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TRANS FATTY ACIDS AND THEIR EFFECTS ON LIPOPROTEINS IN HUMANS

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CONTENTS

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ABSTRACT

Trans fatty acids raise plasma low-density lipoprotein (LDL) cholesterol levels in volunteers when exchanged for *cis* unsaturated fatty acids in the diet. In addition, *trans* fatty acids may lower high-density lipoprotein (HDL) cholesterol levels and raise triglyceride and lipoprotein(a) levels in plasma. *Trans* and *cis* unsaturated fatty acids are thus not equivalent, and diets aimed at reducing the risk of coronary heart disease should be low in both *trans* and saturated fatty acids.

INTRODUCTION AND SCOPE

This chapter describes *trans* fatty acids and explains how they arise in foods. It also reviews the effects of dietary *trans* fatty acids on serum lipoprotein levels and lipoprotein metabolism in humans. Our emphasis is on *trans* monounsaturated fatty acids with a chain length of 18 carbon atoms, as these are the most prevalent class. Epidemiological data on *trans* fatty acids and coronary heart disease are briefly touched on, as are policy implications. The general toxicology of *trans* fatty acids and of partially hydrogenated vegetable oils is not discussed because it has been reviewed in depth elsewhere (40), and *trans* fatty acids appear to be fairly innocuous, at least by conventional toxicological testing in animals. The effect of *trans* fatty acids on essential fatty acid metabolism and prostaglandin synthesis has also been reviewed elsewhere (9).

STRUCTURE, FORMATION, AND ASSAY OF *TRANS* FATTY ACIDS

Structure

In the diets of developed nations, some 20–25% of total daily calories are provided by fatty acids that contain one or more double bonds. These double bonds are typically positioned 3, 6, 7, or 9 carbon atoms from the terminal methyl group, and their geometry is usually *cis,* i.e. the two hydrogen atoms on the carbons adjacent to the double bond usually point in the same direction (Figure 1). However, some 1–10 g of daily fatty acid intake (33) consist of fatty acids with one or more double bonds in the *trans* configuration, the so-called *trans* fatty acids (Figure 1). Some of these derive from dairy fat and ruminant meats, but the bulk are provided by partially hydrogenated vegetable or fish oils. Edible fats that contain *trans* fatty acids usually also contain fatty acids with *cis* double bonds in unnatural positions; these are called positional *cis* isomers.

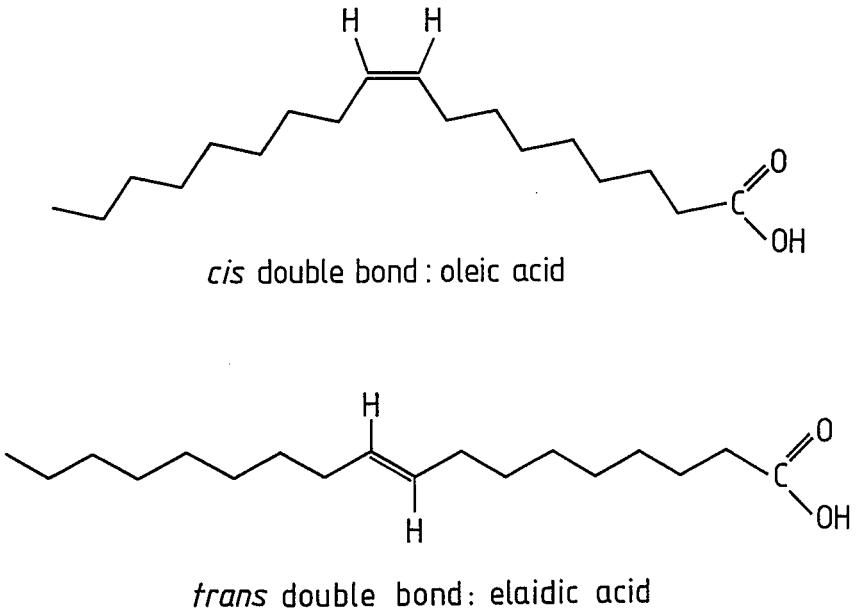

Figure 1 Structure of *cis* and *trans* monounsaturated C18 fatty acids. Both oleic (*cis*) and elaidic (*trans*) acid have their double bond in the Δ9/n-9 position. Partially hydrogenated fats also contain positional isomers of these two fatty acids, and in fact the most prevalent *trans* fatty acid in partially hydrogenated vegetable oils is *trans*-Δ10-octadecenoic acid (C18:1Δ10/n-8) rather than elaidic acid (39).

Nomenclature

In the official International Union of Pure and Applied Chemistry (IUPAC) nomenclature, the position of double bonds in fatty acids is counted from the carboxyl end of the molecule. Thus vaccenic acid, the major *trans* isomer in milk (14), is *trans*-11- or *trans*-Δ11-octadecenoic acid. Physiologists find it more convenient to use the or "n minus" system, which counts from the methyl end of the molecule, so that numbers remain the same when carbon atoms are added to or removed from the carboxyl end during metabolism and when double bonds are inserted or removed. Addition of the n– designation to formulas for *trans* fatty acids underscores the relationship between *cis* fatty acids and the *trans* fatty acids formed from them. For example, vaccenic acid, *trans*-C18:1-Δ11/n-7, arises in the rumen of the cow through biohydrogenation of linoleic acid, *cis, cis*-C18:2n-6,n-9. We therefore give both the systematic (Δ) and the n– designation of double bonds.

Properties

The number, geometry, and position of double bonds in fatty acids affect the melting behavior of the fats of which they form part. Triglycerides high in saturated fatty acids will easily stack in a crystal lattice and are therefore solid at room temperature. *Cis* unsaturated double bonds introduce bends in the molecule that hinder crystal formation, which explains why oils are liquid. The spatial structure of *trans* fatty acids is in between that of saturated fatty acids and *cis* unsaturated fatty acids. As a result, oleic acid or *cis*-C18:1Δ9/n-9 melts at 13°C; its *trans* isomer elaidic acid (*trans*-C18:1Δ9/n-9) melts at 44°C; and stearic acid (C18:0), which is straight and saturated, melts at 72°C.

