Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans

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Abstract The objective of this study was to compare the effects of linoleic acid (*cis,cis*-C18:2(n–6)) and its hydrogenation products elaidic (*trans*-C18:1(n–9)) and stearic acid (C18:0) on serum lipoprotein levels in humans. Twenty-six men and 30 women, all normolipemic and apparently healthy, completed the trial. Three experimental diets were supplied to every subject for 3 weeks each, in random order (multiple cross-over). The Linoleate-diet provided 12.0% of total energy intake as linoleic acid, 2.8% as stearic acid, and 0.1% as *trans* fatty acids. The Stearate-diet supplied 3.9 energy % as linoleic acid, 11.8% stearic acid, and 0.3% *trans* fatty acids. The *Trans*-diet provided 3.8 energy % as linoleic acid, 3.0% stearic acid, and 7.7% as monounsaturated *trans* fatty acids, largely elaidic acid (*trans*-C18:1(n–9)). Other nutrients were constant. Fasting blood was sampled at the end of each dietary period. Mean (±SD) serum LDL cholesterol was 109 ± 24 mg/dl (2.83 ± 0.63 mmol/l) on the Linoleate-diet. It rose to 116 ± 27 mg/dl (3.00 ± 0.71 mmol/l) on the Stearate-diet (change, 7 mg/dl or 0.17 mmol/l, $P = 0.0008$) and to 119 ± 25 mg/dl (3.07 ± 0.65 mmol/l) on the *Trans*-diet (change, 9 mg/dl or 0.24 mmol/l, $P < 0.0001$). High density lipoprotein (HDL) cholesterol decreased by 2 mg/dl (0.06 mmol/l, $P < 0.0001$) on the Stearate-diet and by 4 mg/dl (0.10 mmol/l, $P < 0.0001$) on the *Trans*-diet, both relative to linoleic acid. Our findings show that 7.7% of energy (mean, 24 g/day) of *trans* fatty acids in the diet significantly lowered HDL cholesterol and raised LDL cholesterol relative to linoleic acid. Combination with earlier results (Mensink, R. P., and M. B. Katan. 1990. *N. Engl. J. Med.* **323:** 439–445) suggests a linear dose–response relation. Replacement of linoleic acid by stearic acid also caused somewhat lower HDL cholesterol and higher LDL cholesterol levels. Hydrogenation of linoleic acid to either stearic or *trans* fatty acids produces fatty acids that may increase LDL and decrease HDL cholesterol relative to linoleic acid itself.—**Zock, P. L., and M. B. Katan**. Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. *J. Lipid Res.* 1992. **33:** 399–410.

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Monounsaturated *trans* fatty acids with a length of 18 carbon atoms are formed when vegetable oils, rich in linoleic acid, are partially hydrogenated to produce fats with better texture and stability (1). Although the intake of these *trans* fatty acids, mainly elaidic acid and its isomers (**Fig. 1**), is much lower than that of saturated fatty acids, it is still considerable in industrialized countries. Estimated average consumption of *trans* fatty acids is 3–4% of daily energy intake in the United States (2).

Early studies on the effects of *trans* fatty acids on blood lipids produced conflicting results. Some studies (3–5) suggested that *trans* fatty acids, as compared to their *cis* isomer oleic acid, elevate serum total cholesterol levels, while others (6–8) could not confirm this. In a previous study conducted at our Department it was found that *trans* monounsaturated fatty acids were hypercholesterolemic compared with oleic acid, and that their effect was about half that of a mixture of saturated fatty acids (C12:0, C14:0, and C16:0) (9). In this study *trans* fatty acids not only raised the serum level of atherogenic low density lipoprotein (LDL) cholesterol, but also lowered high density lipoprotein (HDL) cholesterol (9). However, the amount of *trans* fatty acids given was quite high (11% of daily energy intake), and doubts have been voiced as to whether these findings could be extrapolated to lower levels of intake (10).

A product of further hydrogenation of linoleic acid (Fig. 1) is stearic acid, the completely saturated analogue of elaidic acid. Stearic acid has been found not to raise the serum cholesterol level relative to carbohydrates and to exert effects on serum lipids similar to those of oleic acid (11–14). Like *trans* fatty acids and other saturated fatty acids, stearic acid is a rigid molecule whose presence adds firmness to foods. Although stearic acid has a higher melting point than *trans* fatty acids or palmitic acid (C16:0) and has a

Abbreviations; HDL, high density lipoproteins; LDL, low density lipoprotein.

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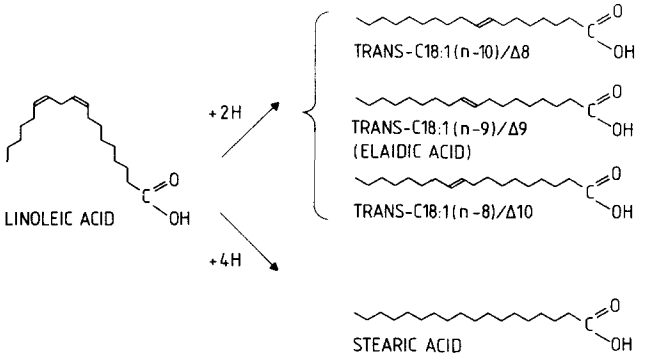

Fig. 1. Structure of linoleic acid and of some products of hydrogenation. Partially hydrogenated soybean oil usually contains a spectrum of positional *trans*-C18:1 isomers with C18:1(n–8)(Δ^{10}) being the most abundant (31).

waxy nature, a limited increase in the stearic acid content at the expense of other saturated fatty acids or *trans* fatty acids has been suggested as a way for the edible-fats industry to produce semi-solid and solid fats with less of a cholesterol-raising potential (15).

Therefore we compared the effects of *trans* fatty acids on serum lipoprotein and apolipoprotein levels in healthy men and women with those of stearic acid and of linoleic acid, the parent fatty acid from which *trans* fatty acids and stearic acid are derived during the hydrogenation of soybean and other linoleic acid-rich oils.

