Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein[a] levels in humans

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Abstract Serum lipoprotein[a] (Lp[a]) is a strong risk factor for coronary heart disease. We therefore examined the effect of dietary fatty acid composition on serum Lp[a] levels in three strictly controlled experiments with healthy normocholesterolemic men and women. In Expt. I, 58 subjects consumed a control diet high in saturated fatty acids for 17 days. For the next 36 days, 6.5% of total energy intake from saturated fatty acids was replaced by monounsaturates plus polyunsaturates (monounsaturated fatty acid diet; n = 29) or by polyunsaturates alone (polyunsaturated fatty acid diet; n = 29). Both diets caused a slight, nonsignificant, increase in median Lp[a] levels, with no difference between diets. In Expt. II, 10% of energy from the cholesterol-raising saturated fatty acids (lauric, myristic, and palmitic acid) was replaced by oleic acid or by *trans*-monounsaturated fatty acids. Each of the 59 participants received each diet for 3 weeks in random order. The median level of Lp[a] was 26 mg/l on the saturated fatty acid diet; it increased to 32 mg/l ($P < 0.020$) on the oleic acid diet and to 45 mg/l ($P < 0.001$) on the *trans*-fatty acid diet. The difference in Lp[a] between the *trans*-fatty acid and the oleic acid diets was also highly significant ($P < 0.001$). Expt. III involved 56 subjects; all received 8% of energy from stearic acid, from linoleic acid, or from *trans*-monounsaturates, for 3 weeks each. All other nutrients were equal. Median Lp[a] levels were 69 mg/l on both the stearate diet and linoleate diet, and rose to 85 mg/l ($P < 0.01$) on the *trans* diet. Changes in Lp[a] were positively related to initial levels. Samples from Expt. I had been stored for 43 months, those from Expt. II for 31 months, and samples from Expt. III for 14 months at −40°C or lower. Comparison of 19 paired samples suggested that storage may have caused an overall decrease of 5–12% in Lp[a] levels, with no effect on the order of ranking of Lp[a] within studies. These short-term experiments suggest that diets high in *trans*-monounsaturated fatty acids may increase serum levels of Lp[a].—**Mensink, R. P., P. L. Zock, M. B. Katan, and G. Hornstra.** Effect of dietary *cis* and *trans* fatty acids on serum lipoprotein[a] levels in humans. *J. Lipid Res.* 1992. **33:** 1493–1501.

Supplementary key words saturated fatty acids • oleic acid • linoleic acid

Lipoprotein[a] (Lp[a]) is a macromolecular complex, made up of apolipoprotein B, cholesterol, and other lipids, and a protein called apo[a]. Apo[a] shows sequence homology to plasminogen (1). The Lp[a] concentration in the blood is largely under genetic control and does not change much with age (2, 3). Most subjects have Lp[a] levels below 150 mg/l, but levels in some may well exceed 400 mg/l (2, 3). Such subjects have a markedly increased risk for coronary heart disease, a relationship that is not confounded by serum low-density or high-density lipoprotein (LDL or HDL) cholesterol levels (3, 4).

In spite of the structural resemblance between Lp[a] and LDL (5), determinants of serum Lp[a] levels are distinctly different from those of LDL. Although niacin used with neomycin has been reported to decrease Lp[a] levels (6), attempts to modify Lp[a] by drug (7) or diet (8, 9) have not been very successful. Recently, however, we have shown that dietary fatty acid composition may affect Lp[a]: replacement of the habitual fat in the Dutch diet by palm oil resulted in a significant decrease in serum Lp[a] levels (10). We therefore decided to analyze Lp[a] levels in serum samples from three controlled studies on diet and lipoproteins. Expt. I (11) involved replacement of saturated fatty acids by monounsaturated (oleic acid) or polyunsaturated fatty acids (linoleic acid). Expt. II (12) compared the cholesterol-raising saturated fatty acids with *cis*-monounsaturated fatty acids (oleic acid) and with *trans*-monounsaturated fatty acids, and Expt. III (13) dealt with stearic acid, linoleic acid, and the *trans* fatty acid, elaidic acid.

METHODS

Subjects and methods

Most subjects were young, normolipidemic, nonobese students. They were all apparently healthy as indicated by a medical questionnaire, and by the absence of anemia,

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glucosuria, and proteinuria. The experimental protocols, which had been approved by the Ethical Committee of the Department of Human Nutrition, Agricultural University, Wageningen, were fully explained to the subjects and their informed consent was obtained. No monetary inducement was offered, except for the food, which was provided at no expense.

Diets consisted of conventional mixed solid foods. All foodstuffs were supplied individually according to each person's energy requirement. On weekdays at noon, hot meals were served at the Department of Human Nutrition in Wageningen. All other food was provided daily as a package. Food for the weekend and instructions for its preparation were provided on Fridays. In addition to the food supplied, each subject had to choose from a list food items that were free of fat and cholesterol. These items provided 9% of total daily energy intake. Body weight was recorded twice weekly and energy intake was adjusted when necessary to prevent changes in weight.

Subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of oral contraceptives. They recorded in diaries any sign of illness, medication 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.

