EFFECT OF DIETARY TRANS FATTY ACIDS ON HIGH-DENSITY AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN HEALTHY SUBJECTS

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Abstract *Background.* Fatty acids that contain a trans double bond are consumed in large amounts as hydrogenated oils, but their effects on serum lipoprotein levels are unknown.

Methods. We placed 34 women (mean age, 26 years) and 25 men (mean age, 25 years) on three mixed natural diets of identical nutrient composition, except that 10 percent of the daily energy intake was provided as oleic acid (which contains one cis double bond), trans isomers of oleic acid, or saturated fatty acids. The three diets were consumed for three weeks each, in random order.

Results. On the oleic acid diet, the mean (±SD) serum values for the entire group for total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol were 4.46±0.66, 2.67±0.54, and 1.42±0.32 mmol per liter (172±26, 103±21, and 55±12 mg per deciliter), respectively. On the trans-fatty-acid diet, the subjects' mean HDL cholesterol level was 0.17 mmol per liter (7 mg per deciliter) lower than the mean value on the diet high in oleic acid ($P<0.0001$; 95 percent confidence interval, 0.13 to 0.20 mmol per liter). The HDL cholesterol level on the saturated-fat diet was the same as on the oleic acid diet. The LDL cholesterol level was 0.37 mmol per liter (14 mg per deciliter) higher on the trans-fatty-acid diet than on the oleic acid diet ($P<0.0001$; 95 percent confidence interval, 0.28 to 0.45 mmol per liter) and 0.47 mmol per liter (18 mg per deciliter) higher on the saturated-fat diet ($P<0.0001$; 95 percent confidence interval, 0.39 to 0.55 mmol per liter) than on the oleic acid diet. The effects on lipoprotein levels did not differ between women and men.

Conclusions. The effect of trans fatty acids on the serum lipoprotein profile is at least as unfavorable as that of the cholesterol-raising saturated fatty acids, because they not only raise LDL cholesterol levels but also lower HDL cholesterol levels. (N Engl J Med 1990; 323:439-45.)

RECENT studies have emphasized the importance of monounsaturated fatty acids in reducing saturated-fat intake and thereby lowering the serum level of the atherogenic low-density lipoprotein (LDL) cholesterol.[1,2] These studies have focused on oleic acid, a monounsaturated fatty acid with the cis configuration; however, many foods also contain trans fatty acids. These are unsaturated fatty acids in which the carbon moieties on the two sides of a double bond point in opposite directions. Most natural fats and oils contain only cis double bonds, in which the carbon moieties lie on the same side (Fig. 1). Trans fatty acids are found in small amounts in the fats of ruminants; for example, 100 g of milk fat contains 4 to 8 g of trans fatty acids.[3] Much larger amounts are found in certain types of margarines, margarine-based products, shortenings, and fats used for frying.[4,5] These trans fatty acids are formed when vegetable and marine oils, rich in polyunsaturated fatty acids, are hardened by a process called hydrogenation to produce fats that have the firmness and plasticity desired by food manufacturers and consumers.[6] The estimated average daily intake of trans fatty acids is 8 to 10 g, or 6 to 8 percent of total daily fat consumption, in the United States[7] and 17 g in the Netherlands.[8] Intake may be much higher in persons who eat large amounts of hard fats and margarines or foods prepared with or fried in such fats. The pressure to reduce the use of saturated tropical fats such as palm oil may cause an increase in the consumption of trans fatty acids, because for the edible-fat industry they are the best alternative to saturated fatty acids for the production of semisolid and solid fats.

The most abundant trans fatty acids in the diet are elaidic acid and its isomers,[7] which are fatty acids with 18 carbon atoms and one double bond (Fig. 1). Two studies suggested that these trans fatty acids, as compared with their cis isomer oleic acid, elevate serum total cholesterol levels.[9,10] However, this effect was not confirmed in a third study.[11] We have now studied the effects of trans fatty acids — specifically, elaidic acid and its isomers — on serum lipoprotein and apolipoprotein levels in healthy women and men.

METHODS

Subjects

Forty-eight women and 27 men, most of them students, applied for enrollment in the study. None had a history of atherosclerotic disease, and all were apparently healthy, as indicated by the results of a medical questionnaire. None had anemia, glycosuria, or proteinuria. One woman was being treated for hypertension with a β-adrenergic blocking agent; none of the other subjects were taking medication known to affect serum lipids. The protocol and goals of the study were fully explained to the subjects, who gave their written consent. No payment was given, except the free food in the study diets. Approval for the study had previously been obtained from the ethics committee of the department.

We accepted all 27 men. Because only 62 subjects could participate, we then added 1 woman who was married to a participant and selected another 34 women at random. They included the woman with hypertension, who did not change the dosage of her medicine throughout the study. One woman and two men withdrew before the study began. Thus, 25 men and 34 women started the study, and all finished it successfully. Before the beginning of the study diets, the subjects' fasting serum lipid levels ranged from 3.40 to 7.15 mmol per liter (mean, 4.75 mmol per liter [184 mg per deciliter]) for total cholesterol, from 0.63 to 2.38 mmol per liter (mean, 1.30 mmol per liter [50 mg per deciliter]) for high-density lipoprotein (HDL) cholesterol, and from 0.36 to 2.54 mmol per liter (mean, 0.96 mmol per liter [85 mg per deciliter]) for triglycerides.