SUBJECTS AND METHODS

Subjects

Out of 110 persons who responded to calls via local newspapers, 45 women and 42 men were randomly selected for a screening procedure. Two women and 1 man were excluded for medical reasons and 2 men and 2 women withdrew during the screening. The remaining 80 volunteers, 39 men and 41 women, had no history of atherosclerotic disease, and all were apparently healthy, as indicated by a medical questionnaire. None had anemia, glycosuria, proteinuria, or hypertension, and none were taking medications known to affect blood lipids. Because only 62 subjects could be enrolled in the trial, 31 men and 31 women were randomly selected to take part. Three men withdrew before the study began. The protocol and aims of the study were fully explained to the subjects, who gave their written consent. No reward was given, except for the free food in the study diets. Approval for the study had been obtained from the Ethics Committee of the Department. During the study 2 men withdrew, one because of personal reasons and one because of illness. One woman withdrew because of pregnancy. All analyses are based on the 26 men and 30 women who completed the trial. Before the beginning of the study diets, the subjects' fasting serum lipid levels ranged from 3.31 to 6.66 mmol/l for total cholesterol (mean, 4.84 mmol/l or 187.2 mg/dl), from 0.87 to 2.04 mmol/l for HDL cholesterol (mean, 1.38 mmol/l or 53.4 mg/dl), and from 0.39 to 1.58 mmol/l (mean, 0.94 mmol/l or 83.2 mg/dl) for triglycerides. The men's age ranged from 19 to 48 years (mean, 25 years), and they weighed between 60 and 84 kg (mean 73 kg). Their body mass index ranged from 18.5 to 26.0 kg/m^2 (mean, 21.5 kg/m^2). The women were between 18 and 49 years old (mean, 24 years), and weighed between 51 and 80 kg (mean, 63 kg); their body mass index ranged from 17.9 to 27.5 kg/m^2 (mean, 21.5 kg/m^2). Thirteen women used oral contraceptives, and 4 men and 4 women smoked.

Design and statistical analyses

The trial ran from January 29 until April 2, 1990. The study had a multiple crossover design and consisted of three consecutive periods. Each subject followed the three study diets for 3 weeks each. Our experience agrees with that of earlier workers (11, 16) in that serum lipid and lipoprotein levels stabilize within 2 weeks after a dietary change (17, 18). One diet was high in linoleic acid (Linoleate-diet), one was high in stearic acid (Stearate-diet), and the third was high in elaidic acid (*Trans*-diet). Before the trial, the participants were categorized according to sex. Male and female subjects were then divided into six groups so that each group had a nearly identical number of subjects from both sexes. Women who used oral contraceptives were equally distributed over the six treatment sequences. Each group received the diets in a different order (**Fig. 2**). In this way, variation due to residual effects of the previous diet or to drift of variables over time were eliminated (19). We attempted to blind the participant as to the sequence of their diets,

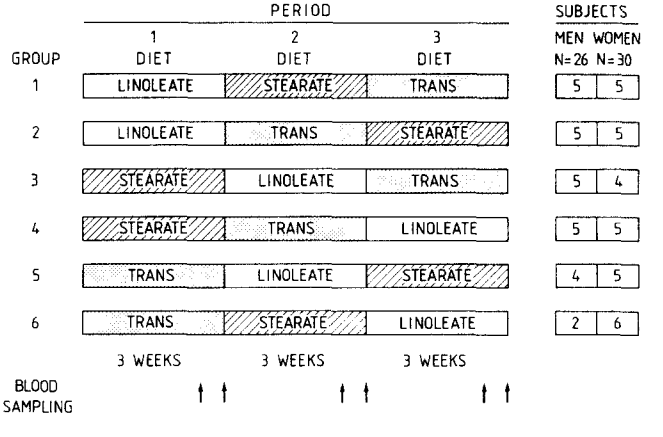

Fig. 2. Experimental design.

but at the end of the trial it turned out that all subjects had recognized the linoleic acid-rich diet, and most of them had also correctly identified the *trans* and stearic acid-rich diets. However, it is highly unlikely that awareness of the nature of the treatment could have affected the outcome of the study.

The data were analyzed with the General Linear Models (GLM) of the Statistical Analyses System (20). When the analyses indicated a significant effect of diet ($P < 0.05$), the Tukey method was used for pairwise comparisons of the diets and for calculation of 95% confidence limits for the differences between two diets (20). This method encompasses a downward adjustment of significance limits for multiple testing and, as a result, only *P*-values of less than 0.02 were considered significant.

Diets

Before the trial started, the participants recorded their habitual diet for 2 working days and 1 weekend day to allow us to estimate their energy and nutrient intake. The food records were coded and the nutrient composition and energy content of the diets were calculated with use of the Netherlands Nutrient Data Base (21). The study diets consisted of conventional solid foods, and menus were changed daily during each 3-week cycle. The planned nutrient content of the three diets was similar, except for 8% of total energy, which was provided by linoleic acid, stearic acid, or elaidic acid. The linoleate group received a commercially available margarine high in linoleic acid (Becel, Van den Bergh foods, Rotterdam, The Netherlands). In addition, high-linoleic acid sunflower oil was used in the preparation of various dishes. For the *Trans*-diet, high-oleic acid sunflower oil (Trisun, SVO Enterprises, Wickliffe, OH) was hydrogenated with a sulfurized nickel catalyst (Pricat 9908, Unichema, Emmerich, Germany) under conditions that favored formation of *trans* rather than saturated fatty acids. This hydrogenation procedure was similar to that applied for our previous study (9, 22). We used this procedure rather than hydrogenation of a linoleic acid-rich oil to avoid the formation of *trans*-isomers of C18:2 and to maximize the yield of *trans*-C18:1. Seventy-five parts of the hydrogenated fat were mixed with 25 parts of the unaltered oleic acid-rich oil, and used to produce a margarine and a shortening high in elaidic acid. Fats for the Stearate-diet were produced by interesterification of a mixture of 41 parts of fully hydrogenated high-linoleic acid sunflower oil (Chempri, Raamsdonksveer, The Netherlands), 50 parts of high-oleic acid sunflower oil, and 9 parts unmodified of high-linoleic acid sunflower oil.

Thus, the basic source of all fatty acids in which the three diets differed was sunflower oil. The special margarines and shortenings were developed and manufactured by the Unilever Research Laboratory (Vlaardingen, The Netherlands). **Table 1** gives the fatty acid composition of margarines and oil used in the three diets. Positional isomers of monounsaturated C18:1-fatty acids were identified by gas–liquid chromatography using a capillary Sil-88 column.