Formation Of Trans Fatty Acids

Small amounts of *trans* fatty acids arise in the first stomach of ruminant animals as intermediates in the hydrogenation (saturation) of dietary unsaturated fatty acids by the hydrogen produced during bacterial fermentation. As a result, the fat in butter, cheese, milk, beef, and mutton contains ~2–8% *trans* fatty acids by weight (10, 42). *Trans* fatty acids are formed in much higher proportions during the industrial hydrogenation of vegetable or fish oils. This process was developed at the beginning of this century to meet increasing demands for margarine. Hard fats were needed in order to produce margarines that resembled butter, but the vegetable and whale oils available were too soft, and the supply of beef tallow and lard was insufficient. Hydrogenation, or "hardening," provided the answer to this dilemma. In the presence of a nickel catalyst, hydrogen bubbled through an oil will add to or "saturate" double bonds; alternatively, formation of metastable reaction intermediates allows double bonds to rotate, thereby converting the *cis* into the *trans* geometry. The composition of the end product can be controlled by choosing the proper catalyst, temperature, stirring speed, and hydrogen pressure. Changing these conditions allows the creation of a variety of partially hydrogenated oils that possess the melting range, stability, and mouth feel required for incorporation into a particular food. If hydrogenation is taken to completion, this process results in a fully hydrogenated fat that contains neither *cis* nor *trans* fatty acids but is made up completely of saturated fatty acids, primarily stearic acid.

Quantification in Foods

The two major assay methods for *trans* fatty acids are infrared absorption spectroscopy and capillary gas-liquid chromatography. The latter technique is gaining in popularity; it is now widely available and allows identification of individual fatty acids, whereas infrared absorption spectroscopy does not. However, in complex mixtures of isomeric fatty acids such as are present in foods, all fatty acid isomers rarely are resolved by gas chromatography. As a

result, *cis* and *trans* isomers may overlap, and results may be biased. Preseparation of *cis* and *trans* isomers by argentation chromatography may solve the problem but is laborious. The problem is even more pronounced for the multitude of isomers of C20:1, C20:2, and C22 unsaturated fatty acids in hydrogenated fish oil. Gas chromatography is therefore unsuitable for routine assessment of the *trans* fatty acid content of foods containing partially hydrogenated fish oil.

Infrared spectrometry is less sensitive than gas chromatography (although sensitivity may be increased with Fourier transform machines) and does not distinguish individual *trans* fatty acids or detect positional *cis* isomers. This method is nonetheless invaluable as a check on results obtained by gas chromatography.

In general, the routine assay of *trans* fatty acids in foods is more complicated than that of other fatty acids, and figures in food tables and consumption figures for *trans* fatty acids may be unreliable.

TRANS FATTY ACIDS AND LIPOPROTEINS

Early Studies: Trans Fatty Acids and Total Serum Cholesterol

Several investigators have examined the effects of partially hydrogenated fats on serum total cholesterol levels in humans. Most studies (2, 7, 11, 25, 50), but not all (28, 29), indicated that serum cholesterol levels were increased after the consumption of partially hydrogenated vegetable oils. However, results are often difficult to interpret owing to a poor description of the intakes of the various classes of fatty acids; to simultaneous changes in intake of *trans*, saturated, and polyunsaturated fatty acids; to small numbers of subjects; and to feeding periods too short for serum cholesterol levels to attain a new equilibrium. We limit the present discussion to studies with reasonably large groups of subjects and with experimental periods of 14 days or more.

Anderson et al (2) fed 23 men low-fat mixed diets supplemented with butter fat, a mixture of olive and corn oil, or partially hydrogenated corn oil in random order for 3 weeks each. The latter two diets differed only in that *cis* fatty acids were exchanged for *trans* fatty acids, with saturated fatty acids remaining constant. *Trans* fatty acids in the partially hydrogenated corn oil diet accounted for 14.7% of daily energy (11% from *trans* monounsaturated fatty acids and 3.7% from *trans* isomers of linoleic acid). *Trans* levels in the other two diets were reported to be zero (the small amounts of *trans* levels in butter probably escaped detection). Average serum cholesterol level was 0.5 mmol/liter (21 mg/dl) higher with the *trans* diet than with the *cis* diet (Figure 2) and an additional 0.6 mmol/liter (24 mg/dl) higher with butter. Thus the effect of *trans* fatty acids on total serum cholesterol levels was about half that of saturated

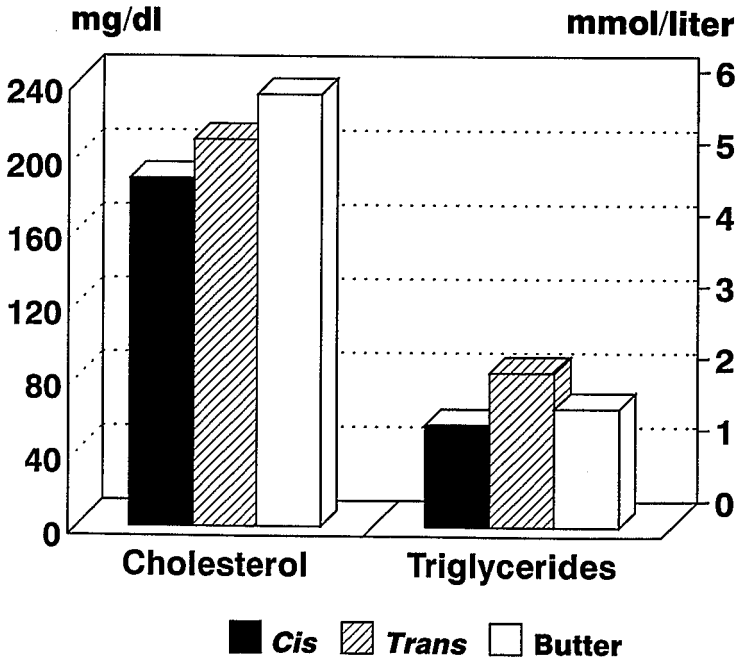

Figure 2 Effect of *trans* fatty acids and butter on serum lipids in the study of Anderson et al (2). Total mono- and polyunsaturated fatty acids were similar in the *cis* and *trans* diets, but in the *cis* diet all unsaturated fatty acids had the *cis* configuration, whereas in the *trans* diet *trans* monounsaturated fatty acids provided 11% of calories and *trans* isomers of linoleic acid provided 3.7% of calories.

fatty acids. The diet high in *trans* fatty acids also caused a marked rise in serum triglyceride levels (Figure 2).