The men were between 19 and 52 years of age (mean, 25). They

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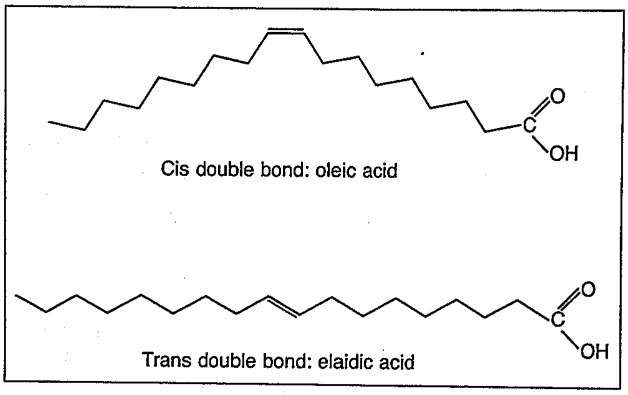

Figure 1. Structure of Cis and Trans Fatty Acids.
Cis double bonds produce a bend in the molecule that impairs crystallization and keeps the oil liquid. To convert vegetable and marine oils into fats of various degrees of plasticity, manufacturers of edible fat straighten out cis unsaturated fatty acids by converting them to trans isomers or saturated fatty acids in a process called hydrogenation.

weighed 65 to 87 kg (mean, 75) and their body-mass index (the weight in kilograms divided by the square of the height in meters) ranged from 18.4 to 25.4 (mean, 22.0). The women were between 19 and 57 years of age (mean, 26). They weighed 54 to 90 kg (mean, 64), and their body-mass index ranged from 17.4 to 29.7 (mean, 22.0). Eight women used oral contraceptives, and two women, but none of the men, smoked.

Design and Statistical Analysis

Each participant followed the three diets for three weeks each, with no washout period between the diets. In our experience, serum lipoprotein levels stabilize within two weeks after a dietary change.[12,13] One diet was high in oleic acid, another high in trans isomers of oleic acid, and the third high in saturated fat, notably lauric acid (C12:0) and palmitic acid (C16:0). Before the study began, the subjects were categorized according to sex, and women were also categorized according to the use of oral contraceptives. The subjects were then randomly divided into six groups so that each group had a nearly identical number of subjects from each category. Each group then received the diets in a different order. In this way, variation due to residual effects of the previous diet or to the drift of variables over time could be assessed and eliminated.[14]

The data were analyzed with the General Linear Models procedure of the Statistical Analysis System.[15] When the analysis indicated a significant effect of a diet ($P<0.05$), the Bonferroni method was used for a pairwise comparison of the diets. Since this procedure involved three simultaneous comparisons, the upper limit of statistical significance was set at one third of the customary level of 0.05 — i.e., 0.017. All P values are two-tailed.

Diets

Before the study began, the subjects weighed their food and recorded their habitual diet for two working days and one weekend day to allow us to estimate their energy and nutrient intake. The food records were coded and the composition of the diets calculated with use of the 1986 edition of the Netherlands Nutrient Data Base.[16]

The diets followed during the study consisted of conventional solid foods, and the menus were changed daily during each three-week cycle. The nutrient content of the three diets was similar, except for 10 percent of total energy, which was provided by oleic acid, trans isomers of oleic acid, or saturated fatty acids. The oleic acid group received special bread enriched with olive oil and a margarine made of a variety of sunflower oil high in oleic acid (Trisun, SVO Enterprises, Wickliffe, Ohio). This oleic acid–rich sunflower oil made up 85 percent of the fat in the margarine: the remaining 15 percent was composed of equal volumes of lightly hydrogenated palm oil and palm-kernel oil that were mixed and then interesterified. The sunflower oil contributed 45 percent of all monounsaturated fatty acids in the oleic acid diet, olive oil contributed 21 percent, and rapeseed oil low in erucic acid, 10 percent. For the trans-fatty-acid diet, the same oleic acid–rich sunflower oil was isomerized so that half the oleic acid molecules were converted to the trans configuration, while avoiding the isomerization of linoleic acid (Table 1). Seventy-eight parts of this hydrogenated fat were subsequently mixed with 10 parts of the unaltered oleic acid–rich sunflower oil, 10 parts of regular sunflower oil, and 2 parts of rapeseed oil low in erucic acid, yielding a margarine and a shortening with high levels of trans fatty acids. This shortening was also used to prepare a special bread containing 8 g of shortening per 100 g. The saturated-fat diet included another special kind of margarine and shortening, this one high in lauric acid and palmitic acid (Table 1). These fats were made by interesterifying 24.5 parts of lightly hydrogenated palm oil and 35.5 parts of lightly hydrogenated palm-kernel oil. This mixture was then added to 40 parts of the oleic acid–rich sunflower oil to prepare the shortening and margarine. The special fats and margarines were developed and manufactured by the Unilever Research Laboratory (Vlaardingen, the Netherlands). Other typical items consumed during the study were bread, full-fat cheese, low-fat meat for sandwiches, milk or yogurt (low-fat varieties were used for the oleic acid and trans-fatty-acid diets), fruit, cookies, jam or honey, potatoes, cooked vegetables, salad garnished with egg yolk, gravy, and occasionally, an egg.