The diets were formulated at 24 levels of energy intake ranging from 5.5 to 17.5 MJ (1315 to 4185 kcal) per day. All foodstuffs were weighed out for each individual subject. On week days at noon, hot meals were served and eaten at the department. All other food was supplied daily as a package. Food for the weekend and guidelines for its preparation were provided on Fridays. In addition to the food supplied, subjects were allowed a limited number of items free from fat and cholesterol. The energy intake from these free-choice items was fixed for each level of energy intake and ranged from 7 to 13% of total energy intake. Subjects were urged not to change their selection of free-choice items throughout the trial. They were asked to maintain their usual pattern of physical activity and not to change their smoking habits, consumption of coffee, or use of oral contraceptives. The participants recorded in diaries any sign of illness, medication used, the selection and use of free-choice items, the amount and type of coffee consumed, 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 study diet were collected on each of the 63 trial days for an imaginary participant with a daily energy intake of 11 MJ (2630 kcal), stored at –20°C,

TABLE 1. Fatty acid composition of the margarines and oil used in the three study diets

Fatty Acid	Linoleate-Diet	Stearate-Diet	*Trans*-Diet
	g per 100 g of fatty acid		
Saturated	18.1	52.9	12.1
Lauric acid (C12:0)	1.5	0.1	0.0
Myristic acid (C14:0)	0.6	0.1	0.0
Palmitic acid (C16:0)	7.6	5.6	3.6
Stearic acid (C18:0)	7.0	45.5	6.6
Monounsaturated	18.8	37.4	84.2
Cis-C18:1	18.4	37.0	43.6[a]
Trans-C18:1	0.1	0.1	40.2[b]
Polyunsaturated	62.5	9.5	2.1
Linoleic acid (*cis,cis*-C18:2)	61.9	9.4	1.9
Trans-C18:2	0.5	0.0	0.2
Others	0.5	0.2	1.6

Commercially available linoleic acid-rich margarine and regular sunflower oil provided 37% of the total fat content of the Linoleate-diet, high-stearic acid margarine and shortening provided 48% of the total fat content of the Stearate-diet, and high-elaidic acid margarine and shortening provided 44% of the total fat content of the *Trans*-diet.

[a]Positional *cis* isomers: C18:1(n–4), 0.1 g; C18:1(n–5), 0.2 g; C18:(n–6), 0.4 g; C18:1(n–7), 1.3 g; C18:1(n–9), 38.5 g; C18:1(n–12), 3.1 g.

[b]GLC showed three positional isomers, predominantly C18:1(n–9)(Δ^9) (elaidic acid) plus traces of C18:1(n–8)(Δ^{10}) and C18:1(n–10)(Δ^8).

and pooled and analyzed after the study. These analyzed values of the food supplied were combined with the values for the free-choice items (See Table 2).

Body weights without shoes, jackets, and heavy sweaters were recorded twice weekly. The level of energy intake was adjusted when necessary, as indicated by weight changes. Over the 63 days of the trial average body weight fell by 0.2 ± 1.1 kg (range, −2.5 to +2.0 kg). The mean difference in body weight at the end of the dietary periods was 0.1 ± 0.7 kg (range −1.7 to 1.4 kg) between the Linoleate-diet and the Stearate-diet, 0.0 ± 0.8 kg (range −2.1 to 1.8 kg) between the Linoleate-diet and the *Trans*-diet, and 0.1 ± 0.7 kg (range −1.8 to 2.4 kg) between the *Trans*-diet and the Stearate-diet.

Blood sampling and analyses

Before the trial each participant had been assigned a random number that was then used for labeling blood and serum tubes. In this way the laboratory technicians were kept unaware of the subject's diet sequence. Blood samples were taken after a 12-h fast on days 1, 17, and 21 (period 1), days 38 and 42 (period 2), and on days 59 and 63 (period 3). All venipunctures of each subject were performed by the same technician, in the same location, and at the same time of the same day of the week. Serum was obtained by low-speed centrifugation within 1 h of venipuncture, stored at −80°C, and analyzed enzymatically for total and HDL cholesterol and triglycerides (23, 24). All samples from a particular subject were analyzed within the same run. The coefficient of variation within runs was 1.5% for total cholesterol, 1.6% for HDL cholesterol, and 2.9% for triglycerides. Mean bias with regard to the target values of three serum pools provided by the Centers for Disease Control (Atlanta, GA) (25) was 0.14 mmol/l for total cholesterol and 0.04 mmol/l for triglycerides. The mean bias with regard to target values of four serum pools obtained from the Solomon Park Research Laboratories (Kirkland, WA) was 0.09 mmol/l for HDL cholesterol. LDL cholesterol was calculated using the Friedewald equation (26). Apolipoproteins were assayed by Dr. M. Sandkamp at the Institut für Klinische Chemie and Laboratoriumsmedizin, Münster, Germany (Head: Prof. Dr. G. Assmann), using a turbidimetric method on microtiter plates, as described (27). All samples from the same proband were measured on the same day within one series. The coefficient of variation within the series was 3.5% for apolipoprotein A-I and 3.6% for apolipoprotein B. The two lipoprotein and apolipoprotein values obtained at the end of each dietary period were averaged for data analyses.

For each subject, the fatty acid composition of serum cholesteryl esters was determined in samples obtained at the end of each dietary period (days 21, 42, and 63) as described earlier (28), with the following modifications: cholesteryl esters and triglycerides were separated with Bond-Elut solid phase extraction columns (Analytichem International, Harbor City, CA), the component fatty acids were methylated with 4% (v/v) H_2SO_4 in methanol, and a capillary Sil-88 column was used for gas chromatographic analysis. The results are expressed as a proportion by weight of all fatty acids detected.