Complete duplicate portions for one imaginary participant with a daily energy intake of 10 MJ or 11 MJ (2390 kcal or 2630 kcal) were collected daily for each diet. The duplicates were stored at −20°C, and were pooled and analyzed after the study. The free-choice items consumed were coded and their composition was calculated using the Netherlands Nutrient Data Base (14). The analyzed values of the duplicate diets were combined with the calculated values for the free-choice items.

Fasting blood samples were collected between 7:15 AM and 10:00 AM. Blood was allowed to clot for 1 h and serum for lipid and lipoprotein measurements was obtained by low-speed centrifugation and stored at −40°C or lower until analysis.

Lp[a] measurements

Serum Lp[a] levels were analyzed by enzyme linked immunosorbent assay (ELISA) using a commercial kit (TintElize® Lp[a], Biopool, Umea, Sweden). The assay utilizes polyclonal antibodies raised against purified Lp[a]. The assay uses micro-test wells coated with affinity-purified goat anti-apo[a] antibodies, which bind the Lp[a] particles present in the test sample. After incubation, anti-apo[a] antibodies conjugated to a peroxidase are added. These antibodies bind to the apo[a] of the immobilized Lp[a] from the test sample. Unbound conjugated antibodies are washed off, and 1,2-phenylenediamine dihydrochloride is added. This is converted by peroxidase into a compound with a specific absorbance at 492 nm. A calibration curve is obtained from human standard plasma samples that are provided with the kit and represent eight different Lp[a] concentrations between 0 and 600 mg/l, expressed in terms of particle mass. In all assays the correlation coefficient for the calibration curve was over 0.990. Addition of 3–1300 mg/l of human plasminogen to an Lp[a]-free medium or to a human plasma containing 140 mg/l of Lp[a] did not change the assay response (Biopool Ltd, personal communication, 1991). A control serum pool was analyzed in each run. The average Lp[a] level for this pool was 245 mg/l and the inter-assay and intra-assay coefficients of variation were both 5.3%. However, due to a lack of international standardization, absolute levels of Lp[a] may not be directly comparable with those in other studies (15). For each study, all samples of one subject were analyzed on the same plate. Samples had been stored at −40°C or lower for 14–43 months.

In a separate run, 29 pairs of serum and of citrated plasma, that had been stored for 1–3 weeks at −80°C, were analyzed. Lp[a] levels ranged between 6 and 670 mg/l for the serum and from 6 to 637 mg/l for the plasma. Plasma samples were on average 11 mg/l lower than serum samples. The correlation coefficient was 0.99. These results suggest that the assay response is not materially changed when the clotting cascade has been activated, and that Lp[a] can be validly assayed in serum.

Design and diets

Expt. I was carried out from 4 October to 27 November 1987 (11). Fifty-eight healthy men and women first consumed a control diet high in saturated fat for 17 days. For the next 36 days, 14 of the men and 15 of the women received a diet enriched with olive oil and sunflower oil (monounsaturated fatty acid diet). The other 13 men and 16 women received a diet enriched with sunflower oil alone (polyunsaturated fatty acid diet). For the monounsaturated fatty acid group, 6.5% of total energy intake provided by saturated fatty acids on the control diet was replaced by a mixture of oleic acid (*cis*-C18:1, n–9) plus linoleic acid (*cis,cis*-C18:2, n–6) and for the polyunsaturated fatty acid group by linoleic acid alone. The intake of other nutrients was kept constant.

At the start of the study the mean (± SD) serum total cholesterol level of the subjects was 5.00 ± 0.79 mmol/l, and their mean body mass index was 21.6 ± 2.0 kg/m². During the study body weight increased by 0.2 ± 1.3 kg in the monounsaturated fatty acid group and by 0.2 ± 1.0 kg in the polyunsaturated fatty acid group. Three men and four women smoked. Nine women used oral contraceptive agents.

Blood was sampled on days 14 and 17 (control period) and on days 50 and 53 (test period). For Lp[a] analyses equal volumes were pooled per subject per diet period. The response to the monounsaturated fatty acid or the polyunsaturated fatty acid diet was calculated as the

change from the end of the control period to the end of the test period. This design only allows comparison of differences between the effect of monounsaturated and polyunsaturated fatty acids; the absolute changes relative to the control diet high in saturated fatty acids may have been biased by unknown drifts with time.

Expt. II was performed from 26 September to 28 November 1988 (12). Twenty-five men and 34 women consumed three different diets for 3 weeks each, in random order. The composition of the diets was identical, except for 10% of daily energy intake which was provided by either a mixture of the cholesterol-raising saturated fatty acids lauric, myristic, and palmitic acid, by oleic acid, or by *trans* isomers of oleic acid. The fat blend high in saturated fatty acids was composed of 55 parts of an interesterified mixture containing 40% of palm oil and 60% of palm kernel oil, 5 parts of an interesterified mixture containing equal volumes of fully hardened palm oil and palm kernel oil, and 40 parts of high oleic acid sunflower oil (TRISUN). The oleic acid group received olive oil and a margarine made with of 85 parts of sunflower oil high in oleic acid and 15 parts of a mixture of equal volumes of interesterified fully hydrogenated palm oil and palm kernel oil. For the *trans*-fatty acid diet, the high oleic acid sunflower oil was hydrogenated under conditions that promoted isomerization, and 78 parts were then mixed with 10 parts of the unaltered oleic acid-rich sunflower oil, 10 parts of regular sunflower oil, and 2 parts of low erucic acid rapeseed oil.

