Dietary *trans* fatty acids and their impact on plasma lipoproteins

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MB KATAN, RP MENSINK, A VAN TOL, PL ZOCK. Dietary *trans* fatty acids and their impact on plasma lipoproteins. Can J Cardiol 1995;11(Suppl G): 36G-38G. Foods contain isomers of unsaturated fatty acids that have double bonds in unusual configurations (*trans* instead of *cis*) or unusual positions, or both. Such fatty acids arise through biohydrogenation in the rumen of cows and sheep or catalytic hydrogenation in industrial hardening of oils. The effects of *trans*-monounsaturates on lipoproteins in man are opposite to those of their *cis*-isomer, oleic acid: *trans* fatty acids raise low density lipoprotein (LDL) cholesterol and lipoprotein Lp(a) and lower high density lipoprotein (HDL) cholesterol, all in a dose-dependent fashion. *Trans* fatty acids raised serum cholesteryl ester transfer activity in 52 of 55 volunteers (mean change 18%, P<0.02), and lowered the ratio of cholesteryl esters to triglycerides in HDL. Lecithin cholesterol acyl transferase was unchanged. The effects of *trans* fatty acids on HDL and LDL may thus be mediated through cholesterol ester transfer protein.

Key Words: *Dietary* trans *fatty acids, Lipoproteins*

Acides gras *trans* diététiques et leur influence sur les lipoprotéines plasmatiques

RÉSUMÉ : Les aliments renferment des isomères d'acides gras insaturés qui ont des doubles liaisons de configuration inhabituelle (*trans* plutôt que *cis*), des positions inhabituelles ou les deux. Ces acides gras proviennent de la biohydrogénation qui survient dans le rumen des vaches et des moutons ou par l'hydrogénation catalytique lors du durcissement industriel des huiles. Les effets des monoinsaturés de type *trans* sur les lipoprotéines chez l'homme s'opposent à ceux de leurs cis-isomères, l'acide oléique : les acides gras *trans* provoquent une élévation du cholestérol LDL et de la lipoprotéine Lp(a) et abaissent le cholestérol HDL de façon dose-dépendante. Les acides gras *trans* ont élevé l'activité de transfert du cholestéryl ester sérique chez 52 volontaires sur 55 (modification moyenne 18 %, P<0,02) et ont abaissé le ratio cholestéryle ester : triglycérides dans le HDL. L'acyltransférase du cholestérol lécithinique est demeuré inchangé. Les effets des acides gras *trans* sur le HDL et le LDL peuvent donc être modifiés par la protéine de transfert de l'ester cholestérol.

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Isomeric fatty acids are unsaturated fatty acids in which one or more of the double bonds have an unusual spatial geometry, namely *trans* instead of *cis*, or an unusual position along the length of the molecule, or both. In foods, geometric and positional isomers almost invariably occur together. As it is analytically easier to distinguish *trans* from *cis* isomers than one positional isomer from another, food analyses usually report *trans* fatty acids only rather than total isomeric fatty acids. Wherever this paper discusses effects of dietary *trans* fatty acids, the positional *cis* isomers are implicitly included.

Small amounts of *trans* fatty acids are continuously formed in the rumen of cows and sheep. *Trans* fatty acids arise here 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 and milk contains some 2 to 8% by weight of *trans* fatty acids, most of the remainder being saturated. Much higher proportions of *trans* fatty acids are formed during the industrial hydrogenation of vegetable oils. Such partially hydrogenated vegetable oils have a particular melting range, stability and mouth feel that makes them suitable for incorporation into a wide range of foods, including baked goods and hard margarines. It should be stressed that only particular types of margarines are high in *trans*, namely brick- or stick-type hard margarines made from partially hydrogenated oils. In contrast, typical levels of *trans* in soft margarines are some 3% of total fatty acids in The Netherlands and 11% in North America. Several brands of soft tub margarines sold in Europe and Canada have *trans* levels of 1 % or less, and are also low in saturated fatty acids (1,2).