The diets were formulated at 28 levels of energy intake ranging from 5.5 to 20.0 MJ (1315 to 4780 kcal) per day. The intake of protein, carbohydrates, alcohol, cholesterol, and dietary fiber did not differ among the diets. In addition, the consumption of the various saturated and polyunsaturated fatty acids was the same on the trans-fatty-acid diet as on the oleic acid diet (Table 2).

All foodstuffs were weighed for each subject. On weekdays at noon, hot meals were served in the department and eaten in our presence. All other food was provided daily as a package. Food for the weekend and guidelines for its preparation were provided each Friday. In addition to the foods supplied, the subjects were allowed to eat a limited number of items free of fat and cholesterol. These free-choice items were listed and accorded points corresponding to their energy values, with one point equaling 41.8 kJ (10 kcal). Each subject was required to consume daily food totaling a specific number of points that varied slightly with energy intake and ranged from 7 to 11 percent of the total daily energy intake. The subjects were urged not to change their selection of free-choice items between diet periods.

The subjects were asked to maintain their usual patterns of activity, smoking habits, and use of oral contraceptives. They recorded in diaries any sign of illness, medications used, the free-choice items selected, and any deviations from their diets. Inspection of the diaries did not reveal any deviations from the protocol that might have affected the results.

Duplicate portions of each diet for one imaginary participant with a daily energy intake of 10 MJ (2390 kcal) were collected during each of the three periods, stored at −20°C, pooled according to diet, and analyzed after the study. The free-choice items consumed were coded, and their composition was calculated with use of the Netherlands Nutrient Data Base.[16] The analyzed values of the duplicate diets were combined with the calculated values for the free-choice items (Table 2).

Body weights without shoes or heavy clothing were recorded twice a week, and energy intake was adjusted when necessary. During the 63 days of the study, the average body weight (±SD) decreased by 0.1±1.0 kg (range, −2.2 to 2.7), but this decrease was not attributable to a specific diet.

Blood Sampling and Analysis

Before the study all subjects were assigned a random number that was then used for labeling blood and serum or plasma tubes. In this

Table 1. Fatty-Acid Composition of the Margarines Used in the Three Diets.

Fatty Acid	Oleic Acid Diet	Trans-Fatty-Acid Diet	Saturated-Fat Diet
	grams per 100 g of fatty acid		
Saturated	22.5	13.3	44.0
Lauric acid (C12:0)	2.7	0.2	14.2
Myristic acid (C14:0)	1.5	0.1	5.4
Palmitic acid (C16:0)	6.6	3.9	15.4
Stearic acid (C18:0)	9.6	7.2	6.2
Monounsaturated	71.1	77.7	48.6
Cis-C18:1	70.7	32.1*	47.9
Trans-C18:1	0.3	45.8†	0.6
Polyunsaturated	6.5	8.1	7.8
Linoleic acid (cis-cis-C18:2)	6.2	7.8	7.4
Others	0.1	1.1	0.0

*Distribution of positional cis isomers per 100 g of fatty acid: C18:1(n−10/Δ8), 8.5 g; C18:1(n−9/Δ9), 15.8 g; C18:1(n−8/Δ10), 3.0 g; C18:1(n−7/Δ11), 2.6 g; C18:1(n−6/Δ12), 1.2 g; C18:1(n−5/Δ13), 0.6 g; and C18:1(n−4/Δ14), 0.3 g.

†Distribution of positional trans isomers per 100 g of fatty acid: C18:1(n−12/Δ6), 0.3 g; C18:1(n−11/Δ7), 0.8 g; C18:1(n−10/Δ8), 4.6 g; C18:1(n−9/Δ9), 13.3 g; C18:1(n−8/Δ10), 10.6 g; C18:1(n−7/Δ11), 9.4 g; and C18:1(n−6/Δ12), 6.7 g.

way, the technicians who performed the chemical analyses were unaware of the subjects' diet sequence.

Blood was sampled after an overnight fast on days 1, 18, and 21 (period 1), days 39 and 42 (period 2), and days 60 and 63 (period 3). Serum was obtained by low-speed centrifugation, stored at −80°C, and analyzed enzymatically for total and HDL cholesterol and triglyceride levels at the end of the study.[18,19] All the samples from each subject were analyzed in one run. The coefficient of variation within one run for control serum samples was 0.9 percent for total cholesterol, 1.3 percent for HDL cholesterol, and 1.7 percent for triglycerides. Accuracy was checked by the analysis of three serum pools of known value provided by the Centers for Disease Control (Atlanta) and, for HDL cholesterol only, of three pools provided by the North-West Lipid Research Clinic.[20] The mean bias with regard to target values of the Centers for Disease Control pools was 0.01 mmol per liter for total cholesterol and 0.08 mmol per liter for total triglycerides. The mean bias with regard to the target value of the North-West Lipid Research Clinic pools for HDL cholesterol was −0.06 mmol per liter. The LDL cholesterol concentration was calculated with use of the Friedewald equation.[21] The two lipoprotein values obtained at the end of each dietary period were averaged for data analyses.