At the start of the study the mean (± SD) serum total cholesterol level was 4.75 ± 0.64 mmol/l, and the mean body mass index 22.0 ± 2.3 kg/m². Body weight increased by 0.1 ± 1.0 kg over the 63 days of the study. Two women, but none of the men, smoked. Eight women used oral contraceptive agents.

Blood was sampled on the 18th and 21st day of each diet period and equal volumes of the two sera samples per subject were pooled.

Expt. III (13) had the same design as experiment II. Between 29 January and 2 April 1990, 26 men and 30 women participated. The difference between the three diets consisted of 8% of energy that was provided by either stearic acid, linoleic acid, or the *trans*-monounsaturated fatty acid, elaidic acid. A fat high in stearic acid was made by interesterification of 41 parts of fully hydrogenated high linoleic acid sunflower oil, 50 parts of high oleic acid sunflower oil and 9 parts of regular high linoleic acid sunflower oil. For the *trans*-fatty acid diet, high oleic acid sunflower oil was hydrogenated so as to favor formation of *trans* fatty acids (13). Seventy-five parts of this fat were mixed with 25 parts of the unmodified oleic acid-rich sunflower oil. For the linoleate diet, regular sunflower oil was the major source of linoleic acid.

The mean initial serum total cholesterol level was 5.05 ± 0.79 mmol/l, and the mean body mass index 21.5 ± 2.1 kg/m². During the 9 weeks of the study, body weight decreased by 0.2 ± 1.1 kg. Four men and four women smoked, and thirteen women used oral contraceptives.

Statistical analyses

In all three studies the distribution of serum Lp[a] levels among the dietary groups as well as the individual responses of Lp[a] to the diets was highly skewed. A square root-transformation rather than a log-transformation proved optimal for normalizing the distribution of Lp[a] levels and responses. In order to check the robustness of the statistical analyses, we analyzed both the original and the normalized data. Absolute Lp[a] concentrations are presented as median levels and square root-transformed values as means. The untransformed individual changes were analyzed with nonparametric tests: the Mann-Whitney rank-sum test for Expt. I was used to examine the difference in change between the monounsaturated fatty acid and the polyunsaturated fatty acid diets, while the Friedman test for Expts. II and III was used to evaluate diet effects. Responses calculated from the square root-transformed Lp[a] levels were analyzed using an unpaired *t*-test for Expt. I and analysis of variance (ANOVA) for Expts. II and III. When a significant diet effect was observed ($P < 0.05$) in Expts. II and III, the three diets were compared pairwise; to correct for multiple comparisons only P values of less than 0.020 were then considered significant (16, 17).

RESULTS

Effect of storage time on Lp[a] levels

Nine subjects had participated in both Expt. I and Expt. II, and ten subjects in both Expt. II and Expt. III. To examine the effect of storage, Lp[a] levels of the nine subjects when they were eating the monounsaturated or polyunsaturated fatty acid diet in Expt. I were compared with their values on the oleic acid diet in Expt. II. For the subjects who participated in both Expt. II and Expt. III, Lp[a] concentrations on the oleic acid diet in Expt. II were compared with levels on the linoleate diet in Expt. III. These diets were chosen because they had similar effects on Lp[a] (see below). Samples from Expt. I had been stored for 43 months, those from Expt. II for 31 months, and samples from Expt. III for 14 months. The nine samples of Expt. I were on average 5.4% lower than the corresponding samples of Expt. II. The ten samples of Expt. II were 12.2% lower than those of Expt. III. Thus, Lp[a] levels had decayed slightly with storage, but the order of ranking remained nearly unchanged (**Fig. 1**). For all 19 pairs combined the correlation coefficient was 0.96.

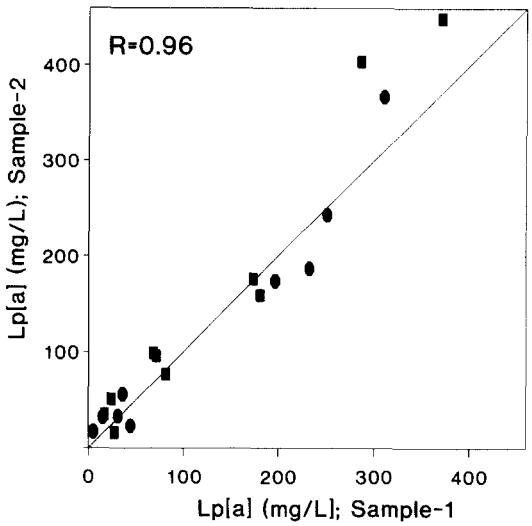

Fig. 1. Effect of storage on serum Lp[a] levels. Nine subjects had participated in both Expt. I and Expt. II. (●), and 10 subjects in both Expt. II and Expt. III (■). To examine the effect of storage, Lp[a] levels of the 9 subjects when on the monounsaturated or polyunsaturated fatty acid diet in Expt. I (sample-1) were compared with their values on the oleic acid diet in Expt. II (sample-2). For the 10 subjects who participated in both Expt. II and Expt. III, Lp[a] concentrations on the oleic acid diet in Expt. II (sample-1) were compared with those on the linoleate diet in Expt. III (sample-2). Samples from Expt. I had been stored for 43 months, those from Expt. II for 31 months, and samples from Expt. III for 14 months.