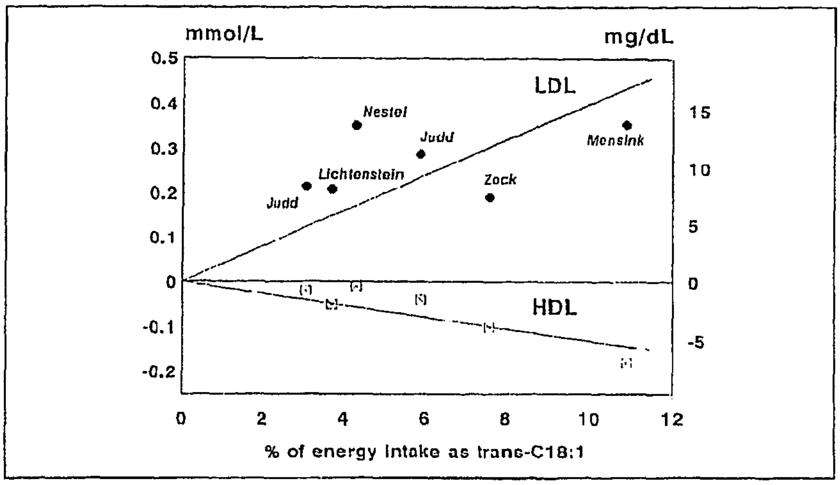

Figure 1) *Effects of monounsaturated* trans *fatty acids* (trans-C18:1) *on lipoprotein cholesterol levels relative to oleic acid* (cis-C18:1). *Data are derived from six dietary comparisons between* trans *monounsaturates and* cis *unsaturated fatty acids (5-7). Differences between diets in fatty acids other than* trans *and* cis *monounsaturates were adjusted for using regression coefficients from a meta-analysis of 27 controlled trials (9). The regression lines were forced through the origin bec ause a zero change in intake will produce a zero change in lipoprotein levels. Regression coefficients per per cent contribution of* trans *fatty acids to total daily energy intake are 0.040 mmol/L per cal% for LDL (R2=0.86, P=0.0028) and –0.013 mmol/L per cal% (R2=0.88, P=0.0019) for HDL cholesterol*

EFFECTS ON SERUM LIPID AND LIPOPROTEIN CONCENTRATIONS IN MAN

The toxicology of partially hydrogenated vegetable oils has been investigated extensively, and no untoward effects have been detected (3). However, attention was drawn to *trans* fatty acids when a study in human volunteers (4) showed that *trans* monounsaturated fatty acids, produced through hydrogenation of oleic acid-rich sunflower oil, lowered high density lipoprotein (HDL) and elevated low density lipoprotein (LDL) cholesterol levels compared with the natural *cis* isomer, oleic acid. A number of studies have appeared since which have largely confirmed these initial findings (5-7). Figure 1 (8) shows the effects of monounsaturated *trans* fatty acids relative to their *cis* isomer oleic acid across five trials. We adjusted for differences in other fatty acids between the *trans* enriched diets and the reference diets, using regression coefficients from a recent meta-analysis (9). Figure 1 suggests that the effect of *trans* fatty acids on HDL increases with the amount consumed. Although more experiments would be needed to define the shape of the dose-response curve more precisely, a linear relation appears satisfactory, and there is no evidence for a threshold below which *trans* fatty acids do not affect HDL cholesterol levels. According to this small meta-analysis, every additional per cent of dietary energy as *trans* fatty acids results in a decrease in HDL cholesterol of 0.013 mmol/L or 0.50 mg/dL (R^2=0.88, P=0.0019) and an increase in LDL cholesterol of 0.040 mmol/L or 1.55 mg/dL (R^2=0.86, P=0.0028). This effect on LDL is similar to that of saturated fatty acids (9).

