Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet

Paul Nestel,[1] Manny Noakes, Bryan Belling, Rosemary McArthur, Peter Clifton, Edward Janus,* and Mavis Abbey

CSIRO Division of Human Nutrition, Adelaide, South Australia, Australia, and St. Vincent's Hospital,* Melbourne, Australia

Abstract The effect of additional dietary *trans* fatty acids (7% energy) on plasma lipids was assessed in a double-blind comparison of four separate diets: 1, enriched with butter fat (lauric-myristic-palmitic); 2, oleic acid-rich; 3, elaidic acid-rich; 4, palmitic acid-rich. The total dietary period was 11 weeks and comprised normal foods plus specific fat supplements. In 27 mildly hypercholesterolemic men, total and LDL cholesterol were significantly lower during the 3-week oleic acid-rich diet, and were similar during the other three diets. For the four diets LDL cholesterol levels were in mg/dl: 1, 163; 2, 151; 3, 165; 4, 161. HDL cholesterol was significantly higher with the palmitic acid-rich diet, 42 mg/dl, compared with elaidic acid, 38 mg/dl, which in turn was not lower than with oleic acid, 38 mg/dl. Plasma elaidic acid concentration rose seven-fold with the *trans* fatty acid diet but did not increase the vulnerability of LDL to oxidative change. The elaidic acid-rich diet led to significant elevations in the level of Lp[a] compared to all the other test diets. The Lp[a] level increased to 296 ± 220 U/l in the elaidic acid-rich period from 235 ± 182 (mean ± SD) in the first ("butter") period ($P < 0.001$) compared with 249 ± 204 in the palmitic acid period ($P < 0.001$) and 236 ± 201 in the oleic acid period (NS). We conclude that 3 weeks consumption of *trans* fatty acid (mainly elaidic) at about 7% energy (probably twice the Australian average) results in LDL cholesterol levels that do not differ from those seen with diets enriched with palmitic acid or butter fat and are higher than when oleic acid is substituted for elaidic acid.—**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.

Supplementary key words plasma cholesterol • elaidic acid • palmitic acid • oleic acid

Commercial hydrogenation of polyunsaturated oils, as well as hydrogenation of linoleic and α-linolenic acids in the rumen of sheep and cattle, lead to the production of *trans* fatty acids. Concern has been expressed over the possible consequences to health brought about by the increased consumption of *trans* fatty acids in recent years. The average amounts currently eaten in countries where hydrogenated fats occur widely in foods has been estimated as at least 4% of total energy (1–3) and possibly two to three times that (4).

Reports on the effects of *trans* fatty acids have been inconsistent. In several of the better controlled studies, Mattson, Hollenback, and Kligman (5) found similar plasma cholesterol concentrations when elaidic acid was partly substituted for oleic acid; Mensink and Katan (6) showed that, at higher intakes of elaidic acid approximating those that prevail in the Netherlands, LDL cholesterol rose and HDL cholesterol fell; and Laine et al. (7) concluded that, provided the linoleic acid content of the diet was high enough, partial hydrogenation of linoleic and oleic acids would not raise the plasma cholesterol level. Two recent authoritative reports by the Food and Nutrition Board in the USA (8) and by the British Nutrition Foundation (2), published before the Mensink and Katan paper (6), concluded that the effect of *trans* fatty acids on the cholesterol level was "neutral".

In the Netherlands study (6), 11% energy was supplied from *trans* fatty acids, substantially more than the average intake in most industrialized countries. The implications for public health and for the edible oil industry are considerable. We have therefore conducted a controlled double-blind study in which we tested a more moderate amount of *trans* fatty acid and in which the elaidic acid-rich diet was compared with an oleic acid-rich diet, as well as with two additional diets enriched with saturated fatty acids. We measured plasma lipid and lipoprotein lipid concentrations including those of Lp[a], as well as the oxidizability of low density lipoproteins.

Abbreviations: SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; MDA, malondialdehyde; TBARs, thiobarbituric acid-reactive substances.

[1]To whom reprint requests should be addressed at: CSIRO Division of Human Nutrition, P.O. Box 10041 Gouger Street, Adelaide SA 5000, Australia.

EXPERIMENTAL METHODS

Subjects

Twenty-seven men found to be mildly hypercholesterolemic previously were recruited from among a larger group who volunteered to undertake diet studies. Their ages ranged from 30 to 63 years (mean ± SD; 46.8 ± 9.6 yr). Body mass averaged 80.16 ± 8.94 kg. Plasma cholesterol concentrations immediately prior to the study ranged from 176 to 283 (221 ± 29) mg/dl.