RESULTS

Diets and dietary adherence

The mean daily intakes of energy and the composition of the three experimental diets as determined by chemical analyses of duplicate diets plus calculated contribution of free-choice items are given in **Table 2**. Energy and nutrients supplied by the free-choice items (mainly carbohydrates and some alcohol and protein) did not differ between the dietary regimes. The intake of protein, carbohydrates, alcohol, cholesterol, phytosterols, and fiber did not change throughout the study. Total fat intake differed somewhat between the *Trans*-diet and the Stearate-diet (3.8 energy %) due to small differences in the intake of oleic acid (*cis*-C18:1) and saturated fatty acids (C12:0, C14:0, C16:0), and also due to the fact that the difference in stearic acid (C18:0) intake turned out to be 8.8% of energy instead of the planned 8%. The percentage of total daily energy from linoleic acid decreased from 12.0% on the Linoleate-diet to 3.9% and 3.8% on the Stearate- and *Trans*-diets, respectively. It was replaced by 9.0% stearic acid on the Stearate-diet and by 7.6% monounsaturated *trans* fatty acids on the *Trans*-diet. At an energy intake of 11 MJ (2630 kcal) per day the *Trans*-diet provided 8.7 g of stearic and 22.2 g of *trans* fatty acids (*trans*-C18:1) per day, while the Stearate-diet supplied 34.2 g of stearic acid and 0.8 grams of *trans* fatty acids.

Gas–liquid chromatography revealed three positional isomers of *trans*-C18:1 in the *trans* fatty acids-rich margarine and shortening; predominantly *trans*-C18:1(n–9) (Δ^9) (elaidic acid) and minor amounts of *trans*-C18:1(n–10) (Δ^8) and *trans*-C18:1(n–8) (Δ^{10}).

The fatty acid composition of the serum cholesteryl esters at the end of the three dietary periods confirmed the subjects' adherence to the diets (**Table 3**). In 54 out of the 56 subjects the proportion of linoleic acid in the cholesterol esters was higher on the Linoleate-diet than on either the Stearate-diet (average change, 6.60 g/100 g; 95% confidence interval, 5.87 to 7.33) or the *Trans*-diet (average change, 8.17 g/100 g; 95% confidence interval, 7.44 to 8.99).

TABLE 2. Mean daily intake of nutrients of subjects while on the high-linoleic acid diet, the high-stearic acid diet, and the high-*trans* fatty acid diet

Energy/Nutrient	Linoleate-Diet	Stearate-Diet	*Trans*-Diet
Energy			
MJ/day	12.0 ± 2.8	11.9 ± 2.7	12.0 ± 2.7
kcal/day	2869 ± 670	2845 ± 646	2869 ± 646
Protein (% of energy)	12.3	12.3	12.8
Fat (% of energy)	41.1	43.5	39.7
Saturated fatty acids	11.0	20.1	10.3
Lauric acid (C12:0)	0.7	0.5	0.5
Myristic acid (C14:0)	0.9	1.0	1.0
Palmitic acid (C16:0)	5.8	5.7	4.8
Stearic acid (C18:0)	2.8	11.8	3.0
Monounsaturated fatty acids	15.8	16.6	23.3
Cis-C18:1	14.7	15.4	14.6
Trans-C18:1	0.1	0.3	7.7[a]
Polyunsaturated fatty acids	12.5	4.3	4.2
Linoleic acid (*cis,cis*-C18:2)	12.0	3.9	3.8
Trans-C18:2	0.0	0.0	0.0
Carbohydrates (% of energy)	46.0	43.5	46.6
Alcohol (% of energy)	0.9	0.9	1.0
Cholesterol (mg/MJ)	33.5	32.6	33.5
β-Sitosterol (mg/MJ)	23.8	25.1	22.6
Other plant sterols (mg/MJ)	3.0	3.9	3.0
Dietary fiber (g/MJ)	3.6	3.9	4.0

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 independent duplicates collected in three different periods during which each diet was consumed by one-third of the subjects. Variations between periods were negligible.

[a]Values for total *trans* fatty acid content as determined by gas–liquid chromatography (GLC) and by infrared spectroscopy (IR) were similar: both GLC and IR indicated 20.2 g of *trans* fatty acids per 100 g of total fatty acids in the *trans* fatty acid diet.

The percentage of stearic acid was increased on the Stearate-diet in 55 out of 56 subjects relative to the Linoleate-diet (change 0.97 g/100 g; 95% confidence interval, 0.86 to 1.09) or to the *Trans*-diet (change 0.88 g/100 g; 95% confidence interval, 0.76 to 0.99). Fifty-four out of the 56 subjects showed a larger proportion of elaidic acid in their cholesteryl esters when on the *Trans*-diet than when on the Linoleate-diet (change 0.81 g/100 g; 95% confidence interval, 0.70 to 0.92) or the Stearate-diet (change 0.83 g/100 g; 95% confidence interval, 0.72 to 0.94). All these differences are highly significant ($P < 0.0001$).

Serum lipids and lipoproteins

Total and lipoprotein cholesterol and triglycerides. **Table 4** gives the serum lipid and lipoprotein levels at the end of each experimental diet period. Compared with levels on the linoleic acid diet, serum total cholesterol increased by 0.15 mmol/l or 5.8 mg/dl ($P = 0.0081$; 95% confidence interval, 0.02 to 0.27 mmol/l) on the Stearate-diet and by 0.16 mmol/l or 6.2 mg/dl ($P = 0.0041$; 95% confidence interval, 0.04 to 0.29 mmol/l) on the *Trans*-diet. LDL cholesterol rose by 0.17 mmol/l or 6.6 mg/dl ($P = 0.0008$; 95% confidence interval, 0.05 to 0.28 mmol/l) on the Stearate-diet and by 0.24 mmol/l or 9.3 mg/dl ($P < 0.0001$; 95% confidence interval, 0.12 to 0.35 mmol/l) on the *Trans*-diet; this latter value was not significantly greater than the increase on the Stearate-diet.

HDL cholesterol decreased by 0.06 mmol/l or 2.3 mg/dl ($P < 0.0001$; 95% confidence interval, 0.03 to 0.10 mmol/l) on the Stearate-diet and by 0.10 mmol/l or 3.9 mg/dl ($P < 0.0001$; 95% confidence interval, 0.06 to 0.13 mmol/l) on the *Trans*-diet. Lower HDL cholesterol levels on the *Trans*-diet than on the Linoleate-diet were seen in 46 of the 56 subjects (**Fig. 3**). The difference of 0.034 mmol/l in HDL cholesterol between the Stearate-diet and the *Trans*-diet just failed to reach significance ($P = 0.0210$; 95% confidence interval, –0.00 to 0.07 mmol/l).

The responses of HDL and LDL cholesterol to the different diets were correlated neither with their initial levels (day 1) nor with the differences in body weight between the dietary regimes. Out of the 12 correlation coefficients calculated, the largest observed was –0.24 ($P = 0.08$) for the response in HDL cholesterol versus the difference in body weight between the *Trans*-diet and the Stearate-diet.

