 30 women randomized 40% energy from fat 21 d	linoleate < trans ≈ stearate mmol/L (mg/dL CHOL 4.74 < 4.90 ≈ 4.89 183 < 190 ≈ 189 LDL 2.83 < 3.07 ≈ 3.00 110 < 119 ≈ 116 HDL 1.47 > 1.37 ≈ 1.41 57 > 53 ≈ 55)

Study	% energy as trans	Study details	Results rating of study groups Cholesterol concentrations in mmol/L	
Judd, Clevidence, Muesing, Wittes, Sunkin, and Podczasy 1994	mod 4% high 7%	29 men 29 women randomized natural food 39% energy from fat 42 d	oleic < mod trans ≈ high trans < sats mmol/L	(mg/dL
			CHOL 5.26<5.46≈5.52<5.61	204<211≈214<217
			LDL 3.34<3.54≈3.60<3.64	129<137≈139<141
			HDL 1.42≈1.40≈1.38<1.47	55 ≈ 54 ≈ 53 < 57)

TABLE 16A

Percent Energy from Specific Fatty Acids in Studies from Table 5

Vergroesen 1972	*high cis mono*	*high trans mono*	*high palmitic*
c12-16:0	4	4	17
c18:0	1	1	2
c18:1 cis	28	14	14
c18:1 trans	—	13	—
c18:2 cis	4	3	4
c18:2 trans	—	—	—
Cholesterol/d (provided lay egg yolk) —/250 —/250 —/250			

Vergroesen, 1975

High Poly	*oleic*	*elaidic*	*lauric + myristic*
c12-14:0	—	—	15
c16:+c18:0	5	5	5
c18:1 cis	21	7	7
c18:1 trans	—	14	—
c18:2 cis	14	14	14
c18:2 trans	—	—	—
Cholesterol (egg yolk in all formulas) = 115 mg/1,000 Kcal			

Vergroesen, 1975

Low Poly	*oleic*	*elaidic*	*lauric + myristic*
c12-14:0	—	—	15
c16:0-18:0	5	6	5
c18:1 cis	31	16	16
c18:1 trans	—	14	—
c18:2 cis	4	4	4
c18:2 trans	.1	—	—
Cholesterol (egg yolk) in all formulas = 115 mg/1,000 Kcal			

Mattson, 1975

Mattson 1975	*high-cis*	*high-trans*
c16:0	5	4
c18:0	5	6
c18:1 cis	22	8
c18:1 trans	—	13

c18:2 cis	6	3
c18:2 trans	—	4
cholesterol (dried egg yolk) in all formulas = 500 mg/d		

Mensink, 1990

Mensink 1990	*oleic*	*trans*	*sat*
c12-16:0	5	5	14
c8:0	3	4	4
c18:1 cis	23	13	13
c18:1 trans	—	11	2
c18:2 cis	4	4	3
Cholesterol (mg/1,000 Kcal) 146		134	141

Zock, 1992

Zock 1992	*linoleate*	*trans*	*stearate*
c12-16:0	7	6	7
c18:0	3	3	12
c18:1 cis	15	15	15
c18:1 trans	—	8	—
c18:2 cis	12	4	4
c18:2 trans	—	—	—
Cholesterol (mg/1,000 Kcal) 140		136	140

Judd, 1994

Judd 1994	*Oleic*	*Mod Trans*	*High Trans*	*Sat*
c12-16:0	11	10	10	16
c18:0	3	3	3	3
c18:1 cis	17	14	11	11
c18:1 trans	1	4	7	1
c18:2 cis	6	6	6	6
Cholesterol intake 135 mg/1,000 Kcal				

c16:0, 38% *cis* c18:1). Half of each fat group received egg yolk cholesterol while the other half received cholesterol-free formulas. The group fed trans plus cholesterol had significantly higher total cholesterol concentrations than the oleic acid group. No significant differences between the other groups were observed. These results questioned whether a fatty acid-dietary cholesterol interaction existed. However, Mattson, Hollenbach and Kligman (89) employing a parallel design of cholesterol containing liquid formulas, found that a *cis*-mono fat achieved equivalent cholesterol concentrations as a trans-mono fat.