Informed consent was obtained and Human Ethics Committee approval was granted.

Experimental design

The study comprised four periods totalling 11 weeks. All subjects began with a 2-week control period which on average resembled their own diet and was simulated with a mixture of fats. This period was followed by an oleic acid-enriched diet period and two further dietary periods in random order: the elaidic acid-enriched and the palmitic acid-enriched diets. The three test diets were each of 3 weeks' duration.

All four periods comprised a background common diet, containing 15% energy as fat from dairy products, meat, bread, and cereals. Four different fat supplements contributing 20% energy were prepared as margarines (see Table 1) to provide a total of 35% energy from fat. Biscuits and potato crisps were also prepared using the margarines so that the contribution from each of the test foods was not unusually large. The subjects were also counselled how to obtain 15% energy from protein and 50% from carbohydrate.

The subjects were instructed individually by a dietitian. The background diet was obtained from the range of foods normally eaten and fat intake was kept constant by instructing subjects to identify and quantify fat intake by means of simplified food tables. Diets were individualized on the basis of energy requirements that were estimated by use of equations predicting basal metabolic rates and adjusted for physical activity determined by interview.

Dietary compliance and any changes in body weight were monitored regularly. Subjects were asked to return uneaten food, which occurred rarely and was taken into account. Subjects were provided with digital electronic scales and instructed to record all food and beverage intake for 3 consecutive days (including 1 weekend day) during each study period as well as recording fat intake on a daily basis. Detailed food records were kept for a total of 12 days and coded and analyzed. Nutrient intakes were calculated by a computer data base of foods in which nutrient composition was based on McCance and Widdowson's *The Composition of Foods* (9) modified to include Australian foods from published sources, commercial sources, and direct food analysis (10).

The fatty acid composition of the fat supplements (margarines) is shown in **Table 1**. For the first or habitual diet the supplement was made from butter, palm oil, and canola oil. It contained about 1% energy from *trans* fatty acid. The oleic acid-rich diet was adjusted through a canola oil-based supplement (about 2% energy from *trans* fatty acids). The high elaidic acid diet was constructed from a supplement comprising canola, linseed, and safflower oils plus hardened canola/palmolein. The supplement for the fourth, or palmitic acid-rich, diet contained predominantly palm oil with lesser contributions from canola, linseed, and safflower oils to match approximately the linoleic and linolenic acid content of the elaidic acid test mix.

TABLE 1. Composition of test blends

Fatty Acids	Major Fats and Oils in Diets			
	Habitual Palm Butter Sunflower	Oleic Acid-Rich Canola	Elaidic Acid-Rich Canola Linseed Safflower Hardened-Canola/Palmolein	Palmitic Acid-Rich Canola Linseed Sunflower Palm
	g/100 g fatty acids			
Linoleic	11	16	21	17
α-Linolenic	1	6	9	9
Capric	1	<1	<1	<1
Lauric	2	<1	<1	<1
Myristic	8	<1	<1	1
Palmitic	30	10	12	31
Stearic	9	4	4	3
Oleic	28	55	25	38
Elaidic	2	6	27	

Long chain fatty acids comprised the remainder, except in the habitual diet, where short chain fatty acids from butter fat contributed also.

Composition of diets as eaten

Table 2 shows the nature of the diets calculated from the food diaries. The intakes of energy, fat, protein, carbohydrate, fiber, and cholesterol were not significantly different between dietary periods and close to the amounts counselled to be eaten.

The two monounsaturated fatty acid-enriched diets were comparable with respect to energy derived from monounsaturated fatty acids, saturated fatty acids (SFA), and polyunsaturated fatty acids (PUFA). This was crucial to the experimental design, so that the only significant difference resided in the type of monounsaturated fatty acid: 16% energy from oleic acid and 1.5% from elaidic acid (oleic acid-rich) versus 8% oleic and 7% elaidic (elaidic acid-rich). The two SFA-rich diets differed from the two monounsaturated fatty acid-rich diets with respect to the whole fatty acid mix. The experimental design of the supplements allowed two separate comparisons for elaidic acid: the fatty acid mix resembled that in the oleic acid diet if elaidic acid behaved like oleic, or it resembled that in the palmitic acid diet if elaidic behaved like a saturated fatty acid.

Measurements

Blood was drawn from fasting subjects on 2 consecutive days at the end of the first habitual period and three times at the end of each of the three other periods. The values in each test period were averaged.