Expt. I: Monounsaturates versus polyunsaturates

Total fat intake of the participants was similar for all three diets (**Table 1**). The proportion of energy from the cholesterol-raising saturated fatty acids (lauric acid, myristic acid, and palmitic acid) decreased by 4.3% on the monounsaturated fatty acid diet and by 5.3% on the polyunsaturated fatty acid diet. The intake of stearic acid decreased by slightly less than 1% on both test diets. These decreases were compensated for by an increased intake of oleic acid (4.3%) and linoleic acid (3.6%) on the monounsaturated fatty acid diet and by linoleic acid alone (8.5%) on the polyunsaturated fatty acid diet.

In the group that switched from the control diet high in saturated fatty acids to the monounsaturated fatty acid diet, the median Lp[a] concentration increased by 7 mg/l, from 84 to 91 mg/l (range of individual changes: −34 to 89 mg/l; **Table 2**). The median value of the polyunsaturated fatty acid group rose by 3 mg/l, from an initial 37 to 40 mg/l (range of changes: −32 to 46 mg/l). The difference in response between the two diet groups was not significant (Mann-Whitney test: $P=0.852$, t-test: $P=0.815$).

Initial Lp[a] levels in subjects on the control diet high in saturated fatty acids were markedly, though not significantly (Mann-Whitney test: $P=0.224$, t-test: $P=0.219$), higher in the monounsaturated fatty acid than in the polyunsaturated fatty acid group. As changes might occur only at relatively high levels, subjects were divided into two groups of 29 each, according to their Lp[a] concentration on the control diet high in saturated fatty acids. Of the 29 subjects with the highest initial Lp[a] levels, 17 were later to receive the monounsaturated fatty acid diet, and the remaining 12 the polyunsaturated fatty acid diet. For the monounsaturated fatty acid group, initial median Lp[a] levels on the control diet were 151 mg/l (range: 45–340 mg/l) and decreased by 1 mg/l to 150 mg/l (range of changes: −34 to 89 mg/l). In those who switched to the polyunsaturated fatty acid diet, Lp[a] declined by 8 mg/l, from 199 mg/l (range: 47–235 mg/l) to 191 mg/l (range of changes: −32 to 46 mg/l). Again, the difference in changes between the two groups did not reach statistical significance (Mann-Whitney test: $P=0.929$, t-test: $P=0.647$).

LDL cholesterol levels (Table 2) decreased by 0.59 mmol/l [23 mg/dl] on the monounsaturated and by 0.46 mmol/l [18 mg/dl] on the polyunsaturated fatty acid diet ($P=0.05$ for difference between diets). Adherence to the diets was confirmed by changes in the fatty acid composition of serum cholesteryl esters. The proportion of oleic acid in serum cholesteryl esters decreased by 0.8% on the monounsaturated fatty acid group and by 4.6% on the polyunsaturated fat acid diet ($P < 0.001$). The level of linoleic acid increased by 4.2% and by 8.7%, respectively ($P < 0.001$).

Changes in Lp[a], or the lack of changes, were similar in men and women (Table 2), and in the five subjects who had suffered intercurrent illness (11) when compared with the other 54 subjects.

TABLE 1. Dietary fatty acid intakes in Experiment I

	Control Diet	Monounsaturated Fatty Acid Diet	Polyunsaturated Fatty Acid Diet
	% of daily energy intake		
Total fat	36.7	37.4	37.6
Saturated fatty acids	19.3	12.9	12.6
Lauric acid (C12:0)	1.2	0.7	0.9
Myristic acid (C14:0)	3.2	1.4	1.3
Palmitic acid (C16:0)	9.3	6.8	6.2
Stearic acid (C18:0)	4.1	3.2	3.4
Monounsaturated fatty acids	11.5	15.1	10.8
Cis-C18:1	10.2	14.5	10.2
Polyunsaturated fatty acids	4.6	7.9	12.7
Linoleic acid (*Cis,cis*-C18:2,n-6)	4.1	7.7	12.6

Values are based on chemical analyses of duplicate diets. All 58 subjects first consumed the control diet high in saturated fatty acids for 17 days; for the next 36 days 14 men and 15 women received the monounsaturated fatty acid diet, and 13 men and 16 women the polyunsaturated fatty acid diet. The intake of protein (13% of daily energy intake), carbohydrates (48–49%), alcohol (1–2%), cholesterol (33–36 mg/MJ), and other nutrient was virtually identical on all three diets.