In a second study by Vergroesen and Gottenbos (90), dietary cholesterol was added to all of the formulas. Six different diets were fed, half were low in poly (10% of total fatty acids) and half were high in poly (34% of total fatty acids). The subjects were further divided into three fatty acid diet groups, one high in lauric + myristic 37% of total fat), one high in oleic 78% of total fat) and one high in trans 35%of total fat). In this study, the lauric + myristic fat achieved higher total cholesterol concentrations than the trans fat; the oleic fat achieved the lowest cholesterol concentrations with little difference between high poly and low poly groups.

Following publication of these studies, the general consensus was that trans fatty acids were equivalent to *cis*-monounsaturates in that they did not effect cholesterol concentrations. The cholesterol raising effect of hydrogenated fats compared to their parent oils was attributed to the effects of hydrogenation in reducing the polyunsaturated fatty acid content of the parent fat. Three 1990's studies have questioned this interpretation of the data.

The first study by Mensink and Katan (91), comparing 3 natural food diets enriched either in oleic, trans, or saturated fatty acids, found that oleic acid produced 0.24 mmol/L (10 mg/dL) lower total and 0.38 mmol/L (15 mg/dL) lower LDL cholesterol concentrations than trans; trans produced 0.28 mmol/L (11 mg/dL) lower total cholesterol concentrations than saturates ($p < 0.001$). In addition, trans feeding lowered HDL cholesterol concentrations by 0.17 mmol/L (7 mg/dL) and increased Lp(a) concentrations by 16 mg/L compared to either oleic or saturated feedings. The Mensink and Katan study suggested that trans fatty acids had a cholesterol raising effect compared to oleic but this effect was not as great as saturates; in addition trans fatty acids had an HDL cholesterol lowering effect.

A second study by Zock and Katan (92) compared linoleate with trans and stearate. Linoleate produced a 0.16 mmol/L (7 mg/dL) lower total and 0.24

mmol/L (9 mg/dL) lower LDL cholesterol concentrations than either trans or stearate; the effects of trans on total and LDL cholesterol concentrations was equivalent to stearate. These results, which suggested a cholesterol raising effect for stearate, are in contrast to other studies which have found that stearate feeding produces similar lipid concentrations as oleic feeding (93). In the Zock and Katan study (92), trans feeding produced lower HDL cholesterol concentrations than linoleate feeding; stearate feeding produced equivalent HDL cholesterol concentrations to trans feeding. Taken together, the two studies from the Netherlands (91,92) suggested that trans fatty acids produce a cholesterol raising effect compared to either linoleic acid or oleic acid; its cholesterol raising effect for total cholesterol concentrations is less that saturates but equivalent to stearic acid. Its effects on LDL cholesterol concentrations are equivalent to stearate but less than c12:0 - c16:0 saturates.

In a study by Judd et al. (94), the cholesterol raising effects of diets rich in either oleic acid or saturated fatty acids were compared to diets containing moderate *trans* (4% of energy) and high *trans* (7% of energy). Oleic acid produced lower total and LDL cholesterol concentrations than either trans period. Moderate trans produced nearly equivalent total and LDL cholesterol concentrations to high trans; both trans diets produced lower total and LDL cholesterol concentrations than the saturate rich diet. The Judd et al. study found that the saturate rich diet produced higher HDL cholesterol concentrations than the moderate trans, the high trans and the oleic diet. The high trans diet produced significantly lower HDL cholesterol concentrations than the oleic diet.

In summary, the majority of the fatty acids studies comparing trans with *cis*-monounsaturates found that trans-monounsaturated fatty acids raised total cholesterol concentrations compared to *cis*-monounsaturated fatty acids; one study comparing trans with polyunsaturated fatty acids found than trans-monounsaturated fatty acids raised total and LDL cholesterol concentrations compared to linolenic acid. Two of the six studies compared trans fatty acids with cholesterol raising saturates (c12:0 - c16:0); both studies found that trans was not as potent as saturates in raising total and LDL cholesterol concentrations.