The HDL to LDL cholesterol ratio was 0.55 on the Linoleate-diet, 0.50 on the Stearate-diet, and 0.47 on the *Trans*-diet; all three values were significantly different from each other ($P < 0.0037$ for each comparison). The level of serum triglycerides was 0.09 mmol/l or 8.0 mg/dl higher on the Stearate-diet than on the Linoleate-diet ($P = 0.0074$; 95% confidence interval, 0.01 to 0.17 mmol/l). Triglyceride values did not differ significantly between the *Trans*-diet and the Stearate-diet ($P = 0.22$; 95% confidence interval, 0.04

TABLE 3. Fatty acid composition of serum cholesteryl esters at the end of the three dietary periods

Fatty Acid	Linoleate-Diet	Stearate-Diet	*Trans*-Diet
		g per 100 g of fatty acids	
C14:0	2.71 ± 0.85	2.29 ± 1.18	2.52 ± 1.05
C16:0[a]	8.75 ± 0.55	8.37 ± 0.64	9.10 ± 0.64
C16:1[a]	1.72 ± 0.77	1.97 ± 0.72	2.52 ± 0.62
C18:0	0.77 ± 0.11	1.74 ± 0.38[b]	0.87 ± 0.29
cis-C18:1[a]	15.06 ± 1.57[b]	20.16 ± 1.44	20.62 ± 1.40
Trans-C18:1	0.13 ± 0.18	0.12 ± 0.19	0.94 ± 0.30[b]
Cis,cis-C18:2[a]	61.01 ± 3.92[b]	54.41 ± 3.23	52.84 ± 3.35
C20:4	6.36 ± 1.36	6.79 ± 1.32	6.42 ± 1.05
Other	3.51 ± 0.98	4.16 ± 0.81	4.17 ± 0.81

Values are means ± SD. The 26 men and 30 women consumed each diet for 3 weeks each, in random order.
[a]Significantly different between each of the diets, $P < 0.02$.
[b]Significantly different from both other diets, $P < 0.0001$.

to –0.12) or between the *Trans*-diet and the Linoleate-diet ($P = 0.14$; 95% confidence interval, –0.03 to 0.13 mmol/l).

Apolipoproteins. **Table 5** shows the mean apolipoprotein levels and their ratios at the end of each dietary period. Relative to levels on the Linoleate-diet, serum apoB rose by 3.4 mg/dl on the Stearate-diet (95% confidence interval, 1.7 to 5.0 mg/dl), and by 5.0 mg/dl (95% confidence interval, 3.4 to 6.6 mg/dl) on the *Trans*-diet. All three values are significantly different from each other ($P < 0.0184$ for each comparison). The apoB to LDL cholesterol ratio was essentially the same for each diet. Mean apoA-I level was 2.0 mg/dl higher on the Linoleate-diet than on the *Trans*-diet ($P = 0.0119$; 95% confidence interval, 0.1 to 4.0 mg/dl). The difference in serum apoA-I between the Linoleate- and the Stearate-diet periods of 1.3 mg/dl ($P = 0.1044$; 95% confidence interval, –0.6 to 3.3 mg/dl), and between the Stearate- and the *Trans*-diet periods of 0.7 mg/dl ($P = 0.3586$; 95% confidence interval, –1.3 to 2.7 mg/dl) were not significant. The mean apoA-I to HDL cholesterol (mg/mmol) ratio increased from 856 on the Linoleate-diet to 887 mg/mmol on the Stearate-, and to 904 mg/mmol on the *Trans*-diet ($P < 0.0081$ for each comparison). The ratio of apoA-I to apoB was 1.85 ± 0.36

TABLE 4. Serum lipid and lipoprotein cholesterol levels at the end of the three dietary periods

	Linoleate-Diet	Stearate-Diet	*Trans*-Diet
		mmol per liter	
Total cholesterol			
Men	4.66 ± 0.73	4.93 ± 0.82[a]	4.85 ± 0.65[a]
Women	4.81 ± 0.71	4.85 ± 0.74	4.95 ± 0.78
All	4.74 ± 0.72	4.89 ± 0.77[a]	4.90 ± 0.72[a]
HDL-cholesterol			
Men	1.34 ± 0.18	1.28 ± 0.17[a]	1.25 ± 0.17[a]
Women	1.58 ± 0.28	1.52 ± 0.26[a]	1.48 ± 0.25[a]
All	1.47 ± 0.27	1.41 ± 0.25[a]	1.37 ± 0.24[a]
LDL-cholesterol			
Men	2.90 ± 0.71	3.16 ± 0.74[a]	3.14 ± 0.65[a]
Women	2.78 ± 0.55	2.87 ± 0.66	3.01 ± 0.66[a]
All	2.83 ± 0.63	3.00 ± 0.71[a]	3.07 ± 0.65[a]
HDL/LDL ratio			
Men	0.50 ± 0.16	0.44 ± 0.14[a]	0.42 ± 0.11[a]
Women	0.59 ± 0.16	0.56 ± 0.16[a]	0.51 ± 0.15[a,b]
All	0.55 ± 0.16	0.50 ± 0.16[a]	0.47 ± 0.14[a,b]
Triglycerides			
Men	0.92 ± 0.31	1.07 ± 0.41[a]	1.00 ± 0.35
Women	0.96 ± 0.40	1.01 ± 0.36	1.00 ± 0.31
All	0.95 ± 0.36	1.04 ± 0.38[a]	1.00 ± 0.32

Values are means ± SD. The 26 men and 30 women consumed each diet for 3 weeks each, in random order. To convert values for total, HDL, and LDL cholesterol to mg/dl, multiply by 38.67. To convert values for triglycerides to mg/dl, multiply by 88.54.
[a]Significantly different from the Linoleate-diet, $P < 0.02$.
[b]Significantly different from Stearate-diet, $P < 0.02$.