For each subject, the fatty-acid composition of erythrocyte membranes was determined in duplicate in samples obtained at the end of each dietary period (days 21, 42, and 63), as described earlier, except that we used a capillary Sil-88 column.[22] The results were expressed as a proportion by weight of all fatty acids detected. For the apolipoprotein analyses, equal volumes of the two plasma samples obtained at the end of each dietary period were pooled. Apolipoprotein A-I was measured by immunoturbidimetry,[23] with sheep antiserum. Apolipoprotein B was measured in plasma in all samples by radial immunodiffusion with antiserum raised in goats.[24] All samples for each subject were analyzed in duplicate on the same plate, and the results of each diet period were averaged. The secondary calibration pools for apolipoprotein B were calibrated with the use of a pool of known value provided by the Centers for Disease Control (Pool IUIS/NHLBI/CDC 1883) as the primary standard. The coefficient of variation within one run was 2.5 percent for apolipoprotein A-I and 3.9 percent for apolipoprotein B.

Results

The mean daily intakes of energy and nutrients on the three experimental diets are shown in Table 2. Total fat and cholesterol intakes did not differ among diets during the study. The percentage of total energy from oleic acid decreased from 23.0 percent on the oleic acid diet to 12.6 percent on the trans-fatty-acid diet and to 12.8 percent on the saturated-fat diet. It was replaced by energy from either trans fatty acids — specifically, elaidic acid and its positional isomers — or saturated fatty acids alone.

The changes in the fatty-acid composition of the erythrocyte membranes during the three dietary periods confirmed the subjects' adherence to the diets. The mean (±SD) percentage of cis-C18:1 was 13.2±0.8 on the oleic acid diet, 11.6±0.8 on the trans-fatty-acid diet, and 12.1±0.8 on the saturated-fat diet. These values are significantly different from each other ($P<0.0001$ for each comparison). The mean percentage of trans-C18:1 was 0.9±0.3 on the oleic acid and the saturated-fat diets and 2.6±0.5 on the trans-fatty-acid diet ($P<0.0001$).

As compared with its level on the oleic acid diet (Table 3), the serum total cholesterol value was 0.26 mmol per liter (10 mg per deciliter) higher on the trans-fatty-acid diet ($P<0.0001$; 95 percent confidence interval, 0.17 to 0.35 mmol per liter) and 0.54 mmol per liter (21 mg per deciliter) higher on the saturated-fat diet ($P<0.0001$; 95 percent confidence interval, 0.45 to 0.63 mmol per liter). The difference between

Table 2. Mean Daily Intake of Energy and Nutrients of Subjects on the Oleic Acid Diet, the Trans-Fatty-Acid Diet, and the Saturated-Fat Diet.*

Energy/Nutrient	Oleic Acid Diet	Trans-Fatty-Acid Diet	Saturated-Fat Diet
Energy			
MJ/day	11.6±2.8	11.5±2.7	11.4±2.9
kcal/day	2780±669	2751±650	2734±703
Protein	13.1	13.3	14.0
Fat	39.6	40.2	38.8
Saturated fatty acids	9.5	10.0	19.4
Lauric acid (C12:0)	0.5	0.4	3.4
Myristic acid (C14:0)	0.5	0.7	2.7
Palmitic acid (C16:0)	4.7	4.3	8.1
Stearic acid (C18:0)	3.0	3.6	3.5
Monounsaturated fatty acids	24.1	24.2	14.7
Cis-C18:1	23.0	12.6	12.8
Trans-C18:1	0.0	10.9	1.8
Total trans†	0.0	11.0	0.8
Polyunsaturated fatty acids	4.6	4.6	3.4
Linoleic acid (cis-cis-C18:2)	4.0	4.2	2.9
Carbohydrates	46.3	45.6	46.1
Monosaccharides and disaccharides	26.3	25.3	24.0
Polysaccharides	19.9	20.2	22.0
Alcohol	1.1	0.9	1.3
Cholesterol (mg/MJ)‡	35.0	31.9	33.6
Dietary fiber (g/MJ)‡	4.1	4.1	4.3

*Unless otherwise indicated, values are expressed as percentages of total daily energy intake. Values are based on chemical analyses of duplicate diets plus the calculated contribution of free-choice items (see Methods). Each value represents the mean of three different periods, during which each diet was consumed by one third of the subjects. Differences in dietary composition between periods and between subjects were negligible, and standard deviations are therefore not given. Specially prepared experimental fats (Table 1) provided 25 percent of the total fat intake in the oleic acid diet, 65 percent in the trans-fatty-acid diet, and 39 percent in the saturated-fat diet.