Plasma total cholesterol and triglyceride concentrations were determined by enzymatic methods (11, 12) on an automated analyzer (Cobas Bio, Hoffmann-La Roche, Basel, Switzerland). High density lipoprotein (HDL) cholesterol was determined after precipitating low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol with PEG 6000 (13). LDL cholesterol was calculated using the equation of Friedewald, Levy, and Fredrickson (14).

TABLE 2. Daily nutrient intakes during the four periods calculated from food diaries

Nutrient	Dietary Period			
	Habitual	Oleic	Elaidic	Palmitic
Energy (MJ/day)	10.7 ± 1.4	10.4 ± 1.2	10.4 ± 1.2	10.7 ± 1.3
(kcal/day)	2547 ± 339	2452 ± 290	2459 ± 277	2549 ± 297
Protein (g)	90 ± 15.9	88 ± 17.2	85 ± 14.6	91 ± 14.5
% Energy	14 ± 2	14 ± 2	14 ± 2	14 ± 2
Fat (g)	101 ± 12.7	97 ± 8.9	102 ± 10.3	104 ± 10.5
% Energy	36 ± 4	36 ± 4	37 ± 3	37 ± 3
Saturated FA				
Total	16.5 ± 2.3	9 ± 1.3	10 ± 1.7	14 ± 1.6
C10:0	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
C12:0	0.6 ± 0.2	0.2 ± 0.2	0.3 ± 0.3	0.3 ± 0.3
C14:0	2.3 ± 0.4	0.6 ± 0.1	0.8 ± 0.2	0.9 ± 0.2
C16:0	8.4 ± 1.0	5.2 ± 0.7	4.9 ± 0.6	9.8 ± 0.9
C18:0	3.8 ± 0.5	2.3 ± 0.4	3.7 ± 0.7	2.3 ± 0.4
Monounsaturated FA				
Total	11.9 ± 1.9	19 ± 1.9	17 ± 1.7	14 ± 1.8
C18:1 total	8.4 ± 0.8	17.8 ± 1.5	15.2 ± 1.4	12.9 ± 1.4
C18:1[a]	<1	1.4 ± 0.4	5.7 ± 0.7	<1
Polyunsaturated FA				
Total	4 ± 0.6	7 ± 0.7	9 ± 0.8	8 ± 0.9
C18:2	3.4 ± 0.6	5.3 ± 0.7	6.6 ± 0.6	5.7 ± 0.8
C18:3	0.4 ± 0.05	1.4 ± 0.1	2.3 ± 0.02	2.1 ± 0.02
Carbohydrate (g)	278 ± 70	313 ± 64	304 ± 55	316 ± 58
% Energy	46 ± 6	51 ± 6	49 ± 5	49 ± 5
Dietary fibre (g)				
Total NSP[b]	23.7 ± 6.7	22.0 ± 7.1	22.2 ± 7.3	21.3 ± 6.4
Soluble NSP	9.4 ± 2.2	8.6 ± 2.2	8.5 ± 2.0	8.5 ± 1.5
Insoluble NSP	14.2 ± 5.0	13.4 ± 5.4	13.7 ± 5.8	12.8 ± 5.3
Cholesterol (mg)	288 ± 74	157 ± 55	168 ± 41	186 ± 53

Values given as mean ± SD.

[a]The *trans* fatty acid values (elaidic) are derived from the measured amounts in the supplements. Australian food data bases do not include values for *trans* fatty acids; up to a further 1% energy was possibly derived from the low fat (15% energy fat) background diet.

[b]NSP, non-starch polysaccharide.

Plasma Lp[a] levels were measured by radioimmunoassay (Pharmacia, Uppsala, Sweden). Apo[a] is cleaved from apoB with dithiothreitol and measured by an immunoradiometric assay, using two different monoclonal antibodies to apo[a] and human purified apo[a] as standards. Coefficients of variation: intra-assay 8.0% at 40 U/l and 2.7% at 124 U/l; interassay 7.6% at 199 U/l and 5.1% at 680 U/l (1 U/l = 0.7 mg/l).

Plasma fatty acids were analyzed by gas chromatography in each subject during all four dietary periods (15). The capillary column [100 m × 0.22 mm i.d. BP × 70(SGE)] had the capacity to resolve *trans* fatty acids. The *trans* fatty acids in the test fats were quantified by gas chromatography.