In contrast, McOsker and coworkers (29) did not report a significant effect of partial hydrogenation on serum cholesterol levels. The 36 healthy subjects who completed the study were divided into seven groups. Each group received four of the seven experimental fats at a level of 41% of daily energy intake. Two diets containing cholesterol are not discussed here. The other five cholesterol-free liquid-formula diets were fed for 8 weeks. They provided 0.0, 5.9, 6.2, 7.3, and 8.4%, respectively, of total energy as *trans* fatty acids at the expense of a mixture of all other fatty acids. Serum cholesterol levels were 4.10, 4.10, 4.09, 4.21, and 4.32 mmol/liter, respectively. Because these values did not differ significantly, the authors concluded that partial hydrogenation does not change the cholesterolemic characteristics of vegetable oils. However, the statistical power of the study was such that it could only detect differences of >0.31 mmol/liter (29).

Table 1 Effects of dietary *trans* and *cis* monounsaturated fatty acids (*trans*-C18:1 and *cis*-C18:1) on serum total cholesterol levels in two experiments employing liquid formula diets

	Mattson et al (28)	Vergroesen et al (50)			
		I	II	III	IV
Days on diet	28	20	20	28	28
Cholesterol intake (mg/day)	500	0	250	220	220
Trans-diet groups					
Number of subjects	13	9	9	12	12
Trans-isomers (% of energy)	18	14	14	14	14
Trans-C18:1 (% of energy)	14	13	13	14	14
Cis-C18:1 (% of energy)	8	14	14	16	7
Cis-diet groups					
Number of subjects	17	9	9	12	12
Cis-C18:1 (% of energy)	23	28	28	31	22
Δserum cholesterol, *trans-cis* (mmol/liter)	−0.04	−0.20	0.55	0.35	0.50

Two groups of investigators used liquid-formula diets to compare *trans* and *cis* monounsaturated fatty acids side by side. Vergroesen and coworkers (50) reported that in the presence of dietary cholesterol, *trans* C18:1 is hypercholesterolemic, but Mattson et al (28) found that *trans* monounsaturated fatty acids had no effect on serum cholesterol compared with the *cis* isomer oleic acid (Table 1). The discrepancy between these two well-controlled studies remains unexplained despite thorough scrutiny (6).

Finally, Laine et al (25) compared the effects of corn oil with those of lightly hydrogenated soy oil on serum lipids and lipoproteins in 12 subjects. Approximately 7.2% of energy from linoleic plus some linolenic acid was exchanged for 5.4% of energy as *trans* fatty acids plus some oleic acid. Total cholesterol levels increased by 0.25 mmol/liter, low-density lipoprotein (LDL) cholesterol levels by 0.34 mmol/liter, and total triglyceride levels by 0.05 mmol/liter. High-density lipoprotein (HDL) cholesterol levels were unchanged.

In summary, the early studies described above reported that *trans* unsaturated fatty acids either had no effect or raised cholesterol levels compared with *cis* unsaturated fatty acids. Some studies also reported that *trans* unsaturated fatty acids raised triglyceride levels.

Recent Studies of LDL and HDL Cholesterol

EFFECTS IN INDIVIDUAL STUDIES The earlier studies did not provide a consistent explanation for the cholesterolemic effects of *trans* fatty acids. In addition,

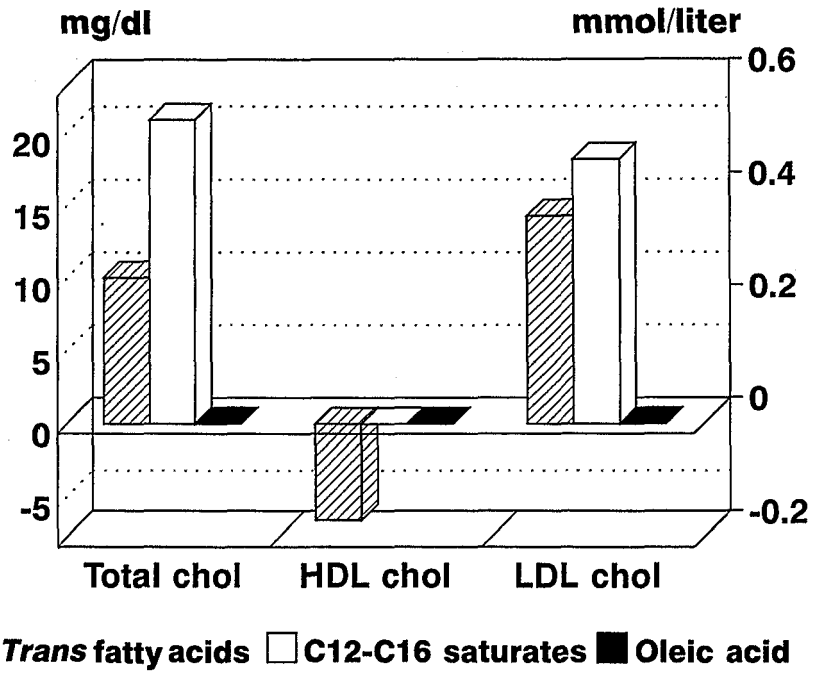

Figure 3 Mean responses of total, HDL, and LDL cholesterol levels of 59 subjects to diets high in *trans* fatty acids (*hatched bars*) or in C12–C16 saturated fatty acids (*open bars*) relative to levels with a diet high in oleic acid (30).