†Determined by infrared spectroscopy and expressed in terms of elaidic acid.[17] Trans fatty acids in the saturated-fat diet were largely derived from milk and cheese; those in the trans-fatty-acid diet were derived almost entirely from the experimental hydrogenated fat.

‡To convert values to amounts per 1000 kcal, multiply by 4.184.

Table 3. Serum Lipid and Lipoprotein Levels after Three Weeks of a Diet High in Oleic Acid, Trans Monounsaturated Fatty Acids, or Saturated Fatty Acids.*

Lipid/Lipoprotein	Oleic Acid Diet	Trans-Fatty-Acid Diet	Saturated-Fat Diet	P Values for Comparison† Oleic Acid vs. Trans FA	Oleic Acid vs. Sat FA	Trans FA vs. Sat FA
	mmol per liter					
Total cholesterol						
Men	4.23±0.72	4.47±0.77	4.79±0.82	<0.0001	<0.0001	<0.0001
Women	4.63±0.57	4.90±0.64	5.15±0.59	<0.0001	<0.0001	<0.0001
All	4.46±0.66	4.72±0.72	5.00±0.71	<0.0001	<0.0001	<0.0001
LDL cholesterol						
Men	2.59±0.61	2.93±0.65	3.05±0.66	<0.0001	<0.0001	0.0086
Women	2.73±0.48	3.12±0.58	3.20±0.50	<0.0001	<0.0001	0.0997
All	2.67±0.54	3.04±0.61	3.14±0.57	<0.0001	<0.0001	<0.0001
HDL cholesterol						
Men	1.24±0.29	1.10±0.23	1.28±0.28	<0.0001	0.0544	<0.0001
Women	1.55±0.32	1.37±0.27	1.53±0.31	<0.0001	0.5411	<0.0001
All	1.42±0.32	1.25±0.29	1.42±0.32	<0.0001	0.5293	<0.0001
Triglycerides						
Men	0.86±0.42	0.99±0.50	1.00±0.63	0.0267	0.0379	0.8807
Women	0.78±0.29	0.91±0.30	0.91±0.32	<0.0001	<0.0001	0.9613
All	0.81±0.35	0.94±0.40	0.94±0.47	<0.0001	<0.0001	0.8680

*Plus–minus values are means ±SD. The 25 men and 34 women were on the diets for three weeks each in random order. To convert values for total, HDL, and LDL cholesterol to milligrams per deciliter, multiply by 38.67. To convert values for triglycerides to milligrams per deciliter, multiply by 88.54.

†FA denotes fatty acids, and SAT saturated.

the trans-fatty-acid diet and the saturated-fat diet of 0.28 mmol per liter (11 mg per deciliter) was also significant (P<0.0001; 95 percent confidence interval, 0.18 to 0.37 mmol per liter). As compared with its level on the oleic acid diet, HDL cholesterol was 0.17 mmol per liter (7 mg per deciliter) lower on the trans-fatty-acid diet (P<0.0001; 95 percent confidence interval, 0.13 to 0.20 mmol per liter); the level was the same on the saturated-fat and oleic acid diets. The HDL cholesterol–lowering effect of trans fatty acids was evident in each of the six diet groups and in 54 of the 59 subjects (Fig. 2). The changes in apolipoprotein A-I levels paralleled those in HDL cholesterol (Table 4). The LDL cholesterol level was 0.37 mmol per liter (14 mg per deciliter) higher on the trans-fatty-acid diet (P<0.0001; 95 percent confidence interval, 0.28 to 0.45 mmol per liter) and 0.47 mmol per liter (18 mg per deciliter) higher on the saturated-fat diet (P<0.0001; 95 percent confidence interval, 0.39 to 0.55 mmol per liter) than on the oleic acid diet (Table 3 and Fig. 3). The plasma apolipoprotein B level was highest on the trans-fatty-acid diet and lowest on the oleic acid diet (Table 4). The ratio of LDL to HDL cholesterol levels was 2.02±0.76 on the oleic acid diet, 2.58±0.94 on the trans-fatty-acid diet, and 2.34±0.79 on the saturated-fat diet; all three values are significantly different from each other (P<0.0001 for each comparison). The ratio of total cholesterol to HDL cholesterol levels increased from 3.31±0.92 on the oleic acid diet to 3.96±1.19 on the trans-fatty-acid diet and to 3.68±1.00 on the