Oxidizability of LDL

Low density lipoprotein (LDL) for oxidation experiments was isolated immediately from fresh plasma by a rapid isolation technique (16). Plasma (0.65 ml) was adjusted to d 1.21 g/ml with solid KBr and layered under 1.33 ml saline (d 1.006 g/ml) in 2-ml tubes. Two tubes were prepared from each plasma sample. The tubes were centrifuged at 100,000 rpm for 30 min at 4°C in a TL 100.2 rotor in an Optima TL100 bench top ultracentrifuge (Beckman Instruments, Palo Alto, CA). LDL that appeared as a distinct band was removed through the side of the tube with a needle and syringe. The LDL samples were immediately dialyzed at 4°C against three changes of buffer (0.2 M sodium phosphate/0.15 M NaCl, pH 7.4) which had been purged with N_2.

Oxidation of LDL was determined as the production of conjugated dienes by continuously monitoring the change in absorbance at 234 nm according to the method of Esterbauer et al. (17). Freshly prepared LDL (0.1 mg protein/ml) was incubated with $CuSO_4$ (final concentration 0.01 mM) for 2 h at 37°C in a Beckman DU65 Spectrophotometer fitted with a Peltier heater (Beckman Instruments, Palo Alto, CA). Control incubations were conducted in the absence of $CuSO_4$. Absorbance at 234 nm was automatically recorded at 2-min intervals. Lag phase, oxidation rate, and maximum diene production were measured as previously described (17). Briefly, oxidation rate was determined from the linear portion of the curve, length of the lag phase was determined from the intercept of lines drawn through the linear portion of the curve and the initial slope, and the maximum diene concentration was determined from the intercept of lines drawn through the linear portion of the curve and the final slope using the extinction coefficient for conjugated dienes at 234 nm (29,500 $M^{-1} \cdot cm^{-1}$). After incubation the reaction was stopped by addition of EDTA (final concentration 0.1 mM) and 4-methyl-2,6-di-ter-butylphenol (BHT) (final concentration 0.04 mM).

Malondialdehyde (MDA) concentration was determined by measuring thiobarbituric acid-reactive substances (TBARs) in the LDL incubation mix after oxidation. Absorbance at 535 nm was measured on a Cobas-Bio centrifugal analyzer (Roche Diagnostica, Nutley, NJ). Concentration was calculated using the extinction coefficient for MDA (1.56×10^5 $M^{-1} \cdot cm^{-1}$).

Statistical analysis

Mean differences among treatments were analyzed by paired *t*-test with Bonferroni correction. Statistical significance was defined as $P < 0.002$; all *t*-tests were two-tailed. No period effect was noted and hence the order of the studies did not influence outcome. Plasma Lp[a] levels had a skewed distribution and were log-transformed before a paired *t*-test was performed. A Wilcoxon log rank test was also used on untransformed data and the results were very similar to the paired *t*-test results (18).

RESULTS

Dietary compliance

Body weights did not differ significantly during any dietary period, nor were changes in body weight significant. The food diaries showed that all subjects complied well. The patterns of food consumption as shown in Table 2 revealed that the objectives of the dietary interventions had been achieved. Total fat did not differ among the four periods. *Trans* fatty acid intake rose fourfold in the elaidic rich-diet, and was reflected in a large increase in plasma elaidic acid (see later).

Plasma and lipoprotein lipids (Table 3)

Plasma cholesterol and LDL cholesterol concentrations were significantly ($P < 0.001$) lower during the oleic acid-rich diet period compared with each of the other three periods. On the other hand, the HDL cholesterol concentration (mainly due to HDL_3) was highest with the palmitic acid-rich diet. Plasma triglyceride concentrations were similar during the four periods.

Individuals were constant in their response to the four diets with a correlation of $r = 0.68$ ($P < 0.001$) between the fall in plasma cholesterol from the habitual saturated fatty acid diet to the oleic acid-rich diet and the rises in plasma cholesterol from the oleic acid-rich diet to the *trans* fatty acid diet; and the rises during the *trans* fatty acid and palmitic acid diets were significantly correlated ($r = 0.54$, $P < 0.01$).

The elaidic acid-rich diet led to significant elevations in the level of Lp[a] compared to all the other test diets. The Lp[a] level increased to 296 ± 220 U/l (mean ± SD) in the elaidic acid-rich diet period in comparison with 235 ± 182 in the habitual saturated fatty acid diet period ($P < 0.001$) and 249 ± 204 in the palmitic acid diet period ($P < 0.001$) and 236 ± 201 in the oleic acid diet period (not significant).