effects on LDL and HDL cholesterol levels were examined in only one trial. Mensink & Katan (31) therefore decided to investigate this issue using a large number of subjects and a multiple crossover design. In a controlled trial, 59 healthy males and females were fed a diet high in monounsaturated *trans* fatty acids, a second diet high in oleic acid, and a third diet high in a mixture of the saturated fatty acids lauric, myristic, and palmitic acid, which raise cholesterol levels (30). Every subject received each diet in random order, for 3 weeks each. The *trans* C18:1 isomers in the *trans* diet accounted for 11% of total energy intake, an amount 2–4 times higher than in a typical Western-style diet (10, 17). Total cholesterol levels increased from 4.46 mmol/liter with oleic acid to 4.72 mmol/liter with the *trans* diet and to 5.00 mmol/liter with the saturated fatty acid diet (Figure 3). The rise in cholesterol levels with the *trans* diet was about one half that with the saturated fatty acid diet. This result is in line with the earlier studies of Anderson et al (2) and Vergroesen et al (50). Surprisingly, the *trans* fatty acids had a marked effect on the distribution of serum cholesterol over LDL and HDL particles: *Trans* fatty acids raised LDL

cholesterol levels by 0.37 mmol/liter, whereas HDL cholesterol levels were 0.17 mmol/liter lower than they were with the oleic acid and the saturated fatty acid diets (Figure 3). The ability of *trans* fatty acids to lower HDL cholesterol levels was seen in 54 of the 59 subjects. The authors concluded that *trans* fatty acids have unfavorable effects on serum lipoproteins because they simultaneously increased LDL and decreased HDL cholesterol levels.

Zock & Katan subsequently investigated the effects of a more modest dose of *trans* C18:1 in 26 men and 30 women (52), employing a design similar to that used by Mensink & Katan (30). Eight percent of energy as *trans*-C18:1 was exchanged for either linoleic acid, the parent fatty acid from which *trans* monounsaturated fatty acids are formed during partial hydrogenation, or stearic acid (C18:0), the product of complete hydrogenation of linoleic acid. Like *trans* fatty acid, stearic acid is a rigid molecule, the presence of which may add firmness to food fats. Figure 4 shows the changes in total and lipoprotein cholesterol levels with diets high in *trans* fatty acid or stearic acid relative to cholesterol levels with the linoleic acid diet. LDL cholesterol levels rose from 2.83 mmol/liter with linoleic acid to 3.00 mmol/liter with stearic acid and to

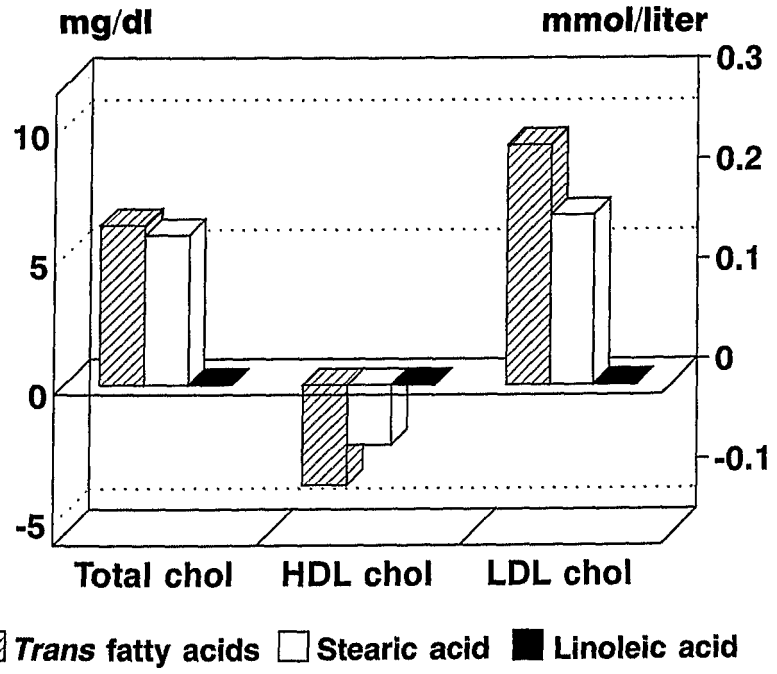

Figure 4 Mean responses of total, HDL, and LDL cholesterol levels of 56 subjects to diets moderately high in *trans* fatty acids (*hatched bars*) or stearic acid (*open bars*) relative to levels with a diet high in linoleic acid (52).

3.07 mmol/liter with *trans* fatty acids. In contrast, HDL cholesterol levels decreased by 0.06 mmol/liter with stearic acid and by 0.10 mmol/liter with *trans* fatty acids. This study thus confirmed that, relative to *cis* unsaturated fatty acids, *trans* monounsaturated fatty acids can raise LDL cholesterol levels and lower HDL cholesterol levels. The differences between the stearic acid and *trans* fatty acid diets were not statistically significant. Stearic acid has a considerably lesser effect on total serum cholesterol than do C12–C16 saturated fatty acids (4, 8, 12, 15, 22, 23) and has been proposed as a favorable alternative to *trans* fatty acids for lending firmness to margarines and shortenings (13). However, our finding that stearic acid and *trans* fatty acids both lower HDL cholesterol levels suggests that substituting stearic acid for *trans* fatty acids does not confer much of an advantage.

Nestel and coworkers in Adelaide, Australia performed two studies on the effects of *trans* C18:1 on plasma lipoprotein levels (35, 36). In the first study, 26 mildly hypercholesterolemic men consumed an average Australian diet rich in saturated fatty acids and two different oil-blend diets in a three-period crossover design. One blend was comprised of sunflower oil and a mix of partially hydrogenated cottonseed and soybean oils, and the other consisted of sunflower oil and partially hydrogenated canola oil and palm olein. The fatty acid composition of the two test blends was approximately the same; they contained 7 g more *trans* C18:1 per 100 g fat, 15 g more linoleic acid, and 19 g less palmitic acid than the control fat per 100 g fat. Both experimental blends significantly lowered total and LDL cholesterol levels. HDL cholesterol levels were not significantly lower in the fats containing more *trans* C18:1 (35). However, the multiple exchanges of fatty acids between the diets make it difficult to ascribe the differences in lipoprotein levels to a particular fatty acid.