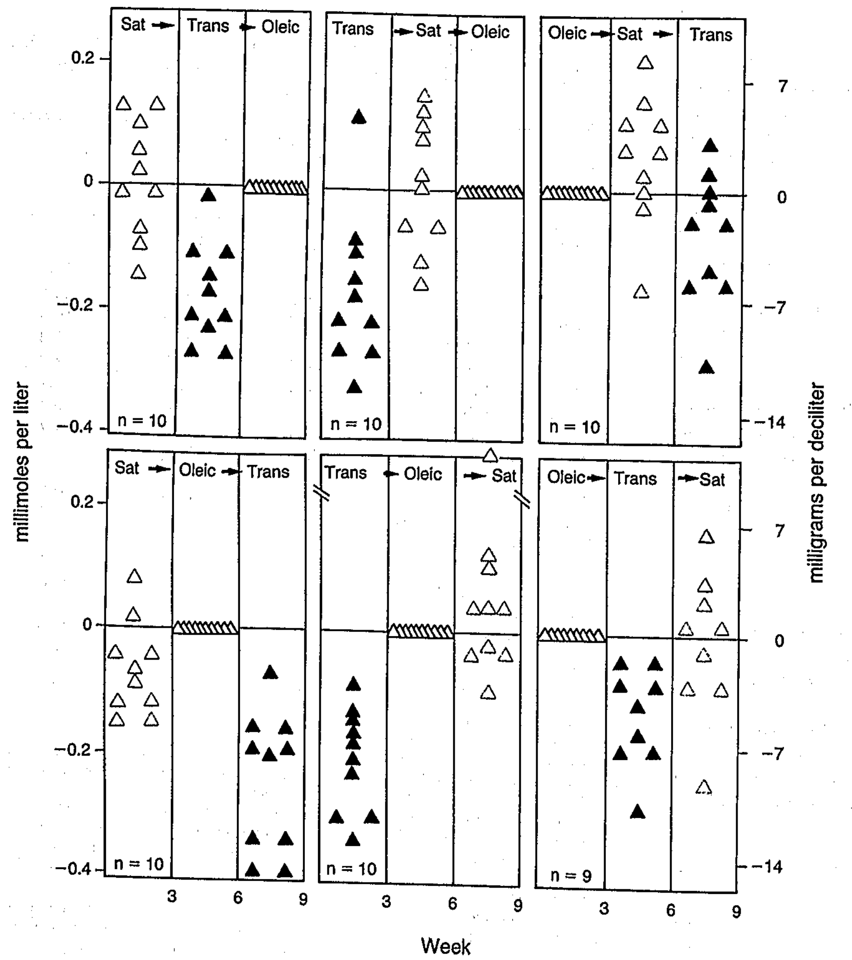

Figure 2. Individual Changes in Serum HDL Cholesterol Levels on a Diet High in Trans Fatty Acids (Trans) or Saturated Fatty Acids (Sat), as Compared with a Diet High in Oleic Acid.

Each point denotes the HDL cholesterol level of a subject on a particular diet minus his or her level when consuming the diet high in oleic acid. Each subject was on each diet for three weeks in random order.

saturated-fat diet. These values are also significantly different from each other (P<0.0001). The ratio of apolipoprotein A-I to apolipoprotein B levels was 1.46±0.32 on the oleic acid diet, 1.19±0.27 on the trans-fatty-acid diet, and 1.32±0.31 on the saturated-fat diet. These values are significantly different from each other (P<0.0001). The level of triglycerides was 0.13 mmol per liter (12 mg per deciliter) higher on both the trans-fatty-acid and saturated-fat diets than on the oleic acid diet (P<0.0001; 95 percent confidence interval, 0.06 to 0.20 mmol per liter).

The ratio of apolipoprotein B to LDL cholesterol levels was 356±40 on the oleic acid diet, 357±48 on the trans-fatty-acid diet, but 334±35 on the saturated-fat diet, which was significantly lower (P<0.0001) than the other two values. The ratio of apolipoprotein A-I to HDL cholesterol levels was 965±129 on the oleic acid diet and 960±112 on the saturated fat diet, and it increased to 1009±127 on the trans-fatty-acid diet; this value was significantly higher than the other two ratios (P<0.0001).

All these effects were observed to a similar extent in men and women. In addition, the responses to the various diets did not differ between the 8 women using oral contraceptives and the other 26 women.

Discussion

The few previous studies that specifically examined the effects of trans fatty acids on total serum cholesterol levels yielded conflicting results. Mattson et al.[11] did not find a hypercholesterolemic effect of trans-C18:1 as compared with oleic acid. Studies by Vergroesen[9] and Vergroesen and Gottenbos,[10] however, suggested that trans isomers of oleic acid have a cholesterol-raising effect, although it is less strong than that of saturated fatty acids. The present study confirmed this report: we found that trans fatty acids were hypercholesterolemic as compared with oleic acid and that their effect was about half that of a

Table 4. Plasma Apolipoprotein A-I and Apolipoprotein B Levels after Three Weeks of a Diet High in Oleic Acid, Trans Monounsaturated Fatty Acids, or Saturated Fatty Acids.*

Apolipoprotein	Oleic Acid Diet	Trans-Fatty-Acid Diet	Saturated-Fat Diet	P Values for Comparison† Oleic Acid vs. Trans FA	Oleic Acid vs. Sat FA	Trans FA vs. Sat FA
	mg per liter					
Apolipoprotein A-I						
Men	1204±160	1129±154	1247±186	0.0002	0.0474	<0.0001
Women	1423±173	1313±178	1407±179	<0.0001	0.3354	<0.0001
All	1330±199	1235±191	1339±197	<0.0001	0.5188	<0.0001
Apolipoprotein B						
Men	934±214	1069±237	1030±247	<0.0001	<0.0001	0.0392
Women	944±118	1073±156	1055±141	<0.0001	<0.0001	0.2025
All	940±164	1071±193	1045±192	<0.0001	<0.0001	0.0169

*Plus–minus values are means ±SD. The 25 men and 34 women were on the diets for three weeks each in random order.
†FA denotes fatty acids, and SAT saturated.