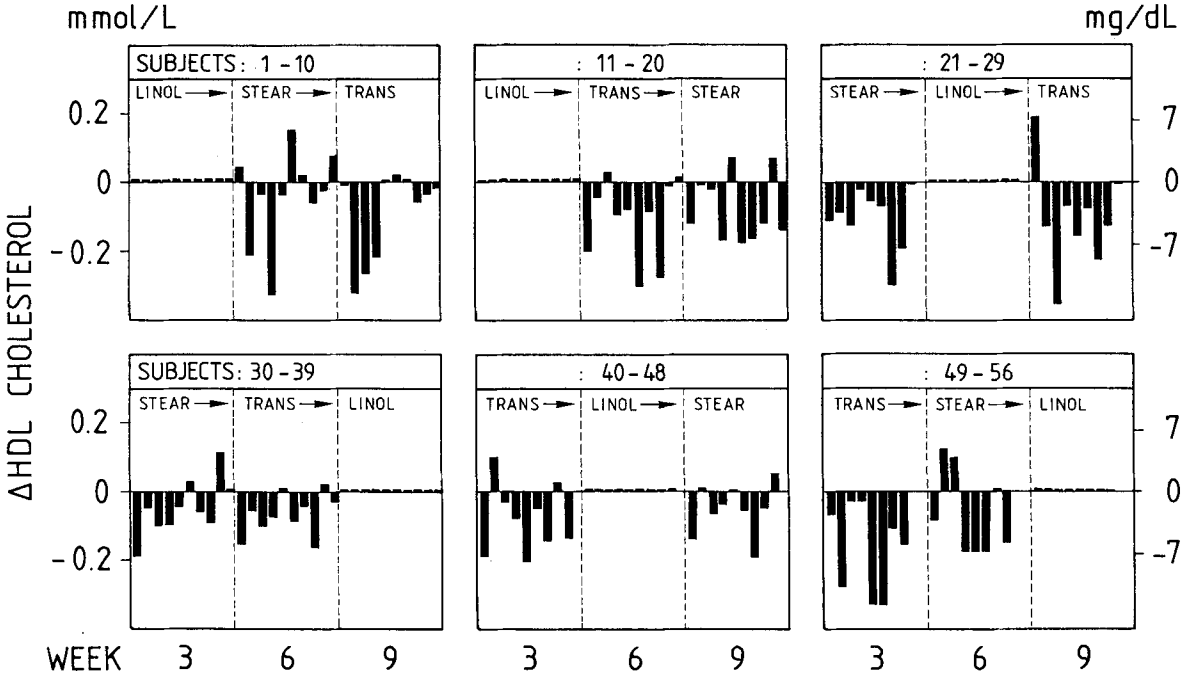

Fig. 3. Individual changes in HDL cholesterol levels on diets high in *trans* fatty acids or stearic acid, relative to a diet high in linoleic acid. Bars indicate the level of each individual subject when on a particular diet minus his or her level when on the Linoleate-diet.

on the Linoleate-diet, 1.75 ± 0.37 on the Stearate-diet, and 1.69 ± 0.33 on the *Trans*-diet. These values are also significantly different from each other ($P < 0.0012$ for each comparison).

Gender effects. As compared with the Linoleate-diet, the mean response of the men on the Stearate-diet was significantly greater than that of the women for total cholesterol (difference in change, 0.22 mmol/l or 8.5 mg/dl $P = 0.0438$) and for apoB (difference in change, 3.7 mg/dl; $P = 0.0155$). The statistical analyses did not reveal any other significant difference between the sexes in the responses of their lipid, lipoprotein, or apolipoprotein levels to the diets.

DISCUSSION

The three fatty acids under study all had 18 carbon atoms. Thus effects of the chain length of the fatty acids under study on serum lipid and lipoproteins can be excluded. Since the experimental diets did not materially differ in nutrients other than *Trans* fatty acids, stearic acid, and linoleic acid, the changes observed between the diets must be due to differences in the number and/or geometry of the double bonds in these fatty acids.

Trans fatty acids

Effects on lipids and lipoproteins. In a previous study conducted at our department it was found that monounsaturated *trans* fatty acids at a dose of 11% of energy (on average, 33 g/day) increased LDL cholesterol by 14.3 mg/dl (0.37 mmol/l) and decreased HDL cholesterol by 6.6 mg/dl (0.17 mmol/l) as compared with the *cis* isomer, oleic acid (9). However, the dose of *trans* fatty acids consumed probably exceeded the range of intakes in free-living subjects. By contrast, the 7.7% of total daily energy intake that was provided by the *trans* fatty acids in the present trial, although still higher than average consumption, may be reached by persons who eat large amounts of hardened vegetable fats and margarines and of products prepared with or fried in such fats (29, 30). Another difference with the previous trial was that we now studied the effects of *trans* fatty acids relative to linoleic acid. If one assumes that linoleic acid and oleic acid differ little in their effects on serum lipids when consumed in moderate amounts (31–34), then the magnitude of changes observed here is compatible with a linear relation between the dose of *trans* monounsaturates consumed and the response of serum lipoprotein concentrations. **Fig. 4** shows, side-by-side, the effects of monounsaturated *trans*-C18:1 fatty acids as observed relative to linoleic acid in the present and relative to oleic acid in the previous study. Although more studies are needed to determine the true relationship between the dose of various types of *trans* fatty acids and their effects on serum lipoproteins, comparison of the results of our two studies suggests that in our hands the effects of *trans* fatty acids

TABLE 5. Serum apoA-I and apoB levels and their ratios at the end of the three dietary periods

	Linoleate-Diet	Stearate-Diet	*Trans*-Diet
		mg/dl	
Apolipoprotein A-I			
Men	115.7 ± 7.8	115.6 ± 8.5	114.9 ± 8.4
Women	131.5 ± 16.8	129.1 ± 13.9	128.4 ± 14.6
All	124.1 ± 15.5	122.8 ± 13.5	122.1 ± 13.8[a]
Apolipoprotein B			
Men	69.5 ± 13.3	74.9 ± 14.4[a]	75.4 ± 12.7[a]
Women	68.8 ± 11.5	70.4 ± 12.2	73.1 ± 12.6[a,b]
All	69.1 ± 12.2	72.5 ± 13.4[a]	74.1 ± 12.6[a,b]
Apolipoprotein A-I/B ratio			
Men	1.73 ± 0.38	1.61 ± 0.37[a]	1.57 ± 0.32[a]
Women	1.95 ± 0.31	1.88 ± 0.33[a]	1.80 ± 0.31[a,b]
All	1.85 ± 0.36	1.75 ± 0.37[a]	1.69 ± 0.33[a,b]

Values are means ± SD. The 26 men and 30 women consumed each diet for 3 weeks each, in random order.
[a]Significantly different from the Linoleate-diet, $P < 0.02$.
[b]Significantly different from the Stearate-diet, $P < 0.02$.

on LDL and HDL cholesterol are proportional to the amounts consumed.