TABLE 3. Plasma and lipoprotein lipids

	Habitual	Oleic	Elaidic	Palmitic
		mg/dl		
Plasma cholesterol	228 ± 29	215 ± 28	229 ± 29	226 ± 28
Difference vs. oleic	13 ± 17[a]		14 ± 14[a]	11 ± 14[a]
Plasma triglyceride	139 ± 50	135 ± 42	142 ± 48	128 ± 44
LDL cholesterol	163 ± 25	151 ± 26	165 ± 29	161 ± 26
Difference vs. oleic	12 ± 14[a]		14 ± 12[a]	10 ± 10[a]
HDL cholesterol	38 ± 6	38 ± 6	38 ± 7	42 ± 6
Difference vs. palmitic	4 ± 4[a]	4 ± 4[a]	4 ± 3[a]	
HDL_2	9 ± 3	9 ± 5	8 ± 3	9 ± 4
Difference vs. palmitic	0 ± 2	0 ± 2	1 ± 2	
HDL_3	29 ± 4	29 ± 4	30 ± 5	32 ± 4
Difference vs. palmitic	3 ± 2[a]	3 ± 3[a]	2 ± 2[a]	

Values given as mean ± SE.
[a] $P < 0.001$.

Plasma fatty acid profile (Table 4)

Plasma fatty acids reflected the dietary changes. *1. Trans* fatty acids (mainly elaidic acid) increased sevenfold with the high *trans* diet over the habitual and high palmitate diets; the oleic acid diet, which contained some elaidic acid, led to a doubling of plasma elaidic acid. *2.* The three diets with added α-linolenic acid, from either canola or linseed oils, gave values for plasma linoleate, α-linolenate, and eicosapentaenoate (presumably derived from α-linolenate) that were similar. In fact, the ratios of linoleic to α-linolenic acids were 31, 24, and 27 for the oleic acid-rich diet, the elaidic acid-rich diet, and the palmitic acid-rich diet, respectively. *3.* Plasma palmitate levels were highest, although only slightly, in subjects on the habitual and high palmitic acid diets. Plasma oleate levels were slightly higher in subjects on the habitual and high oleic acid diets.

Oxidation of LDL (Table 5)

Oxidation of LDL was similar during each diet for all measured parameters (rate of oxidation, diene and malondialdehyde concentrations, and the lag time, which indicates the consumption time of antioxidants).

DISCUSSION

The results of the study are clear: in comparison with the elaidic acid diet, the oleic acid-rich diet led to significantly lower plasma total and LDL cholesterol concentrations. Another conclusion is that the elaidic acid-rich diet gave total and LDL cholesterol concentrations that were comparable to the levels observed with the palmitic acid-rich diet and the other saturated fatty acid diet that simulated the habitual mix of dietary fatty acids.

TABLE 4. Plasma fatty acids (by carbon number, number of double bonds)

Diet	14:0	16:0	16:1	18:0	18:1 *trans*	18:1 *cis* n-9	18:2 n-6	18:3 n-3	20:3 n-6	20:4 n-6	20:5 n-3	22:5 n-3	22:6 n-3
						% of total fatty acids							
Habitual	1.44 ± 0.35	24.71 ± 1.46	2.83 ± 0.89	6.68 ± 0.79	0.22[c] ± 0.13	23.01 ± 2.46	29.64[e] ± 3.96	0.53 ± 0.19	1.45 ± 0.26	5.67 ± 0.90	0.77 ± 0.28	0.57 ± 0.13	1.13 ± 0.25
Oleic acid-rich	1.17 ± 0.29	22.36[a] ± 1.84	2.28 ± 0.85	6.53 ± 0.57	0.42[c] ± 0.17	24.78 ± 2.03	29.82[e] ± 4.01	0.96[f,g] ± 0.29	1.31 ± 0.28	5.98 ± 1.23	0.94 ± 0.36	0.63 ± 0.19	1.23 ± 0.48
Elaidic acid-rich	1.17 ± 0.36	21.70[a] ± 1.66	2.23 ± 0.76	6.20 ± 0.59	1.48 ± 0.57	21.35[d] ± 2.17	33.72 ± 4.52	1.40[f] ± 0.44	1.15 ± 0.19	5.35 ± 0.84	0.99 ± 0.30	0.65 ± 0.15	1.23 ± 0.37
Palmitic acid-rich	1.04 ± 0.27	23.41[b] ± 1.64	2.18 ± 0.70	6.58 ± 0.55	0.23[c] ± 0.10	21.82[d] ± 2.93	32.54 ± 4.04	1.21[f] ± 0.36	1.28 ± 0.22	5.59 ± 0.94	1.05[h] ± 0.27	0.59 ± 0.18	1.17 ± 0.48

Values given as mean ± SD.
[a] $P < 0.001$ compared to habitual.
[b] $P < 0.01$ compared to elaidic.
[c] $P < 0.001$ compared to elaidic.
[d] $P < 0.001$ compared to oleic.
[e] $P < 0.05$ compared to elaidic.
[f] $P < 0.001$ compared to habitual.
[g] $P < 0.001$ compared to elaidic.
[h] $P < 0.05$ compared to habitual.