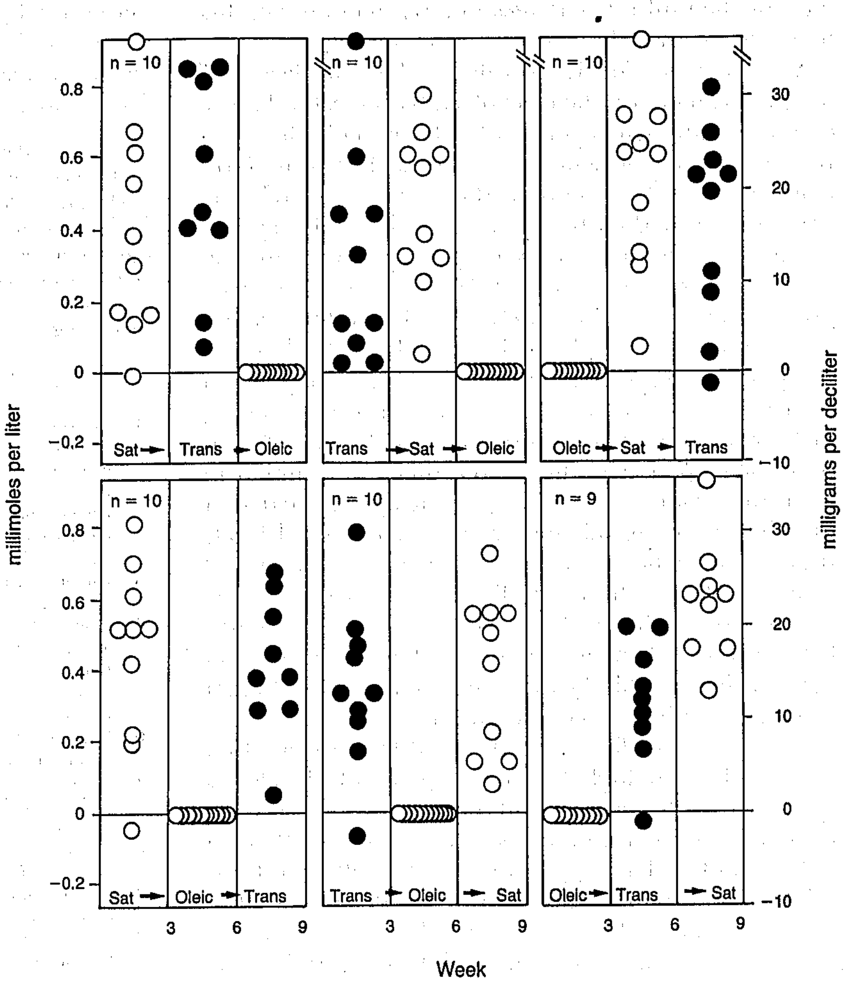

Figure 3. Individual Changes in Serum LDL Cholesterol Levels on a Diet High in Trans Fatty Acids (Trans) or Saturated Fatty Acids (Sat), as Compared with a Diet High in Oleic Acid.

Each point denotes the LDL cholesterol level of a subject on a particular diet minus his or her level when consuming the diet high in oleic acid. Each subject was on each diet for three weeks in random order.

mixture of saturated fatty acids (lauric, myristic, and palmitic acid). However, the effect of trans fatty acids on the lipoprotein risk profile was more unfavorable than is suggested by the small increase in the serum total cholesterol concentration. The level of LDL cholesterol was higher, but the level of HDL cholesterol was lower, and as a result the ratio of LDL to HDL cholesterol was even higher on the trans-fatty-acid diet than on the saturated-fat diet. Laine et al.[25] studied the effects of lightly hydrogenated soybean oil on serum lipoprotein levels in healthy men and women. They found that, as compared with hydrogenated soybean oil, unhydrogenated soybean oil decreased LDL cholesterol levels by 0.52 mmol per liter. The level of saturated fatty acids was the same in the two diets, but the intake of polyunsaturated fat as a percentage of total energy intake was 7 percent lower and the intake of trans fatty acids 3 percent higher on the hydrogenated soybean-oil diet. The hydrogenated oil did not decrease HDL cholesterol levels in that study,[25] but in view of the multiple changes in fatty-acid intake, this does not necessarily contradict our finding.

We have previously postulated that all classes of fatty acids increase the level of HDL cholesterol more or less equally when substituted for carbohydrates in the diet.[26] The present study shows that trans fatty acids may be an exception; the replacement of oleic acid or saturated fat by trans fatty acids as 10 percent of total energy intake decreased HDL cholesterol levels by 0.17 mmol per liter (Table 3), which is even greater than the decline in HDL cholesterol levels that occurs after fat is replaced by carbohydrates.[13]

Apolipoprotein B levels were somewhat higher on the trans-fatty-acid diet than on the saturated-fat diet, but considerably lower on the oleic acid diet. In addition, the responses to the various diets were similar in men and women. We have previously reported that men had a greater change in HDL cholesterol levels than women when saturated fatty acids were removed from the diet.[2] The main difference was that in the present study, the level of stearic acid did not change between the oleic acid and the high–saturated-fat diets. Whether this factor may explain the different results needs further investigation.