The *trans* fatty acids supplied to our subjects consisted largely of C18:1(n–9) (elaidic acid), with traces of C18:1(n–8) and C18:1(n–10). These are also the predominant *trans* fatty acids produced by hydrogenation of vegetable oil in food manufacturing (2, 35). The spectrum of *trans*-C18:1 positional isomers consumed by the subjects in our previous trial was much broader (9, 22). Nevertheless, the observed effects in both trials were largely congruent if the differences in dose are taken into account. We therefore think that our findings will apply to most monounsaturated *trans*-C18:1 fatty acid isomers as present in commercial fats and foods.

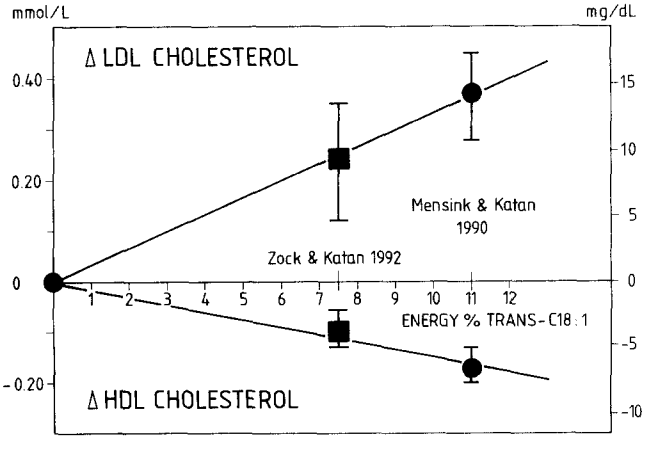

Fig. 4. Effects of *trans* fatty acids on LDL and HDL cholesterol in the present trial compared with those in a previous trial. Circles represent data from a comparison of *trans*-C18:1 with oleic acid (9). Squares are based on the present comparison between *trans*-C18:1 and linoleic acid. Bars denote 95% confidence intervals. In addition to the two experiments, the origin provides a third point, because a zero change in intake will produce zero change in lipoprotein levels.

Public health implications. Based on our two studies as summarized in Fig. 4, it can be speculated that one dietary energy % of *trans* fatty acids in the diet raises LDL cholesterol by about 1.2 mg/dl (0.03 mmol/l) and lowers HDL cholesterol by about 0.6 mg/dl (0.015 mmol/l) relative to oleic or linoleic acid. The current average *trans* intake of 3–4% of energy in the United States might thus cause an elevation of LDL cholesterol by about 4 mg/dl and a depression of HDL cholesterol by about 2 mg/dl relative to *cis*-unsaturates. Although these figures appear modest, the predicted effect on HDL cholesterol is of the same magnitude as the difference in HDL cholesterol between physically active and sedentary people (36) or between individuals at the 10th percentile of body mass index versus those at the 50th percentile (37). For comparison, smoking 10 cigarettes per day will, on average, lower HDL cholesterol by about 2–4 mg/dl (38).

What would be the predicted effect on coronary heart disease risk if the average *trans* fatty acids consumption of 7 to 10 g/day in the United States population were to be completely replaced by oleic and linoleic acid? Both epidemiological data and results of controlled trials suggest that the predicted decrease of 4 mg/dl in LDL cholesterol should lead to a decrease in the average risk for coronary heart disease of 3%. The increase of 2 mg/dl in HDL cholesterol should add another 4–5% reduction in risk (39, 40). Total risk reduction should thus average 7–8%, provided that raising HDL indeed reduces risk, which is a plausible but still unproven assumption (39). A reduction in risk of this magnitude does not appear to justify total elimination of partially hydrogenated oils from the

United States food supply, especially since a sizeable part of current *trans* fatty acid intake is supplied by dairy and beef fat. However, it is possible that selected individuals consume considerably higher amounts of *trans* fatty acids than the average of 3–4% (41), and for them the effects of *trans* fatty acids can be of clinical importance. Obviously, the *trans* fatty acid intake of various segments of the population needs to be assessed, and groups with high intake need to be identified. Even the average intake in the United States is controversial, with quoted figures ranging from 8 to 15 g/day (30). The estimate of 17 g/day for the Netherlands (42) is similarly based on incomplete data and a host of assumptions. Better intake data are thus urgently needed before any public health measures at curbing *trans* intake can even be considered.

Stearic acid

Recent studies (31–34) suggest that the effects of linoleic acid and oleic acid on serum total and LDL cholesterol levels are more similar than previously observed (11, 12). Stearic acid has also repeatedly been reported to produce serum lipid levels similar to those seen on oleic acid (11–14). Thus, one would expect stearic acid and linoleic to be more or less equivalent with respect to their cholesterolemic effect. This was indeed observed for the women in our study, but not for the men, who showed a small but significant rise in total and LDL cholesterol and triglycerides on the Stearate- versus the Linoleate-diet. The increase of 10.4 mg/dl (0.27 mmol/l) in total cholesterol is in line with the equations of Keys, Anderson, and Grande (11) and Hegsted et al. (12), which predict an increase of 9.7–11.6 mg/dl in men when 8 dietary energy % of linoleic acid is replaced by stearic or oleic acid. In the absence of an oleic acid diet period in the present trial, it is impossible to tell whether the difference in cholesterol levels in males of 10.4 mg/dl between the Stearate- and Linoleate-diet periods was due to stearate being somewhat cholesterol-raising or linoleate being somewhat cholesterol-lowering relative to oleic acid. Whatever the relative effects of stearate, oleate, and linoleate, they are much smaller than the 28.6 mg/dl (0.74 mmol/l) increase that can be predicted for a replacement of 8 dietary energy % of linoleic acid by a mixture of lauric (C12:0), myristic (C14:0), and palmitic (C16:0) acids (11). Obviously, stearic acid raises total and LDL cholesterol less than other saturated fatty acids, and it will probably take a number of very large trials to tell whether the effects of stearic and oleic acid differ at all.