OUTPATIENT FEEDING STUDIES

Besides these metabolic diet studies, four outpatient feeding studies comparing the effects of extensive replacement of more saturated fats with hydrogenated fats on serum lipid concentrations have been published (**Table 17**).

TABLE 17
Outpatient Feeding Studies
with Trans fatty Acids

Study	% energy as trans	Study details	Results
Nestel, Noakes, Belling, McArthur, Clifton, and Abbey 1992	2% difference	26 men, mild hypercholesterolemic outpatient diet counselling randomized crossover test fat supplied in biscuits, spread. 42% energy as fat; test fat supplied 60% fat control 18% SFA /3% trans blend1 13% SFA /5% trans blend2 12% SFA /5% trans 14 d control 28 d test	blend1 ≈ blend2 < $control_1$ ≈ $control_2$ **mmol/L** CHOL 5.57 ≈ 5.49 < 5.82 ≈ 5.72 LDL 3.92 ≈ 3.83 < 4.13 ≈ 4.03 HDL 1.10 ≈ 1.11 < 1.11 ≈ 1.15 **(mg/dL** CHOL 216 ≈ 212 < 225 ≈ 221 LDL 152 ≈ 148 < 160 ≈ 156 HDL 43 ≈ 43 ≈ 43 ≈ 45)
Wood, Kubena, Tseng, Martin, and Cook 1993	~ 2% difference	29 men outpatient diet counselling randomized crossover 36 - 38 % energy as fat 60% test fat supplied as spreads, cookies, ice cream, milk fatty acid intake diets unavailable; only test fats measured 42 d	sunflower oil ≈ palm/sunflower mix ≈ palm≈ margarine ≈ crude palm< butter **mmol/L** CHOL 4.84 ≈ 5.12 ≈ 5.15 ≈ 4.99 ≈ 5.15 ≈ 5.17 LDL 3.23 ≈ 3.41 ≈ 3.41 ≈ 3.36 ≈ 3.36 ≈ 3.52 HDL 1.00 < 1.03 ≈ 1.06 > 1.00 < 1.03 ≈ 1.03 **(mg/dL** CHOL 187 ≈ 198 ≈ 199 ≈ 193 ≈ 199 ≈ 200 LDL 125 ≈ 132 ≈ 132 ≈ 130 ≈ 130 ≈ 136 HDL 39 < 40 ≈ 41 > 39 < 40 ≈ 40)
Wood, Kubena, O'Brien, Tseng, and Martin 1993	~ 7% difference	38 men outpatient diet counselling randomized crossover 60% of energy as fat designed to be test fat no data on other intake of participants 42 d	soft margarine < hard margarine ≈ butter + sunflower ≈ butter + olive < butter **mmol/L** CHOL 4.83 < 5.12 ≈ 5.12 ≈ 5.25 < 5.45 LDL 3.26 < 3.46 ≈ 3.49 ≈ 3.59 < 3.75 HDL 1.16 ≈ 1.16 ≈ 1.19 ≈ 1.21 ≈ 1.21 **(mg/dL** CHOL 187 < 198 ≈ 198 ≈ 203 < 211 LDL 126 < 134 ≈ 135 ≈ 139 < 146 HDL 45 ≈ 45 ≈ 46 ≈ 47 ≈ 47)

Study	% energy as trans	Study details	Results
Nestel, Noakes, Belling, McArthur, Clifton, Janus, and Abbey 1992	6% difference	27 men outpatient diet counselling randomized crossover 36 - 37% energy as fat 57% was from test fat as supplied in margarines, biscuits, potato chips 14 d habitual 21 d	oleic < elaidic < palmitic < habitual mmol/L CHOL 5.56 < 5.92 ≈ 5.84 ≈ 5.89 LDL 3.90 < 4.26 ≈ 4.16 ≈ 4.21 HDL 0.98 ≈ 0.98 < 1.09 > 0.98 (mg/dL CHOL 215 < 229 ≈ 226 ≈ 228 LDL 151 < 165 ≈ 161 ≈ 163 HDL 38 ≈ 38 < 42 > 38)

These studies provided participants products made with test fat (e.g. chips, spreads, biscuits, cookies, ice cream, milk); using intensive dietary counselling these products provided 60% of total dietary fat intake. All four studies concluded similarly (95,96,97,98): Hydrogenated fats achieved lower cholesterol concentrations than typical intakes of butter or mixed animal and vegetable fats. The two outpatient studies that further compared hydrogenated fats with liquid oils (96,98) found similar results to that seen in the metabolic ward studies: Hydrogenated fat feeding produced higher cholesterol concentrations than parent fat feeding.