Both the trans-fatty-acid diet and the saturated-fat diet resulted in higher serum triglyceride levels, as compared with the oleic acid diet. In this respect, too, results from other studies are conflicting. Mattson et al.[11] did not find a triglyceride-elevating effect of trans relative to cis monounsaturates. Anderson et al.,[27] however, found that triglyceride levels were higher when subjects were on a diet high in trans isomers of monounsaturated and polyunsaturated fatty acids than when they were on a diet high in butterfat.

The average consumption of trans fatty acids in the United States is 2 to 4 percent of daily energy intake.[7] Linear extrapolation from the results of the present study would suggest that replacing all trans fatty acids in the diet with oleic acid might increase the level of HDL cholesterol by an average of 0.05 mmol per liter (1.8 mg per deciliter) and decrease the level of LDL cholesterol by 0.10 mmol per liter (3.9 mg per deciliter). We do not know, however, whether the effect of trans fatty acids on the levels of HDL and LDL cholesterol is proportional to intake. In addition, we have specifically examined trans fatty acids with 18 carbon atoms and one double bond, the most important class of fatty acids in the diet.[7] We do not know whether our results can be extended to trans fatty acids with a higher number of carbon atoms, such as those formed when fish oil is hydrogenated. Our findings do not even apply unreservedly to all hydrogenated fats and oils rich in trans fatty acids with 18 carbon atoms and one double bond. Although the spectrum of trans fatty acids in our experimental diet was quite similar to that in hydrogenated soybean oil[28] — the major source of trans fatty acids in the American diet — subtle but important differences in composition might exist between the hydrogenated fat we used and that used in actual food production. Nevertheless, for the time being it would seem prudent for patients at increased risk of atherosclerosis to avoid a high intake of trans fatty acids.

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LACK OF EFFICACY OF HYDERGINE IN PATIENTS WITH ALZHEIMER'S DISEASE

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Abstract *Background.* There is no effective pharmacologic treatment for Alzheimer's disease, the most common dementing illness in the United States. Hydergine, a combination of ergoloid mesylates, is the only approved medication for Alzheimer's disease, but despite widespread use its efficacy remains to be established. We conducted a clinical trial of Hydergine-LC, a newer preparation of ergoloid mesylates in the form of a liquid in a capsule (LC) that may have greater bioavailability, to determine its value in patients with Alzheimer's disease.

Methods and Results. Eighty older adults with probable Alzheimer's disease participated in this double-blind, placebo-controlled trial of Hydergine-LC for 24 weeks. The recommended dose of 1 mg orally three times daily was used. Cognition and behavior were evaluated before and after the trial, and the patients were monitored for adverse effects. The medication was safe and well tolerated. The Hydergine-LC group did not perform better after treatment than the placebo group on any test, and its performance was worse ($P<0.01$ and $P<0.02$, respectively) on one cognitive measure (Wechsler Adult Intelligence Scale Digit Symbol Substitution Task) and on one behavioral scale (the Geriatric Evaluation by Relatives Rating Instrument).

Conclusions. Hydergine-LC appears to be ineffective as a treatment for Alzheimer's disease. (N Engl J Med 1990; 323:445-8.)

ALZHEIMER'S disease is the most common dementing illness and may be the fourth leading cause of death in the United States. It severely affects about 1.4 million Americans, and this number is predicted to increase fivefold in the next half century.[1-4] The current direct and indirect annual cost of Alzheimer's disease in the United States is estimated to be $24 billion to $48 billion.[4,5] The disease has no known cause or effective treatment, although a wide variety of therapeutic approaches have been considered.[6] One medication used for Alzheimer's disease is Hydergine (Sandoz brand of ergoloid mesylates), a preparation containing 0.333 mg each of dihydroergocornine mesylate, dihydroergocristine mesylate, and dihydroergocryptine mesylate, the last of which consists of dihydro-α-ergocryptine mesylate and dihydro-β-ergocryptine mesylate in a proportion of 2:1. In 1984, Hydergine was the 11th most widely prescribed drug in the world,[7] and it is the only medication approved by the Food and Drug Administration for Alzheimer's disease. Initially thought to act as a cerebral vasodilator, Hydergine is now considered to be a metabolic enhancer, because it may improve some aspects of neuronal metabolism and neurotransmitter activity.[8]

Although more than 20 double-blind, placebo-controlled trials have shown favorable results with ergoloid mesylates, many clinicians remain skeptical of its efficacy; uncertainties persist about placebo responses, the heterogeneity of study groups, and the clinical importance of the observed beneficial effects.[7] Hydergine-LC, a newer preparation of ergoloid mesylates, delivers the drug as a liquid in a capsule (LC), a form that may have greater bioavailability.[9] To test the safety and efficacy of this new formulation, we conducted a double-blind, placebo-controlled trial of Hy-

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