TABLE 5. Oxidizability of LDL during the four diets

	Habitual Control	Oleic Acid-Rich	Elaidic Acid-Rich	Palmitic Acid-Rich
Oxidation rate, nm/mg protein/m	15 ± 1.9	16 ± 1.9	17 ± 1.8	17 ± 2
Diene concentration, nm/mg protein	427 ± 47	440 ± 26	449 ± 31	438 ± 34
Malondialdehyde, nm/mg protein	69 ± 10	70 ± 10	65 ± 10	69 ± 10
Lag time, min	49 ± 6	51 ± 5	59 ± 7	57 ± 7

Values given as mean ± SD.

This can lead to several interpretations. *1)* Raising the *trans* fatty acid intake from < 2% energy to around 7% energy does not raise the cholesterol level above that which prevails with current saturated fatty acid-rich diets, *2) Trans* fatty acids at this level of intake give higher cholesterol levels than are obtained when oleic acid replaces elaidic acid. Note that the total saturated and unsaturated fatty acids (including elaidic) were approximately similar with the oleic acid- and elaidic acid-rich supplements. A reasonable conclusion is that if the average plasma cholesterol in the industrialized countries is to be lowered further, a reduction in *trans* fatty acid consumption should be recommended as part of the overall reduction in saturated fatty acids.

Another important observation from this study is that elaidic acid-rich diets significantly elevate plasma Lp[a] levels compared to all the other diets studied, even those that produced very similar LDL cholesterol levels. Plasma Lp[a] is a significant risk factor for coronary artery disease (19). This is a novel observation, since Lp[a] concentrations are remarkably resistant to dietary change and indeed to most forms of LDL-lowering therapy (20). Although the increase was only of the order of 30%, it is potentially atherogenic if the findings are confirmed.

Our results confirm those of Mensink and Katan (6) with respect to elaidic acid raising the LDL concentration. In other respects the results differ despite some similarities in design, namely the comparison of diets enriched in either oleic, elaidic, or mixed fatty acids. The major difference relates to HDL cholesterol levels: in the Netherlands study, elaidic acid significantly lowered HDL, unlike in our study in which the only change was an increase in HDL cholesterol with the palmitic acid-rich diet. That palmitic acid enrichment might raise HDL cholesterol has been observed previously (21, 22). One notable difference between the two studies was the substantially higher intake of *trans* fatty acids in the Netherlands trial (6). Our findings do not, therefore, exclude the possibility that very high intakes of elaidic acid (some three times to four times national averages) do lower HDL.

One other study that suggested that *trans* fatty acids raise LDL cholesterol (7) failed to match the PUFA content of the two diets, the higher *trans* fatty acid diet also being reduced in PUFA. In a shorter report by Vergroesen (23) *trans* fatty acids were also found to raise plasma cholesterol.

We differ in one other respect from Mensink and Katan (6). The LDL cholesterol concentrations were similar with the elaidic acid and the two saturated fatty acid diets (Table 3), whereas in the Netherlands experiment, the *trans* fatty acid-induced rise in LDL cholesterol was intermediate between that observed in the oleic acid- and the saturated fatty acid-enriched diets. Since the mixes of fatty acids were not identical, no firm conclusion can be drawn.

The more puzzling discrepancy is between the several studies, including ours and that by Mattson et al. (5) which showed no adverse effect of *trans* fatty acids on plasma cholesterol. In a well designed and controlled experiment, the plasma total cholesterol concentrations were similar during a high oleic-linoleic acid diet and a test diet in which at least 60% of the *cis*-unsaturated bonds were isomerized to partial or total *trans* configurations. If HDL cholesterol is indeed lowered at high intakes of *trans* fatty acids, then an increase in LDL cholesterol might have been missed by Mattson et al. (5) by measuring the total cholesterol concentration alone. Further, the subjects in the Mattson study had unusually low plasma cholesterol levels for adult men (all below 195 mg/dl); by contrast we tested mildly hypercholesterolemic men. We have shown in another study that the "baseline" LDL cholesterol significantly influences the response to a diet rich in saturated fatty acid and cholesterol (24); hence the LDL cholesterol response might have been expected to be greater in our hypercholesterolemic subjects.