Our data do suggest that stearic acid might have some HDL cholesterol-lowering potential relative to linoleic acid, 0.06 mmol/l or 2 mg/dl in the present study. In the study of Bonanome and Grundy (14) a diet enriched with 16 energy % from stearic acid resulted in HDL levels that were 3.9 mg/dl (0.10 mmol/l) lower than those on a diet enriched with oleic acid. This difference was not statistically significant, but at the very least the observations of Bonanome and Grundy (14) on stearic acid and HDL do not contradict ours. Becker et al. (43) compared the effects of saturated fat versus monounsaturated and polyunsaturated fat. Their saturated fat diet supplied 12–13 energy % from stearic acid as opposed to 1 energy % in the two other diets. The stearate-rich saturated fat diet decreased HDL cholesterol by 0.10 mmol/l compared with the monounsaturated and by 0.14 mmol/l compared with the polyunsaturated fat diet. As in the study of Bonanome and Grundy (14), these differences were not statistically significant. Nevertheless, data from both studies are in line with our present observations, and they suggest that stearic acid may be neutral as regards total but not HDL cholesterol. The potential HDL cholesterol-depressing effect of stearic acid obviously needs more study.

Effect of gender on responses of lipid and lipoproteins to diet

Suitability of females for studies on diet and lipids. Most trials on the effects of diet on serum lipid and lipoprotein levels have used men only, partly because coronary heart disease was considered primarily a male disease, but partly also because women were considered less suitable because of confounding effects of the menstrual cycle. In fact, different studies on the influence of the natural menstrual cycle on serum lipids show different effects (44, 45) or only minor if any effects (46), and a recent textbook concluded "the degree of variation observed is relatively small and results of separate studies have sometimes been inconsistent" (47). However, oral contraceptives do influence the concentrations of total and HDL cholesterol in a cyclical manner (46). At first sight one might think that this might confound our findings in the 13 women who used oral contraceptives. This, however, is not the case. Different women entered the study at different points in their cycle, and in addition, diets were fed in random sequence, so that the effects of the contraceptive cycle canceled each other and averaged out. Thus, any woman who had an unusually low HDL cholesterol during week 3 of the Linoleate-diet because she was at the end of her cycle was counterbalanced on average by another woman whose HDL cholesterol was high when she was at week 3 of the Linoleate-diet because she happened to be at the pill-free period of her cycle.

Unlike the means, one would expect the standard deviations of the responses to be biased upwards because cyclic effects add a random positive or negative

term to each lipid data point. The standard deviations of the responses to dietary changes of the women using oral contraceptives were indeed somewhat larger than those of the women not using contraceptives (on average 0.43 mmol/l vs. 0.28 mmol/l for LDL cholesterol, and 0.15 mmol/l vs. 0.10 mmol/l for HDL cholesterol). However, standard deviations of the mean responses of all 30 women combined (13 users plus 17 non-users) were very similar to those of the 26 men (on average 0.38 vs. 0.34 mmol/l for LDL and 0.13 vs. 0.09 mmol/l for HDL cholesterol). This is in accord with our previous experience (18, 32). This would seem to validate the use of female volunteers as experimental subjects in studies of diet and lipids; at the very most, the number of females required to reach a certain statistical power should be adjusted upward when the majority are using oral contraceptives.

Gender effects on response. We found no differences between men and women in the response of serum lipoprotein or apolipoprotein levels to the *trans* fatty acid diet relative to linoleic acid (Tables 4 and 5). In a previous study the effects of replacing oleic acid by *trans* fatty acids were also similar in men and women (9). It therefore appears that the lipoprotein levels of men and women respond similarly to dietary *trans* fatty acids.

The increase in total and LDL cholesterol and triglycerides in men on the Stearate-diet was not observed for the women in our study (Table 4). Compared with the Linoleate-diet, the changes of the men's total cholesterol and apoB levels on the Stearate-diet were significantly larger than those of the women (Tables 4 and 5). HDL cholesterol was decreased to a similar extent in men and women. The HDL/LDL ratio was significantly lowered on the Stearate-diet versus the Linoleate-diet in both men and women, although the effect was very small in the women (Table 4). Whether stearic acid is cholesterol-raising in men but not in women could not be definitively addressed here. Obviously this point needs further investigation.

Consequences for the production of hardened fats

Replacement of 8 dietary energy % of monounsaturated *trans* fatty acids by stearic acid resulted in only minor changes in serum lipid and lipoprotein values. Our data thus do not support the idea that increasing the stearic acid content of hardened fats at the expense of *trans* fatty acids is beneficial for the serum lipid profile; either mode of hydrogenation produced fatty acids that increased LDL and decreased HDL cholesterol relative to linoleic acid itself.

However, *trans* fatty acids and stearic acid probably raise total and LDL cholesterol to a lesser extent than C12-16 saturated acids. Even if the *trans* fatty acids were to be equated with C12-16 saturated acids because of their added unfavorable effect on HDL, the content of *trans* plus cholesterol-raising saturates in butter still far exceeds that in soft margarines. Butter is also very high in cholesterol. Therefore, replacement of butter by soft margarines remains of value in the dietary treatment of hypercholesterolemia. However, manufacturers should consider the option of producing soft margarines free of *trans* fatty acids. Diet margarines with zero *trans* fatty acids made from unmodified sunflower oil and a small amount of a stearate-rich hard stock (48) have been marketed successfully for many years in Europe and are also available in North America.

Although data on the *trans* fatty acid content of brick margarines, vegetable shortenings, and solid vegetable frying fats are incomplete, it is probably much higher than in soft margarines, and the sum of *trans* fatty acids and cholesterol-raising saturates in such fats may approach that in beef tallow or lard. If the unfavorable effects of *trans* fatty acids on serum lipoproteins are borne out by future studies, then the use of so-called "cholesterol-free" solid vegetable fats for cooking or (deep-fat) frying may offer few health benefits over beef tallow or lard, and at that time replacement of such fats by unmodified or very lightly hydrogenated oils should be considered. Use of high-*trans* fats could then be limited to baked goods and other products where suitable replacements are much harder to find.

However, any large-scale displacement of partially hydrogenated vegetable oils from the market should await the outcome of further experiments, which in addition to lipids should also attempt to gauge effects on fibrinogen and on platelet function and aggregation tendency.

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