TABLE 2. Serum lipoprotein[a] and LDL-cholesterol levels in Experiment I

Group	Control Diet	Mono- or Polyunsaturated Fatty Acid Diet
	mg/liter	
Lipoprotein[a][a]		
Monounsaturated fatty acid group		
Men	45 (0–77)	52 (0–160)
Women	142 (0–340)	129 (0–336)
All	84 (0–340)	91 (0–336)
Polyunsaturated fatty acid group		
Men	37 (0–235)	36 (0–240)
Women	43 (0–220)	44 (0–237)
All	37 (0–235)	40 (0–240)
$\sqrt{\text{(Lipoprotein[a])}}$[b]		
Monounsaturated fatty acid group		
Men	6.6 ± 5.0	6.7 ± 4.8
Women	10.9 ± 5.8	11.3 ± 5.8
All	8.8 ± 5.8	8.9 ± 5.7
Polyunsaturated fatty acid group		
Men	6.6 ± 5.1	6.5 ± 5.4
Women	7.3 ± 5.7	7.5 ± 5.7
All	7.0 ± 5.4	7.0 ± 5.5
LDL-cholesterol	*mmol/liter*	
Monounsaturated fatty acid group		
Men	3.05 ± 0.79	2.52 ± 0.73
Women	3.53 ± 0.65	2.89 ± 0.58
All	3.30 ± 0.75	2.71 ± 0.67
Polyunsaturated fatty acid group		
Men	3.62 ± 0.68	3.06 ± 0.68
Women	3.09 ± 0.61	2.73 ± 0.52
All	3.33 ± 0.68	2.87 ± 0.60[c]

Fifty eight subjects first received the control diet high in saturated fatty acids for 17 days; for the next 36 days, 14 men and 15 women received a diet high in monounsaturated fatty acids, and 13 men and 16 women received a diet high in the polyunsaturated fatty acids.

[a] Values are median levels (ranges).

[b] Values are square root-transformed means ± SD.

[c] Denotes a significant difference in change between the two diet groups: $P < 0.05$.

Expt. II: Saturates versus *cis*-monounsaturates versus *trans*-monounsaturates

The intake of the cholesterol-raising saturated fatty acids decreased by 8.5% of energy intake on the oleic acid diet and by 8.8% on the *trans*-fatty acid diet compared with the saturated fatty acid diet (**Table 3**). Stearic acid and total fat intake were similar on all three diets.

Table 4 shows that the median serum Lp[a] level was 26 mg/l on the saturated fatty acid diet and rose to 32 mg/l on the oleic acid diet (range of individual changes: −77 to +110 mg/l; Friedman test: $P=0.020$, ANOVA: $P=0.002$) and to 45 mg/l on the *trans*-fatty acid diet (range of changes: −58 to +254 mg/l; Friedman test and ANOVA: $P < 0.001$). The difference between the median Lp[a] levels on the *trans*-fatty acid and the oleic acid diets was also highly significant (range of changes: −32 to 144 mg/l; Friedman and ANOVA: $P < 0.001$). The effects were observed to a similar extent in men and women.

TABLE 3. Dietary fatty acid intakes in Experiment II

	Saturated Fatty Acid Diet	Oleic Acid Diet	*Trans*-Fatty Acid Diet
	% of daily energy intake		
Total fat	38.8	39.6	40.2
Saturated fatty acids	19.4	9.5	10.0
Lauric acid (C12:0)	3.4	0.5	0.4
Myristic acid (C14:0)	2.7	0.5	0.7
Palmitic acid (C16:0)	8.1	4.7	4.3
Stearic acid (C18:0)	3.5	3.0	3.6
Monounsaturated fatty acids	14.7	24.1	24.2
Cis-C18:1	12.8	23.0	12.6
Trans-C18:1	1.8	0.0	10.9
Polyunsaturated fatty acids	3.4	4.6	4.6
Linoleic acid (*Cis,cis*-C18:2,n-6)	2.9	4.0	4.2

Values are based on chemical analyses of duplicate diets. Fifty-nine subjects received each diet for 3 weeks each in random order. The intake of protein (13–14% of daily energy intake), carbohydrates (46%), alcohol (1%), cholesterol (32–35 mg/MJ), and other nutrients was virtually identical on all three diets.

TABLE 4. Serum lipoprotein[a] and LDL-cholesterol levels in Experiment II

Group	Saturated Fatty Acid Diet	Oleic Acid Diet	*Trans*-Fatty Acid Diet
		mg/liter	
Lipoprotein[a][a]			
Men	22 (0–220)	27 (0–200)	44 (0–297)[c,d]
Women	31 (0–447)	34 (0–484)[c]	48 (0–510)[c,d]
All	26 (0–447)	32 (0–484)[c]	45 (0–510)[c,d]
$\sqrt{\text{(Lipoprotein[a])}}$[b]			
Men	5.6 ± 4.6	5.9 ± 4.6	6.8 ± 4.9[c,d]
Women	5.5 ± 5.9	7.8 ± 5.9[c]	8.6 ± 6.4[c,d]
All	6.5 ± 5.4	7.0 ± 5.4[c]	7.8 ± 5.8[c,d]
LDL-cholesterol		*mmol/liter*	
Men	3.05 ± 0.66	2.59 ± 0.61[c]	2.93 ± 0.65[c,d]
Women	3.20 ± 0.50	2.73 ± 0.48[c]	3.12 ± 0.58[d]
All	3.14 ± 0.57	2.67 ± 0.54[c]	3.04 ± 0.61[c,d]

Twenty five men and 34 women received each diet for 3 weeks each in random order.
[a]Values are median levels (ranges).
[b]Values are square root-transformed means ± SD.
[c]Significantly different from levels on the diet high in saturated fatty acids: $P < 0.020$.
[d]Significantly different from levels on the diet high in oleic acid: $P < 0.020$.