This important issue is therefore not resolved. The study by Mattson et al. (5) appeared to rule out an adverse response from consuming *trans* fatty acids. Our study suggests that the LDL cholesterol response is analogous to that of saturated fatty acids such as palmitic acid and probably also myristic and lauric (Table 3). Based on assumptions by Keys, Anderson, and Grande (25) for their equations, we have compared the calculated changes in plasma cholesterol when elaidic acid is regarded as either monounsaturated or as saturated. We found that when elaidic acid was included among the saturated fatty acids, the calculated and observed changes were close. For

example: changing from oleic to elaidic acid increased plasma cholesterol by 14 mg/dl; this is similar to the "theoretical" value regarding elaidic acid as a saturate (+ 12 mg/dl) compared with elaidic acid as a monounsaturate (1 mg/dl).

Seven percent *trans* fatty acid (as energy) may appear substantial, being about 20 g per day or 2 1/2 times as much as the reported average consumption in the United States (3) and United Kingdom (2). Nevertheless, if 7–8 g is the average daily consumption [and some American sources believe it may be considerably higher (4)], then there would be many people consuming the 20 g used in the present study. The concentration of elaidic acid in adipose tissue from a sample of American subjects was recently reported as 4%, which the authors believe reflected a daily intake of about 5 g (26).

This amount of dietary elaidic acid raised the concentration of the fatty acid in plasma sevenfold. However, even the much more modest elaidic acid content of the oleic acid-rich margarines doubled the plasma elaidic acid concentration relative to that seen with the habitual diet. Despite that, the oleic acid-rich diet gave the lowest LDL cholesterol levels, presumably because any effect from the modest content of elaidic acid was counterbalanced by the high intakes of oleic and polyunsaturated fatty acids. We have shown previously that the cholesterol raising effect of semi-hardened fats and oils can be nullified by increasing their linoleic acid content (27). In that study, *trans* fatty acids contributed 4% energy, saturated fatty acids 12.5%, and *cis* plus *cis-cis* unsaturated fatty acids 22.5%. Under these circumstances the LDL cholesterol fell by 7% compared with a habitual diet (18% saturates, 15% monounsaturates, 6% polyunsaturates).

Dietary *trans* fatty acids are otherwise considered safe (2, 8). In this study we excluded the possibility that elaidic acid may render LDL more oxidizable although there was no prima facie reason for considering this to be likely.

The implications to the edible oil industry will be important if the Netherlands study (6) and our study prove correct. At face value, our study would suggest little benefit from avoiding use of palm oil by substituting isomerized *trans* fatty acids at relatively high levels. However, there is no evidence at present that lower intakes of *trans* fatty acids, resembling current average consumption, also raise LDL cholesterol.

We have compared a *trans* fatty acid-rich diet with one enriched in oleic acid (canola oil) and two others enriched with either palmitic acid (palm oil) or a mix of saturated fatty acids. The design allowed a conclusion to be drawn whether elaidic acid influenced LDL cholesterol like a monounsaturated or a saturated fatty acid. The *trans* fatty acid-enriched diet (7% energy) led to an LDL cholesterol concentration resembling that with the two saturated fatty acid-rich diets and higher than with the oleic acid-rich diet. HDL cholesterol was, however, not lowered with elaidic acid. In addition, the Lp[a] concentration was significantly raised with elaidic acid. Since the average intakes of *trans* fatty acid isomers are probably no more than 4% energy in countries where margarine consumption is high, it will be necessary to carry out dose-response studies to establish the level at which elaidic acid intake is likely to raise LDL cholesterol statistically. The results also suggest that the current public health controversy about the merit of using partly hydrogenated unsaturated oils in preference to palm oil is unresolved. One corollary is that it would be desirable to develop new partly hardened margarines that will not raise LDL cholesterol, yet be suitable for commercial purposes.

The technical assistance of Cheri Doran, Anne Stevens, Julie Priest, and Calli Triantafilidis are gratefully acknowledged. Meadow Lea Foods Australia (Dr. R. Bowrey and Mr. R. Knoke) formulated the margarines and provided support.

Manuscript received 31 December 1991 and in revised form 16 March 1992.