Besides the studies detailed in Table 17, three outpatient studies have compared the simple replacement of butter as a spread to margarine as a spread (99,100,101). In such a study design, the contribution of the test fat to total fat intake is much smaller than that of the studies detailed in Table 17 -- accounting for only 10-30% of total fat intake or 3-9% of total energy intake. All three studies found no significant differences in the total, LDL and HDL cholesterol concentrations when margarine replaced butter as a spreadable fat. The interpretation of these three studies must be done with caution since the differences in percent energy from specific fatty acids were small, the studies had a limited number of subjects (71, 21, 54, respectively) and the studies provided no dietary instructions other than spread substitution.

EFFECTS OF TRANS ON HDL AND Lp(a) CONCENTRATIONS

Whereas the preceding discussion has focussed on the effects of trans fatty acid ingestion on total and LDL cholesterol concentrations, the effects of trans fatty acids on two other lipid factors -- HDL cholesterol and Lp(a) concentrations -- has also been tested. Since these lipid factors may confer additional CHD risk, a summary of the results is discussed below.

HDL CHOLESTEROL CONCENTRATIONS

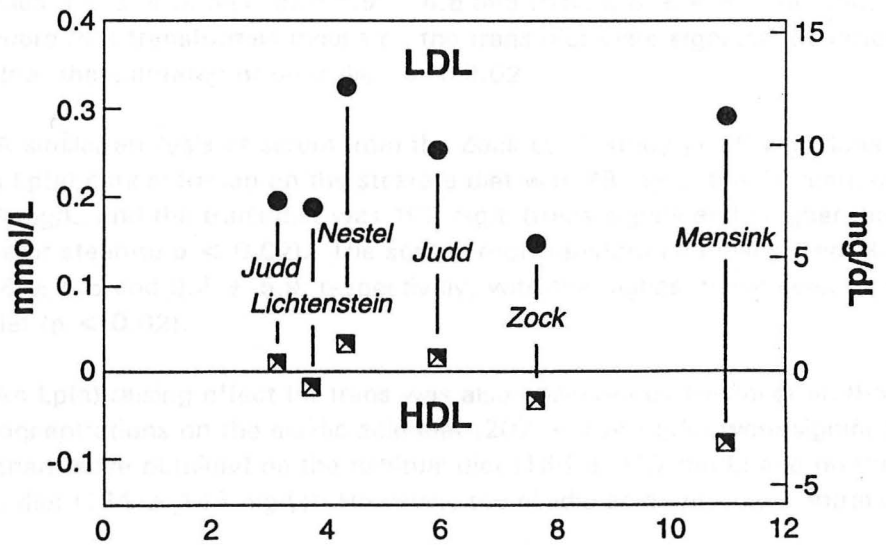
The effects of hydrogenated fats on HDL cholesterol concentrations have been evaluated in six metabolic studies. Mensink and Katan (91) found that feeding 11% of energy as trans significantly decreased HDL cholesterol concentrations 0.17 mmol/L (7 mg/dL; 12%) compared to oleic. Zock and Katan (92) found that feeding 8% of energy as trans significantly decreased HDL cholesterol concentrations 0.10 mmol/L (4 mg/dL; 7%) compared to linoleic feeding. Lerner et al. (81) reported in an abstract that margarine substituted for

butter as the fat used in spreads, cooking and baking resulted in a 6-7% decrease in HDL cholesterol concentrations.