Factors that lower HDL cholesterol levels often will also elevate fasting triglyceride levels; dietary carbohydrates are a case in point (9). In view of the HDL-lowering effect of *trans* fatty acids, one would thus expect a simultaneous rise in triglycerides. This is indeed confirmed by the results of recent trials for which fasting triglyceride levels were reported. In the three largest trials, serum triglycerides were modestly, but significantly, elevated on the *trans* fatty acids diets relative to the oleic or linoleic acid reference diets (4, 6,10). In the other recent studies triglyceride levels were also somewhat higher on *trans* fatty acids, although the changes were not statistically significant within each separate trial (5,7,11). When we apply the same adjustments and linear regression analysis as applied in Figure 1, it emerges that every additional per cent of energy as *trans* fatty acids increases triglyceride levels by 0.013 mmol/L or about 1 mg/dL (R^2=0.60, P=0.04). Thus, in addition to the LDL and HDL lowering effects, monounsaturated *trans* fatty acids appear to raise modestly fasting plasma triglyceride levels.

EFFECTS ON LIPOPROTEIN(a)

Serum lipoprotein(a) [Lp(a)] is a strong and independent risk factor for coronary artery disease. We therefore examined the effect of *trans* fatty acids on serum Lp(a) levels in controlled trials.

In our first experiment (4) 10% 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 three weeks in random order. The median level of Lp(a) was 26 mg/L on the saturated-fat diet; it increased to 32 mg/L (P<0.001) on the oleic-acid diet and to 45 mg/L (P<0.001) on the *trans*-fatty-acid diet (10). The second experiment (12) involved 56 subjects, who all received 8% of energy from either the saturated fatty acid stearic acid, from linoleic acid or from *trans*-monounsaturates, for three weeks each. Median Lp(a) levels were 69 mg/L on both the stearic-acid and linoleic-acid diet, and rose to 85 mg/L (P<0.01) on the *trans*-fatty-acid diet (10).

Our data thus agree with the finding of Nestel et al (5) in that *trans* fatty acids appear to raise Lp(a), even though the effect is quite small relative to genetically determined differences in Lp(a) levels. The effect of *trans* on Lp(a) is relevant for our understanding of lipoprotein metabolism: both saturates and *trans* fatty acids raise LDL levels in plasma, but *trans* fatty acids raise Lp(a), while saturates, if anything, tend to lower it (10). This suggests that dietary effects on LDL and on Lp(a) follow different pathways.

EFFECTS ON CHOLESTERYL ESTER TRANSFER PROTEIN

Human plasma contains a protein which transfers cholesteryl esters from HDLs to lipoproteins of lower density. We hypothesized that this cholesteryl ester transfer protein (CETP) could play a role in the effect of *trans* fatty acids on HDL and LDL levels. We therefore measured the serum activity levels of CETP (using excess exogenous substrate assays) in sera from our second study on *trans* fatty acids. The CETP activities measured after the stearate-diet and the linoleate-diet were identical, despite the higher VLDL+LDL cholesterol levels seen after the stearate-diet. The *trans*-diet was accompanied by an 18% increase in CETP activity if all subjects were analysed together. The increase in CETP activity after the *trans*-diet was seen in 52 out of 55 individuals; one individual showed no effect and two showed a decrease in activity (13).

The increase in CETP activity coincided with a low cholesteryl esters/triglycerides ratio in HDL. This was to be expected because CETP removes cholesteryl esters from HDL and replaces them with triglycerides. The average molar ratio (±SD) was 6.15±1.83 on the *trans*-diet versus 6.97±2.19 on the linoleate-diet and 6.71±2.25 on the stearate-diet (P<0.02).

It was previously reported that high concentrations of elaidic acid (*trans* C18:1n-9), added in vitro, may increase the transfer of cholesteryl esters from HDL to LDL (14). Therefore, *trans* fatty acids could act by increasing the serum levels of CETP, as suggested by our data, or by increasing the efficiency of the transfer proces, as found in the in vitro experiments. Recently, Abbey and Nestel (11) reported increased CETP activity after substitution of *trans*-elaidic acid for *cis*-oleic acid in the diet. A significant increase was only detected using a CETP activity assay employing endogenous lipoproteins, but absent if CETP activity was assayed in lipoprotein-deficient plasma. The CETP activity assay used in the present experiments is independent of endogenous lipoproteins and correlates very well with CETP mass. It is therefore possible that dietary *trans* fatty acids increase the transfer of cholesteryl esters by increasing CETP mass as well as by changing the structure of plasma lipoproteins, resulting in their acting as better substrates for CETP action.