In the second study, 4–5% of energy as *trans* C18:1 was exchanged exclusively for either oleic acid or palmitic acid. Twenty-seven mildly hypercholesterolemic male subjects each consumed the three different diets for 3 weeks each. Compared with the oleic acid diet, the *trans* fatty acid diet raised both total and LDL cholesterol levels by 0.36 mmol/liter, but HDL cholesterol levels were unaffected. Palmitic acid raised total cholesterol levels by 0.28 mmol/liter, LDL cholesterol levels by 0.26 mmol/liter, and HDL cholesterol levels by 0.10 mmol/liter, relative to those of subjects receiving the oleic acid diet (36). Nestel and coworkers concluded that *trans* fatty acids and palmitic acid raise cholesterol levels to approximately the same extent but that *trans* fatty acids, at the levels fed, do not lower HDL cholesterol levels relative to oleic acid (36).

Lichtenstein and coworkers (26) compared the effects of a corn oil–enriched diet with those of a diet rich in partially hydrogenated corn oil in the form of margarine in 14 women and men with moderate hypercholesterolemia. The margarine diet contained ~3.7% more energy as *trans* monounsaturated fatty

acids than the unhydrogenated corn oil diet, at the expense of *cis* mono- and polyunsaturated fatty acids. This diet yielded levels of total and LDL cholesterol 0.28 mmol/liter higher ($P < 0.01$) than those on the unhydrogenated oil diet. Again, no change in HDL cholesterol levels was detected.

Judd and coworkers (19) reported a large, well-controlled trial in 29 men and 29 women. Every subject consumed four different diets for 6 weeks each, in random order. One diet was high in oleic acid, and two were enriched with different amounts of partially hydrogenated soybean oil (3% and 6% of energy as *trans* monounsaturated fatty acids), at the expense of oleic acid. In the fourth diet, 6% of energy was obtained from a mixture of saturated fatty acids, which were substituted for oleic acid. Compared with levels on the oleic acid diet, total cholesterol levels increased by 0.20 mmol/liter with the 3% *trans* diet, by 0.26 mmol/liter with the 6% *trans* diet, and by 0.35 mmol/liter with the saturated fatty acid diet. Changes in LDL cholesterol levels paralleled those in total cholesterol levels; LDL cholesterol levels rose by 0.20, 0.26, and 0.30 mmol/liter with the 3% *trans*, 6% *trans*, and saturated fatty acid diet, respectively. HDL cholesterol levels were not lowered significantly on the 3% *trans* diet, and they decreased by 0.04 mmol/liter on the 6% *trans* fatty acid diet relative to levels in subjects on the oleic acid diet. The magnitude of the effects observed in this study was somewhat greater for LDL cholesterol levels and smaller for HDL cholesterol levels than that seen in the studies of Mensink & Katan (30) and Zock & Katan (52). Nevertheless, the direction of the changes was the same, and the findings of Judd support the concept that *trans* monounsaturated fatty acids raise LDL and lower HDL cholesterol levels.

OVERVIEW OF THE EFFECTS OF *TRANS* FATTY ACIDS ON LDL AND HDL CHOLESTEROL LEVELS The six studies described above consistently showed that diets containing *trans* fatty acids raise LDL cholesterol levels relative to those containing *cis* unsaturated fatty acids. The findings for HDL cholesterol levels are less uniform; *trans* fatty acids either lowered HDL cholesterol levels (19, 30, 52) or had no significant effect (26, 35, 36). The three studies that reported a significant effect of *trans* fatty acids in lowering HDL cholesterol levels involved more subjects and investigated higher intakes of *trans* fatty acids. Thus the effect of *trans* fatty acids on HDL might occur only at higher intakes. However, the statistical power of the studies reporting no effect may have been insufficient to detect a slight drop in HDL cholesterol levels. The authors themselves suggest (26) that the low amounts of total and saturated fat in the background diet might have diminished the responsiveness of HDL cholesterol levels to diet.

In five of the six studies, *trans* monounsaturated fatty acids were isoenergetically exchanged for *cis* unsaturated fatty acids [oleic acid (19, 30, 36), linoleic acid (52), or a mixture of the two (26)]. Figure 5 summarizes the