These three studies are in contrast to three metabolic studies that did not find a consistent HDL effect. Judd et al. (94) found a significant reduction of 0.04 mmol/L (2mg/dL) comparing 7% energy as trans with oleic. However, a nonsignificant reduction in HDL cholesterol concentrations of 0.02 mmol/L (1 mg/dL) was observed when comparing 4% energy as trans with the oleic diet. Laine et al. (87), comparing 3% energy as trans with either corn oil or palm found equivalent HDL cholesterol concentrations on all three feeding periods. Lichtenstein et al. (83), comparing 4% energy as trans with corn oil, found a nonsignificant increase of 0.03 mmol/L (1 mg/dL) in HDL cholesterol concentrations during the margarine feeding period.

What these small differences in HDL cholesterol concentrations can be attributed to remains unclear. Do trans fatty acids have a dose-dependent HDL cholesterol lowering effect? Or are the HDL cholesterol effects observed due to random variation? It should be noted that given the large inter-individual variations in HDL cholesterol concentrations, an n of at least 200 would be needed to have sufficient power to test whether a 3% energy change in trans fatty acids produces a 1-3 mg/dL lowering in HDL cholesterol concentrations. However, several researchers suggest that the HDL lowering effect of *trans* fatty acids is more than suggestive of a dose response.

FIGURE 5



A dose-response effect of *trans* fatty acid ingestion, as estimated by food frequency questionnaires, correlated minimally with HDL cholesterol levels $r=-0.08$, $p=0.03$ but this correlation did not hold after adjustment for energy intake, age, BMI, waist to hip ratio, smoking status, physical activity and alcohol intake (57). In control subjects of the Ascherio study (58), quintiles of *trans* fatty intake did not have a graded reduction in HDL cholesterol levels (the HDL cholesterol in the 5th quintile of intake was 40.7 compared to 37.5 in the lowest quintile of intake). The relationship between *trans* fatty acids was not diminished when the model was adjusted for LDL and HDL, suggesting that the association observed between self-reported *trans* fatty ingestion and CHD does not depend on an HDL lowering action of *trans* fatty acids.

Lp(a)

The effects of trans fatty acids on Lp(a) have been evaluated in only four studies, three suggesting an Lp(a) raising effect of trans while one suggesting a neutral effect.

Analyzing changes in Lp(a) concentrations in the Mensink and Katan Study, 11% of energy as trans resulted in significant increases in the median Lp(a) concentrations (saturated diet 22 mg/L, oleic acid 27 mg/L and trans fatty acids 44 mg/L). Since Lp(a) concentrations do not conform to Gaussian distribution, Lp(a) concentrations were transformed to the square root. Using the square root transformed means ± SD, the values for the three feeding periods were: saturated fatty acids 5.6 ± 4.6, oleic acid 5.9 ± 4.6 and trans 6.8 ± 4.9. The trans fatty acid square root transformed means on the trans diet were significantly different than either the saturated or oleic diet, $p < 0.02$.

A similar analysis of serum from the Zock et al. study (102) was done. The median Lp(a) concentration on the stearate diet was 78 mg/L, the linoleate diet was 84 mg/L, and the trans diet was 103 mg/L (trans significantly higher than linoleate or stearate $p < 0.02$). The square root transformed means were 8.8 ± 5.5, 8.8 ± 5.5 and 9.4 ± 5.9, respectively, with the highest value seen on the trans diet ($p < 0.02$).

An Lp(a) raising effect for trans was also observed by Nestel et al. (95). Lp(a) concentrations on the elaidic acid diet (207 ± 154 mg/L) were significantly higher than those obtained on the habitual diet (166 ± 127 mg/L) and on the palmitic diet (174 ± 143 mg/L). However, the elaidic acid Lp(a) concentrations

were not significantly different than that observed on the oleic diet (165 ± 141 mg/L) suggesting very large individual variation in responsiveness may have contributed to the observed effect. Lichtenstein et al. (83) found that feeding 4% energy as trans did not change the average Lp(a) concentration (corn oil diet 160 ± 210 mg/L; corn oil margarine diet 130 ± 190 mg/L, P = 0.22).