REFERENCES

1. Senti, F. R., editor. 1985. Health Aspects of Dietary *Trans* Fatty Acids. Federation of American Societies for Experimental Biology, Bethesda, MD.
2. *Trans* Fatty Acids—The Report of the British Nutrition Foundation's Task Force: British Nutrition Foundation 1987. London.
3. Hunter, J. E., and T. H. Applewhite. 1991. Reassessment of *trans* fatty acid availability in the US diet. *Am. J. Clin. Nutr.* **54:** 363–369.
4. Enig, M. G., A. Subodh, M. Keeney, and J. Sampugna. 1990. Isomeric *trans* fatty acids in the US diet. *Am. Coll. Nutr.* **9:** 471–486.
5. Mattson, F. H., E. J. Hollenback, and A. M. Kligman. 1975. Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. *Am. J. Clin. Nutr.* **28:** 726–731.
6. Mensink, R. P., and M. B. Katan. 1990. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Engl. J. Med.* **323:** 439–445.
7. Laine, D. C., C. M. Snodgrass, E. A. Dawson, M. A. Ener, K. Kuba, and I. D. Franz. 1982. Lightly hydrogenated soy oil versus other vegetable oils as a lipid-lowering dietary constituent. *Am. J. Clin. Nutr.* **35:** 683–690.
8. National Research Council. 1989. Diet and Health—Implications for Reducing Chronic Disease Risk. Washington, DC, National Academy Press.
9. Paul, A. A., and D. A. T. Southgate. 1978. McCance and Widdowson's The Composition of Foods. 4th ed. Elsevier-North Holland, Amsterdam.
10. Baghurst, K. I., and S. J. Record. 1984. A computerized dietary analysis system for use with diaries or food frequency questionnaires. *Community Health Stud.* **8:** 11–18.
11. Allain, C. C., L. S. Poon, C. S. G. Chon, U. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20:** 470–475.
12. Fossati, P., and L. Principe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin. Chem.* **28:** 2077–2080.

13. Warnick, G. R., T. Nguyen, and A. A. Albers. 1985. Comparison of improved methods for quantification of high density lipoproteins. *Clin. Chem.* **31:** 217–222.
14. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
15. Christie, W. W. 1972. The preparation of alkyl esters from fatty acids and lipids. *In* Topics in Lipid Chemistry. F. D. Gustone, editor. Logos Press, London. 171–197.
16. Chung, B. H., J. P. Segrest, M. J. Ray, J. D. Brunzell, J. E. Hokanson, R. M. Krauss, K. Beaydrie, and J. T. Cone. 1986. Single vertical spin gradient ultracentrifugation. *Methods Enzymol.* **128:** 181–209.
17. Esterbauer, H., G. Striegl, H. Puhl, and M. Rotheneder. 1989. Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic. Res. Commun.* **6:** 67–75.
18. Armitage, P., and G. Berry. 1987. Statistical Methods in Medical Research. 2nd edition. Blackwell, Oxford.
19. Hoefler, G., F. Harnoncourt, E. Paschke, W. Mirtle, K. H. Pfeiffer, and G. M. Kostner. 1988. Lipoprotein Lp[a]: a risk factor for myocardial infarction. *Arteriosclerosis.* **8:** 398–401.
20. Brown, S. A., J. Morrisett, J. R. Patsch, R. Reeves, A. M. Gotto, Jr., and W. Patsch. 1991. Influence of short term dietary cholesterol and fat on human plasma Lp[a] and LDL levels. *J. Lipid Res.* **32:** 1281–1289.
21. Rassias, G., M. Kestin, and P. J. Nestel. 1991. Linoleic acid lowers LDL cholesterol without a proportionate displacement of saturated fatty acid. *Eur. J. Clin. Nutr.* **45:** 315–320.
22. Hayes, K. C., A. Pronczuk, S. Lindsey, and D. Diersen-Schade. 1991. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. *Am. J. Clin. Nutr.* **53:** 491–498.
23. Vergroesen, A. J. 1972. Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. *Proc. Nutr. Soc.* **31:** 323–329.
24. Clifton, P. M., M. Kestin, M. Abbey, M. Drysdale, and P. J. Nestel. 1990. Relationship between sensitivity to dietary fat and dietary cholesterol. *Arteriosclerosis.* **10:** 394–401.
25. Keys, A., J. T. Anderson, and F. Grande. 1965. Serum cholesterol response to changes in the diet. I. Iodine value of dietary fat versus 2S-P. *Metabolism.* **14:** 747–748.
26. London, S. J., F. M. Sacks, J. Caesar, M. J. Stampfer, E. Siguel, and W. C. Willett. 1991. Fatty acid composition of subcutaneous adipose tissue and diet in postmenopausal US women. *Am. J. Clin. Nutr.* **54:** 340–345.
27. Nestel, P. J., M. Noakes, G. B. Belling, R. McArthur, P. M. Clifton, and M. Abbey. 1992. Plasma cholesterol-lowering potential of edible-oil blends suitable for commercial use. *Am. J. Clin. Nutr.* **55:** 46–50.