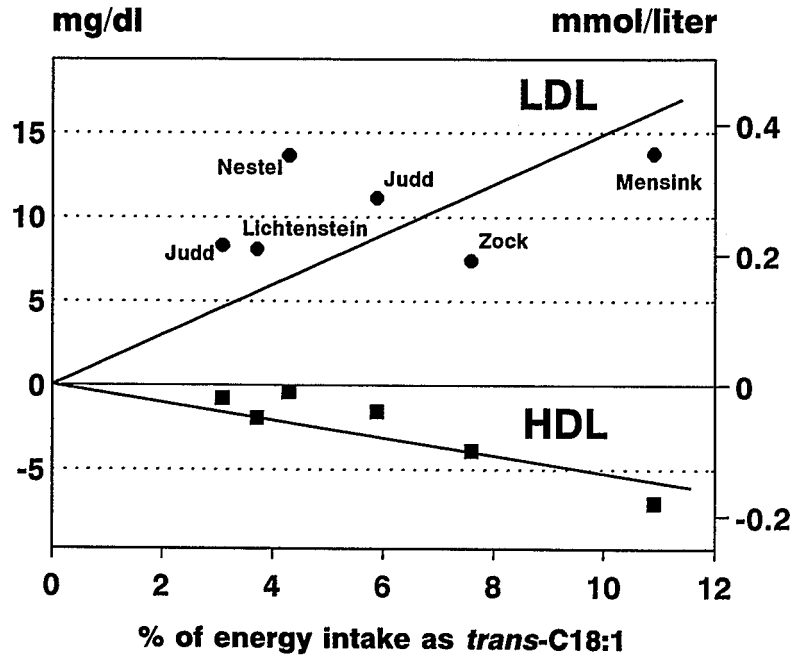

Figure 5 Overview of the effects of exchange of *trans* monounsaturated fatty acids for oleic acid on lipoprotein cholesterol levels. Data are derived from six isoenergetic dietary comparisons between *trans* monounsaturated and *cis* unsaturated fatty acids (19, 26, 30, 36, 52). Within one study, *cis* and *trans* diets sometimes also differed in their content of fatty acids other than *cis* and *trans* monounsaturated fatty acids. For the present analysis we calculated the effects of these differences on lipoprotein levels (32) and subtracted them from the effect of *trans* vs *cis* diets as reported in the original publication. Regression lines were forced through the origin because a zero change in intake will produce a zero change in lipoprotein levels. Regression coefficients are 0.040 mmol/liter for LDL and −0.013 mmol/liter for HDL cholesterol. These are the predicted changes in serum lipoprotein cholesterol levels if 1% of daily energy intake from oleic acid is replaced by *trans* monounsaturated fatty acids. For an average US diet this amounts to almost 3 g of fatty acid per day.

findings of the five trials discussed above (53). We expressed the effects of *trans* monounsaturated fatty acids relative to their *cis* isomer oleic acid and adjusted for small differences in other fatty acids between the *trans*-enriched and reference diets using regression coefficients from Mensink & Katan (32). When the data are combined into a linear model, each additional percent of dietary energy as *trans* fatty acids results in an increase in LDL cholesterol levels of 0.040 mmol/liter or 1.5 mg/dl ($R^2 = 0.86$; $P = 0.0028$) and a decrease in HDL cholesterol levels of 0.013 mmol/liter or 0.4 mg/dl ($R^2 = 0.88$; $P = 0.0019$). The effect of *trans* fatty acids on LDL cholesterol levels is similar to

that of saturated fatty acids, whereas their effect on HDL cholesterol levels is about the same as that of carbohydrates (32). Obviously, more experiments are needed to define the exact shape of the dose-response curve, but the assumption of linearity seems justifiable until proven otherwise.

Thus, diets containing *trans* monounsaturated fatty acids with a chain length of 18 carbon atoms raise LDL cholesterol levels and may modestly lower HDL cholesterol levels relative to diets containing *cis* isomer oleic acid. These effects appear to be roughly proportional to the amounts consumed.

Lipoprotein(a)

Lp(a) particles consist of LDL molecules that have an extra glycoprotein, the so-called apoprotein(a), attached through a disulfide link (49). Despite the structural similarity of the two types of lipoproteins, the determinants of Lp(a) levels are markedly different from those of LDL levels. The effects of diet on LDL cholesterol levels are quite marked (see e.g. 32), whereas Lp(a) concentrations are generally insensitive to dietary change (5). However, several trials indicate that dietary *trans* fatty acids affect Lp(a) levels.

Hornstra and coworkers showed that replacement of the habitual fat in a Dutch diet with palm oil lowered Lp(a) levels in healthy men. They suggested that *trans* fatty acids may increase Lp(a) levels because replacement of the habitual dietary fat with palm oil decreased *trans* fatty acid intake by more than 50% (16).

The first direct test of the effect of dietary *trans* fatty acids on Lp(a) levels was performed by Nestel and coworkers, who measured Lp(a) levels by radioimmunoassay in their second study of *trans* fatty acids and lipoproteins (36). Average Lp(a) concentrations were 296 units/liter with the *trans* fatty acid diet, 249 with the palmitic acid diet, and 236 with the oleic acid diet. Levels with *trans* fatty acids were significantly higher than with palmitic acid, but the difference between *trans* and oleic acid failed to reach statistical significance.

Mensink et al measured Lp(a) levels by enzyme-linked immunosorbent assay (ELISA) (34). In their first trial, which studied the effects of *trans* fatty acids at a dose of 11% of energy (30), median Lp(a) levels were 45 mg/liter with the *trans* fatty acid diet. This level was significantly higher than that obtained with the saturated fat diet (26 mg/liter) or the oleic acid diet (32 mg/liter) (34). Figure 6 (21) shows the distribution of Lp(a) levels in this trial with diets rich in *trans* and saturated fatty acids; Lp(a) levels were higher with the *trans* fatty acid diet in 47 of 59 subjects. In the second trial, ~8% of energy from *trans* fatty acids was exchanged for stearic or linoleic acid (52). Median Lp(a) levels were 85 mg/liter with the *trans* fatty acid diet and 69 mg/liter with both the stearic acid and the linoleic acid diets ($P < 0.01$).

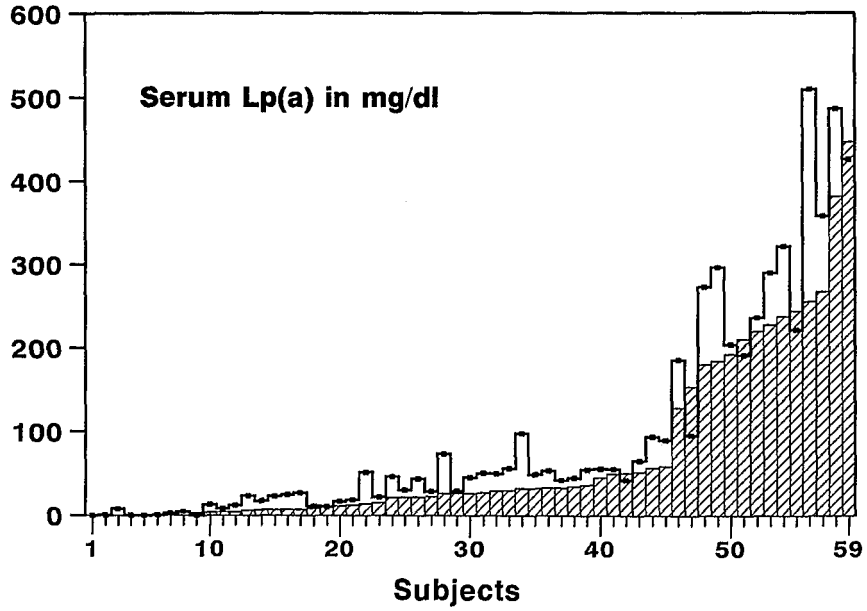

Figure 6 Distribution of serum Lp(a) levels in 59 volunteers while on diets high in C12–C16 saturated fatty acids (*hatched bars*) or in *trans* fatty acids (*solid line*) (21, 34). Subjects were ranked from left to right by their Lp(a) levels on the diet high in saturated fatty acids.