Further interpretation of the Lp(a) findings must be done cautiously. Lp(a) concentrations are not normally distributed in the population. The question can be raised whether an Lp(a) effect is limited to only those with high Lp(a) concentrations. Data on individual responsiveness, presented in the two Netherlands studies (102), suggest that individuals with low Lp(a) concentrations were unlikely to have increases during the trans dietary period. Why an Lp(a) raising effect would be limited to subjects with higher baseline concentrations is puzzling. Responsiveness limited to individuals with higher levels may either be assay artifact or a diet-gene interaction. Higher Lp(a) concentrations have greater intra assay variations than lower concentrations since most assays have greater reliability at lower concentrations (103). Although the four studies above used three different assay techniques (Nestel et al., radioimmunoassay; Zock and Katan, ELISA with two polyclonal antibodies; Lichtenstein et al., ELISA with one polyclonal antibody and one monoclonal antibody that does not cross react with plasminogen), the possibility of an assay artifact contributing to the observed effect cannot be ruled out. Since Lp(a) levels are genetically determined (104), a diet-gene interaction, while still a possibility, cannot be fully assessed until Lpa) assays are standardized.

SUMMARY AND CONCLUSIONS

As with any diet-disease relationship, the relationship between ingestion of hydrogenated fats and risk for coronary disease should be assessed only after considering all available data. Since diet-disease relationships are established on the basis of multiple types of investigation, the assessment of the strength of a specific relationship should be based on the breadth of studies that suggest a relationship.

Evidence from both prospective and case-control nutritional epidemiology studies using self-reported estimate of *trans* fatty acid intake from food frequency questionnaires suggest a positive association between *trans* fatty acid intake and CHD. On the other hand, evidence from multiple case-control studies using the *trans* fatty acid content of adipose tissue as an estimate of *trans* fatty acid intake

show no association. Animal models of diet-induced atherosclerosis suggest that *trans* fatty acids are less atherogenic than saturated fatty acids despite the fact that, in several studies, *trans* fatty acid feeding raised cholesterol levels close to that observed of saturated fat. In human feeding studies, hydrogenated fat feeding will lower total and LDL cholesterol levels when substituted for butter or palm oil; however, hydrogenated fat feeding will raise total and LDL cholesterol levels when *substituted* for an unhydrogenated liquid vegetable oil. The effects of *trans* ingestion on HDL cholesterol levels remains unclear. Taken together, the data do not support the assertation that *trans* fatty acids ingestion promotes CHD.

Despite the fact that further research is needed to clarify the exact role that *trans* fatty acids may play in atherogensis, clinicians can make straightforward recommendations to their patients based on the science as we know it today.

1) Hydrogenated fats will lower total and LDL cholesterol concentrations when substituted for animal fats (butter, lard) and vegetable fats rich in saturates (palm oil, palm-kernel oil, and coconut oil). Given the extensive epidemiologic literature supporting a causal relationship between total cholesterol concentrations and coronary heart disease in humans, hydrogenated fats are a superior substitute for the traditional shortenings of butter, lard and palm oil. Patients should choose margarine over butter and tub margarine is preferable to stick margarine.

2) Hydrogenated fats, if substituted for the parent vegetable oils, will produce some increases in total and LDL cholesterol concentrations. Therefore, patients should use liquid oils low in saturated fatty acids instead of margarine wherever possible.

3) Since margarine only accounts for 1/3 of all *trans* intake (Table 18), efforts to reduce *trans* from non-margarine sources would be made. Since hydrogenated fats are ubiquitous, strategies to reduce total fat will also reduce *trans* intake.

4) Despite the perception that *trans* fatty acids are of major importance, Americans eat 4-10 times as much saturated fat than they do *trans* fat. A diet aimed at reducing the risk of CHD by lowering cholesterol levels is a diet focussed on reducing saturated fat intake.

TABLE 18
***TRANS* CONTENT OF US DIET**
SPECIFIC FOOD SOURCES

	g/d
Household shortenings	0.3
margarines + spreads	2.5
food service fats + oils	1.6
commercial products	2.2
meats + dairy products	1.3
	7.9 g/d

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