To examine the effect of intrinsic serum Lp[a] levels, subjects were ranked according to their levels on the saturated fatty acid diet. **Fig. 2** shows that subjects with the highest innate Lp[a] levels had the greatest changes on both the oleic acid and the *trans*-fatty acid diet. Higher Lp[a] levels on the *trans*-fatty acid diet than on the saturated fatty acid diet were seen in 47 of the 56 subjects, while 40 subjects had higher Lp[a] levels on the oleic acid diet than on the saturated fatty acid diet. Four subjects had no detectable amounts of Lp[a] on any diet.

Serum LDL cholesterol decreased by 0.47 mmol/l [18 mg/dl] on the oleic acid diet ($P < 0.001$) and by 0.10 mmol/l [4 mg/dl] on the *trans*-fatty acid diet ($P < 0.001$) as compared with the saturated fatty acid diet (Table 4). Relative to levels on the oleic acid diet, the proportion of oleic acid in serum cholesteryl esters decreased by 3.9% on the saturated fatty acid diet ($P < 0.02$), and by 7.1% on the *trans*-fatty acid diet ($P < 0.02$). The percentage of *trans*-monounsaturated fatty acids in serum cholesteryl esters was 0.13% on the oleic acid diet, 0.21% on the saturated fatty acid diet ($P < 0.020$), and 1.34% on the *trans*-fatty acid diet ($P < 0.020$).

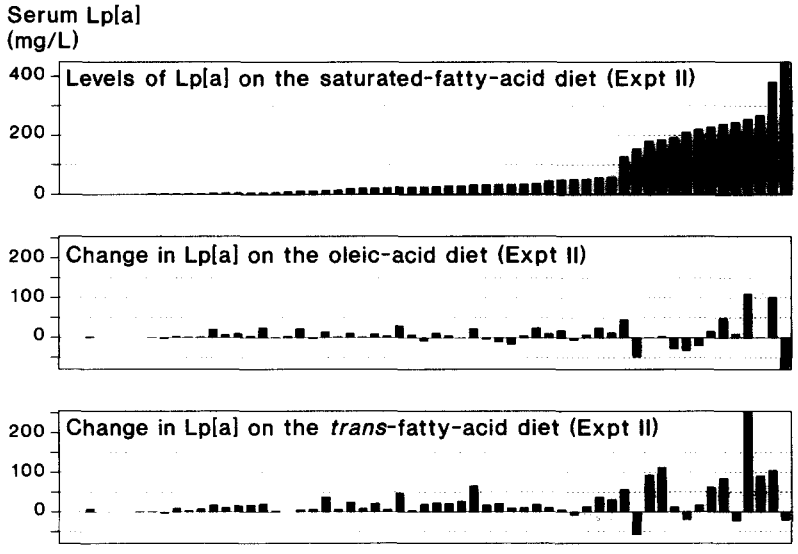

Fig. 2. Individual levels of serum Lp[a] in Expt. II (n = 59) on the diet high in the cholesterol-raising saturated fatty acids, and responses to the diet when 10% of energy from the cholesterol-raising saturated fatty acids was replaced by either oleic acid or *trans*-monounsaturated fatty acids.

TABLE 5. Dietary fatty acid intakes in Experiment III

	Stearate Diet	Linoleate Diet	*Trans*-Fatty Acid Diet
	% of daily energy intake		
Total fat	43.5	41.1	39.7
Saturated fatty acids	20.1	11.0	10.3
Lauric acid (C12:0)	0.5	0.7	0.5
Myristic acid (C14:0)	1.0	0.9	1.0
Palmitic acid (C16:0)	5.7	5.8	4.8
Stearic acid (C18:0)	11.8	2.8	3.0
Monounsaturated fatty acids	16.6	15.8	23.3
Cis-C18:1	15.4	14.7	14.6
Trans-C18:1	0.3	0.1	7.7
Polyunsaturated fatty acids	4.3	12.5	3.8
Linoleic acid (*Cis,cis*-C18:2,n-6)	3.9	12.0	3.8

Values are based on chemical analyses of duplicate diets. Fifty-six subjects received each diet for 3 weeks each in random order. The intake of protein (12–13% of daily energy intake), carbohydrates (44–47%), alcohol (1%), cholesterol (33–34 mg/MJ), and other nutrients was virtually identical on all three diets.

Expt. III: Stearic acid versus linoleic acid versus *trans*-monounsaturates

Intake of the cholesterol-raising saturated fatty acids and of oleic acid differed by less than 1% between the three diets (**Table 5**). Nine percent of energy from stearic acid was replaced by 8.1% of linoleic acid on the linoleate diet and by 7.4% of *trans*-monounsaturated fatty acids on the *trans*-fatty acid diet.