Lichtenstein et al did not observe a rise in Lp(a) levels measured by ELISA in subjects after consumption of *trans* fatty acids (26). This outcome suggests that *trans* fatty acids fed at the level and under the conditions specified do not affect Lp(a) levels. Alternatively, this study may be of limited statistical power owing to the low dose of *trans* fatty acids and the relatively small number of subjects.

Finally, in a study in Norway, Almendingen et al found that when 8 to 8.5% of the dietary energy was *trans* fatty acids, provided either by partially hydrogenated soybean oil or by partially hydrogenated fish oil, Lp(a) levels were significantly elevated relative to those on diets containing butter (1a). *Trans* fatty acids thus appear to be one of the rare dietary factors that influence Lp(a) levels, even though the effect is modest compared with genetically determined differences.

Fasting Triglyceride Levels

High levels of serum triglyceride during fasting are often associated with low HDL cholesterol levels. Thus, in view of the lowering effect of *trans*

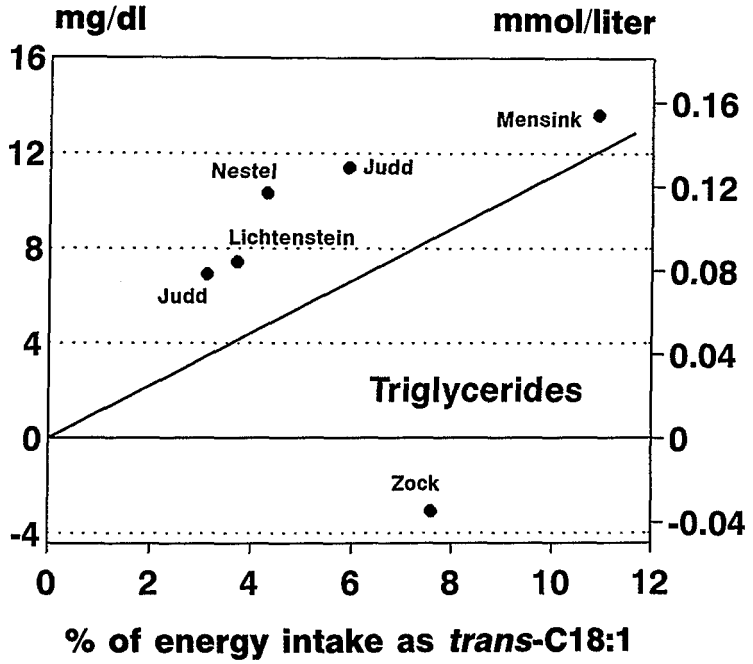

Figure 7 Overview of the effects on fasting levels of triglyceride of replacing oleic acid with *trans* fatty acids (19, 26, 30, 36, 52). Data were adjusted for differences in fatty acids other than *trans* and *cis* monounsaturated fatty acids (32) (see Figure 5). The regression coefficient is 0.013 mmol/liter. This is the predicted change in serum triglyceride level if 1% of daily energy intake from oleic acid is replaced by *trans* monounsaturated fatty acids.

fatty acids on HDL cholesterol levels, one would expect to see a simultaneous rise in triglyceride levels. Indeed, Andersen et al (2) reported such an effect (Figure 2). In the three largest of the most recent trials, serum triglyceride levels were also modestly but significantly elevated with the *trans* fatty acid diet compared with those observed with the oleic or linoleic acid reference diets (19, 30, 52). In other recent studies, triglyceride levels were somewhat higher with *trans* fatty acids, although the changes were not statistically significant in any of the individual trials (26, 35, 36). Figure 7 shows the effect of *trans* monounsaturated fatty acids on serum triglyceride levels relative to oleic acid during five trials. We recalculated all changes using oleic acid as a reference (32). The increase in triglycerides observed by Zock & Katan (52) with the *trans* fatty acid diet relative to that on the linoleic acid diet disappeared when oleic acid was used as a reference. Using the same adjustments and method of analysis as were applied in Figure 5, we found that each additional percent of energy as *trans* fatty acids increases

triglyceride levels by 0.013 mmol/liter, or ~1 mg/dl (R^2 = 0.60; P = 0.04). Thus, in addition to raising levels of LDL cholesterol and Lp(a)and lowering HDL cholesterol levels, *trans* monounsaturated fatty acids appear to modestly raise fasting levels of plasma triglyceride.