Diminished levels of plasma CETP activity are often associated with a low risk lipoprotein profile, while increased CETP levels are found in patients with various forms of hyperlipidemia (15). Also, intravenous injection of CETP into rats or introduction of CETP into mice by transgenesis results in a rise in LDL and a fall in HDL cholesterol (15). These changes are similar to those seen in humans consuming high *trans* fatty acid diets. Experiments with mice and monkeys fed atherogenic diets revealed close correlations between atherosclerosis development, LDL cholesterol concentrations and plasma CETP levels (15). Our present data thus support the notion that the fall in HDL and increase in LDL on *trans* fatty acids are due to increased CETP activity, and that these lipoprotein changes may contribute to atherogenesis.

POLICY IMPLICATIONS

Trans fatty acids raise LDL and lower HDL, and if these changes are due to increased activity of CETP then both the rise in LDL and the fall in HDL might promote atherosclerosis. However, it would be erroneous to conclude that dietary fats high in *trans* fatty acids should now be replaced by fats high in saturates and cholesterol. The role of saturated fat and cholesterol in coronary artery disease has been abundantly documented. Also, intakes of saturated fatty acids far exceed intakes of *trans*. It is therefore preferable to count *trans* fatty acids in with the saturates, and aim for a reduction of the sum of the two. This implies replacement of hard fats by oils and margarines low in both *trans* and saturated fatty acids. Such margarines are widely available in Europe and Canada (1). Their use will make it possible to reduce *trans* intake without reverting to products high in saturated fatty acids and cholesterol.

REFERENCES

1. Kinan MB. Trans fatty acids in margarines. J Am Diet Assoc 1994;10:1097. (Lett)
2. Postmus E, deMan L,deMan JM. Composition and physical properties of North American stick margarines. Can J Food Sc Tech J 1989;22:481-6.
3. Senti FR, ed. Health aspects of dietary trans fatty acids. Bethesda: Federation of American Societies for Experimental Biology, 1988.
4. Mensink RP, Katan MB. Effect of dietary trans fatty acids on high-density and low-density lipoprotein cholesterol in healthy subjects. New Engl J Med 1990;323:439-45.
5. Nestel PJ, Noakes M, Belling GB, et al. Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. J Lipid Res 1992;33:1029-36.
6. Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME, Podczasy JJ. Dietary trans fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. Am J Clin Nutr 1994;59:861-8.
7. Lichtenstein AH, Ausman LM, Carrasco W, Jenner JL, Ordovas JM, Schaefer EJ. Hydrogenation impairs the hypolipidemic effect of corn oil in humans. Arterioscler Thromb 1993;13:154-61.
8. Zock PL, Mensink RP,Katan MB. Dietary trans fatty acids and lipoprotein cholesterol. Am J Clin Nutr 1995;61:617. (Lett)
9. Mensink RP, Katan MB. Effect of dietary fatty acids on serum lipids and lipoproteins – a meta-analysis of 27 trials. Arterioscler Thromb 1992;12:911-9.
10. Mensink RP, Zock PL, Katan MB, Hornstra G. Effect of dietary cis and trans fatty acids on serum lipoprotein[a] levels in humans. J Lipid Res 1992;33:1493-501.
11. Abbey M, Nestel PJ. Plasma cholesterol ester transfer protein activity is increased when trans-elaidic acid is substituted for cis-oleic acid in the diet. Atherosclerosis 1994;106:99-107.
12. Zock PL, Katan MB. Hydrogenation alternatives: Effects of trans fatty acids and stearic acid versus linoleic acid on serum lipids and lipoproteins in humans. J Lipid Res 1992;33;399-410.
13. Van Tol A, Zock PL, Van Gent CM, Scheek L, Katan MB. Dietary trans fatty acids increase serum cholesterylester transfer protein activity in man. Atherosclerosis 1995;115:129-34.
14. Lagrost L. Differential effects of cis and trans fatty acid isomers, oleic and elaidic acids, on cholesteryl ester transfer protein activity. Biochim Biophys Acta 1992;1124:159-62.
15. Tall AR. Plasma cholesteryl ester transfer protein. J Lipid Res 1993;34:1255-74.