In this set of subjects the median Lp[a] level was 69 mg/l on both the stearate and the linoleate acid diets (**Table 6**). It rose to 85 mg/l on the *trans*-fatty acid diet (range of individual changes relative to stearic acid diet: −99 to 230 mg/l; Friedman test: $P=0.006$, ANOVA: $P=0.018$). The difference between the *trans*-fatty acid diet and the linoleate diet was also highly significant (Friedman test: $P < 0.001$, ANOVA: $P=0.018$). Gender did not affect the changes observed.

As shown in **Fig. 3**, changes in Lp[a] on the *trans*-fatty acid diet relative to the stearate diet were related to initial levels. Lp[a] increased on the *trans*-fatty acid diet in 34 out of the 56 subjects, it decreased in 17 subjects, and 5 subjects showed no change.

The mean LDL cholesterol level was 3.00 mmol/l [116 mg/dl] on the stearate diet, 2.83 mmol/l [109 mg/dl] on the linoleate diet ($P < 0.001$), and 3.07 mmol/l [119 mg/dl] on the *trans*-fatty acid diet (Table 6). The proportion of linoleate in serum cholesteryl esters increased by 6.6% on the linoleate diet ($P < 0.020$), but decreased by 1.6% on the *trans*-fatty acid diet ($P < 0.020$) compared with the stearate diet. The proportion of *trans*-monounsaturated fatty acids in serum cholesteryl esters was 0.12% on the stearate diet, 0.13% on the linoleate diet, and 0.94% on the *trans*-fatty acid diet ($P < 0.020$).

Summary of results

These three studies together strongly suggest that *trans*-monounsaturated fatty acids elevate serum Lp[a] levels as compared with oleic acid, linoleic acid, or stearic acid (Expts. II and III). Oleic acid and linoleic acid had similar effects on serum Lp[a] (Expt. I). The cholesterol-raising saturated fatty acids caused marginally lower Lp[a] levels than oleic acid in Expt. II. Changes were related to initial levels, and were similar for men and women.

TABLE 6. Serum lipoprotein[a] and LDL-cholesterol levels in Experiment III

Group	Stearate Diet	Linoleate Diet	*Trans*-Fatty Acid Diet
		mg/liter	
Lipoprotein[a][a]			
Men	78 (0–298)	84 (1–310)	103 (0–351)[d]
Women	63 (0–749)	63 (0–782)	69 (0–891)
All	69 (0–749)	69 (0–782)	85 (0–891)[c,d]
$\sqrt{\text{(Lipoprotein[a])}}$[b]			
Men	8.8 ± 5.5	8.8 ± 5.5	9.4 ± 5.9[c,d]
Women	10.9 ± 7.8	11.0 ± 8.0	11.2 ± 8.2
All	9.9 ± 6.9	9.9 ± 7.0	10.3 ± 7.2[c,d]
LDL-cholesterol		*mmol/liter*	
Men	3.16 ± 0.74	2.90 ± 0.71[c]	3.14 ± 0.65[d]
Women	2.87 ± 0.66	2.78 ± 0.55	3.01 ± 0.66[d]
All	3.00 ± 0.71	2.83 ± 0.63[c]	3.07 ± 0.65[d]

Twenty six men and 30 women received each diet for 3 weeks each in random order.
[a] Values are median levels (ranges).
[b] Values are square root-transformed means ± SD.
[c] Significantly different from levels on the stearate diet: $P < 0.020$.
[d] Significantly different from levels on the linoleate diet: $P < 0.020$.

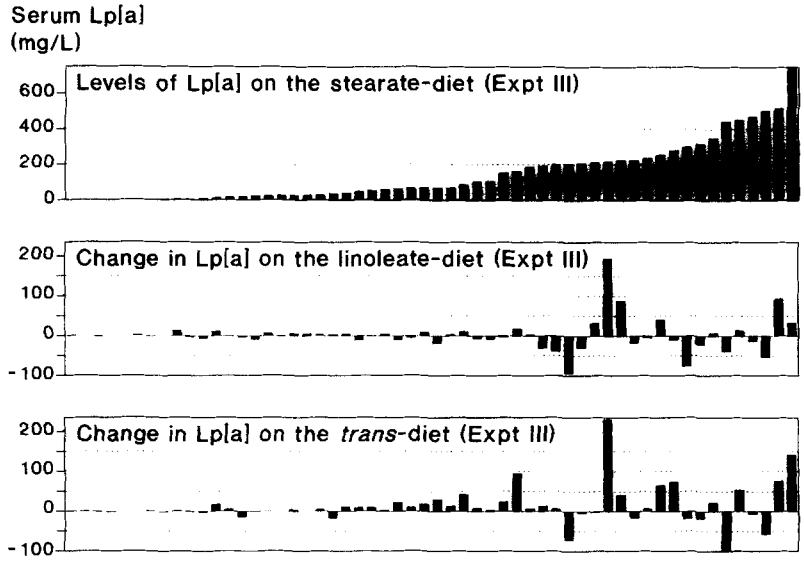

Fig. 3. Individual levels of serum Lp[a] in Expt. III (n=56) on the diet high in the saturated fatty acid stearic acid, and responses to the diet when 8% of energy from stearic acid was replaced by either linoleic or *trans*-monounsaturated fatty acids.