Effects of Different Types of Trans Fatty Acids

Variations in hydrogenation procedures will produce mixtures of *trans* fatty acids that differ in the positions of their double bonds along the carbon chain of the fatty acid molecule. In addition, use of fish oil as a starting material will result in *trans* fatty acids with chain lengths of 20 and 22 carbon atoms instead of the 18 carbon atom isomers that predominate in partially hydrogenated vegetable oils. In the studies from Wageningen (30, 52), the *trans* fatty acids were derived from oleic acid (*cis* C18:1Δ9/n-9), and as a result the distribution of *trans* isomers centered around *trans* C18:1Δ9/n-9 (elaidic acid), with smaller contributions from *trans* C18:1Δ6/n-12 to C18:1Δ12/n-6. Because *trans* fatty acids in partially hydrogenated soybean oil are largely derived from linoleic acid (*cis, cis* C18:2Δ9,12/n-6,n-9), the most prevalent *trans* isomer is *trans* C18:1Δ10/n-8 (39). Reeves (38) suggested that such differences in position of the *trans* bond will modulate the impact of *trans* fatty acids on serum lipoproteins. It was also suggested that *trans* fatty acids from ruminant sources, which consist predominantly of C18:1Δ11/n-7 (vaccenic acid), may have a different impact on coronary risk than those from industrially hydrogenated oils (51). However, hydrogenated soybean oil also contains appreciable amounts of vaccenic acid (19), and trials employing different sources or isomer compositions of *trans* fatty acids obtained similar results (19, 30, 52). Moreover, a recent study by Almendingen et al in Oslo (1a) showed that partially hydrogenated fish oil rich in *trans* isomers of C20:1 and C22:1 markedly lowered plasma HDL cholesterol levels and and raised LDL cholesterol levels and Lp(a) levels in volunteers. This finding suggests that *trans* fatty acids from various sources have qualitatively the same effects on lipoprotein levels.

MECHANISMS

Spady et al (43) have developed a general mechanism for the effects of dietary cholesterol and fatty acids on plasma LDL cholesterol levels, using the hamster as a model. However, the validity of this mechanism in humans has not yet been established, nor does it account for dietary effects on HDL cholesterol levels. Thus, the action of *trans* fatty acids on plasma lipoproteins in humans is not readily explained. However, there are tentative indications that the cholesteryl ester transfer protein (CETP) is involved. CETP is a protein present

in the plasma of humans and certain other mammals that transfers cholesterylesters from HDLs to LDLs in exchange for triglycerides. Lagrost et al (24) suggested that high concentrations of elaidic acid may increase the rate of transfer of cholesteryl esters from HDLs to LDLs when added to plasma in vitro, although this hypothesis was not confirmed in another study (37). Abbey & Nestel (1) reported increased CETP activity in the plasma of volunteers after substitution of elaidic acid for oleic acid in the diet. A significant increase was detected using an assay that employed endogenous lipoproteins, but no effect was seen when CETP activity was assayed in lipoprotein-deficient plasma.

Van Tol et al (49a) measured the serum activity levels of CETP using excess exogenous substrate in sera sampled in a study (52) that compared *trans* fatty acids, stearic acid, and linoleic acid. The assay used was independent of endogenous lipoproteins and correlated well with CETP mass. The *trans* fatty acid diet resulted in an 18% increase in CETP activity (49a), which coincided with the expected lower ratio of cholesteryl esters to triglycerides in HDLs.

Considerable evidence indicates that increased activity of CETP may promote atherosclerosis (for a review see 44). The finding that the drop in HDL cholesterol levels with the *trans* fatty acid diet might be due to increased CETP activity suggests that this change in HDL cholesterol levels may represent a contribution to atherogenesis over and above the contribution made by the increase in LDL cholesterol levels, which is certainly atherogenic. However, the rise in CETP with diets high in *trans* fatty acids has been reported in only two studies. Moreover, the changes observed may have occurred secondary to changes in plasma lipoprotein concentration or composition rather than to true changes in the amount or activity of CETP molecules.

TRANS FATTY ACIDS AND CORONARY HEART DISEASE

The studies described in the above sections indicate that dietary *trans* fatty acids have multiple unfavorable effects on lipoprotein levels and thus on the risk profile for coronary heart disease. Cross-sectional observations showed that in free-living subjects, habitual consumption of *trans* fatty acids is also associated with higher LDL cholesterol levels and lower HDL cholesterol levels (41, 47). The question then becomes whether high intakes of *trans* fatty acids promote coronary heart disease. In the absence of controlled clinical trials, one must rely on epidemiological observations for an answer.

Thomas et al (46) reported that values for *trans* unsaturated fatty acids tended to be higher in the adipose tissue of patients who had died from ischemic

heart disease than in controls, but possible confounders were not taken into account. In the Nurses' Health Study, the intake of *trans* fatty acids as measured by a semiquantitative food frequency questionnaire was an independent predictor of coronary heart disease (51). Several case-control studies also showed a higher intake of *trans* fatty acids (3, 41, 45) or margarines (45, 48) in patients with coronary heart disease than in controls.

These epidemiological studies have been criticized, however, and often on justifiable grounds. Consumption of foods high in *trans* fatty acids may be part of a lifestyle involving many other factors that affect heart disease risk; such confounders are difficult to exclude. On the other hand, the very crudeness of methods for measuring food intake should make us take note when an association manages to make itself heard above the noise of the measuring instrument. The poor precision of methods for determining intakes will cause associations between diet and disease to appear weaker than they really are. This phenomenon of attenuation has been the primary explanation for the absence of associations between saturated fat intake and coronary heart disease within populations (18, 27). The fact that the Nurses' Health Study (51) and other studies (3, 41, 45, 48) found a positive association between *trans* fatty acids and coronary heart disease therefore deserves attention. By themselves, such data cannot prove that high intakes of *trans* fatty acids promote coronary heart disease, but the effects of *trans* fatty acids on plasma lipids and lipoproteins are unmistakable and lend credence to a causal link.

CONCLUSIONS AND RECOMMENDATIONS

Trans fatty acids raise plasma LDL cholesterol levels when exchanged for *cis* unsaturated fatty acids in the diet. They may also lower HDL cholesterol levels and raise triglyceride and Lp(a) levels. These effects appear to be shared by *trans* fatty acids from various sources and with different isomer compositions. The effects of *trans* fatty acids on LDL cholesterol levels are similar to those of saturated fatty acids, and diets aimed at reducing the risk of coronary heart disease should therefore be low in both *trans* and saturated fatty acids. However, in countries with a high incidence of premature coronary heart disease, average intakes of saturated fatty acids are much higher than those of *trans* fatty acids. Dietary prevention of coronary disease should therefore continue to focus on reducing dietary saturated fats and cholesterol; for regulatory and educational purposes, *trans* fatty acids could be conveniently included with the saturated fatty acids.

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