DISCUSSION

The level of Lp[a] is largely under genetic control, and initial studies suggested that, unlike LDL, Lp[a] levels are singularly insensitive to diet. Although this suggestion appears to be correct as far as dietary cholesterol is concerned (9, 18), dietary fat composition did affect Lp[a] concentrations in some (10, 19), but not all (20, 21), studies. Our results suggest that *trans*-monounsaturated fatty acids (*trans*-C18:1) increase Lp[a] levels relative to three other fatty acids with 18 carbon atoms: stearic acid (C18:0), oleic acid (*cis*-C18:1), and linoleic acid (*cis,cis*-C18:2). Compliance with the diets was very good, as indicated by changes in cholesteryl ester fatty acid composition.

We have shown recently in a 2 × 6 week double-blind cross-over trial that replacement of the habitual fat by palm oil lowered Lp[a] in healthy normolipidemic men (10). We suggested that the effect observed was due either to a component in palm oil or to displacement of a component present in the habitual dietary fat. Our present findings are in agreement with the latter suggestion, as replacement of the habitual fat by palm oil decreased the intake of *trans* fatty acids by more than 50% (10). Thus, the apparent Lp[a]-lowering effect of palm oil may have been due to displacement of *trans* fatty acids.

In Expt. II, Lp[a] levels were slightly, though significantly, lower on the diet high in the cholesterol-raising saturated fatty acids than on the oleic acid diet. In Expt. I, however, there was only a nonsignificant increase in median Lp[a] when subjects were switched from saturated fatty acids to oleic or linoleic acid. Apart from not being statistically significant, this slight change could also have been caused by a nonspecific drift with time, as a parallel design was used in Expt. I. Recently, Brown et al. (18) also reported similar Lp[a] levels on a saturated fatty acid diet and a diet high in polyunsaturated fatty acids. Although the level of the cholesterol-raising saturated fatty acids differed by less than 3% of total energy intake between the two diets (18)—the major exchange being between monounsaturated and polyunsaturated fatty acids, which, according to our results (Expt. I), have similar effects on Lp[a]—these findings do not support the evidence for an Lp[a]-lowering effect of saturated fatty acids.

Other studies have suggested that serum Lp[a] levels are not related to serum LDL cholesterol or apolipoprotein B levels (3, 18). This is confirmed by our present findings: depending on the type of fatty acid studied, diet-induced changes in Lp[a] and in LDL cholesterol or apolipoprotein B levels could be either in the opposite direction, as in Expt. II where oleic acid lowered LDL-cholesterol but raised Lp[a] relative to saturates, or in the same direction, as in Expt. III where *trans*-fatty acids raised both LDL-cholesterol and Lp[a] relative to linoleic acid. These observations make it less likely that dietary effects on LDL cholesterol and Lp[a] levels are mediated by the same pathway.

Changes were positively related to intrinsic Lp[a] levels as has also been observed by others (6, 10).

It has been reported that storage of serum samples at −20°C for 6 months (22) or even for up to 7 years does not affect serum Lp[a] levels (23), when ELISA kits were used from the same manufacturer as ours. Our results, however, do suggest that Lp[a] levels decay slightly with

storage. However, in each experiment each subject was sampled on two (Expt. I) or three (Expts. II and III) different diets, and only changes in Lp[a] between diets within one experiment were compared. Thus, a slight overall decay in Lp[a] might underestimate slightly the extent of response of Lp[a] to diet, but is highly unlikely to affect the direction of this response. Also, the significance of responses was similar when tested by two different statistical approaches—the conventional *t*-test and ANOVA versus nonparametric tests—which supports the robustness and consistency of the effects observed.

The experimental diets were consumed for 3–5 weeks. Although 3 weeks is long enough for serum total or LDL lipoprotein cholesterol levels to stabilize (11), we cannot exclude the possibility that the observed changes in Lp[a] are transient or, alternatively, underestimated and not yet at their maximum level. Our results do suggest that the observed effects are proportional to the amounts of fatty acids consumed. The intake of *trans*-monounsaturated fatty acids in Expt. III was about 4% of daily energy intake lower than in Expt. II, while the changes in Lp[a] were also less pronounced (Tables 4 and 6).

In conclusion, these short-term dietary experiments suggest that diets high in *trans*-monounsaturated fatty acids may increase serum levels of Lp[a].

Note added in proof: Recently, Nestel et al. (Nestel, P., M. Noakes, B. Belling, R. McArthur, P. Clifton, E. Janus, and M. Abbey. Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet *J. Lipid Res.* 1992. **33:** 1029–1036) reported that a diet rich in the *trans* fatty acid, elaidic acid, elevated the level of Lp[a] in mildly hypercholesterolemic men. Our findings are in good agreement with this observation.

We thank the volunteers for their cooperation and interest; Ms. Sylvia W. S. Jans, M.Sc. of the Department of Human Biology for analysis of serum lipoprotein[a] levels; and the technical and dietary staff of the Department of Human Nutrition for their assistance. These studies were partly supported by the Netherlands Nutrition Foundation, the Netherlands Ministry of Welfare, Public Health, and Cultural Affairs, and the Commission of the European Communities.

Manuscript received 12 February 1992 and in revised form 12 May 1992.

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