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Dietary *trans* fatty acids increase conjugated linoleic acid levels in human serum

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Conjugated linoleic acid (CLA), fatty acids with 18 carbon atoms and two conjugated cis/trans *double bonds, have shown anticarcinogenic effects in experimental studies. We determined the proportion of CLA (the sum of* cis-9, trans-*11 and* trans-9, cis-*11 CLA) of total fatty acids in the diets and serum samples of healthy subjects who consumed for 5 weeks a diet high in saturated fatty acids mainly from dairy fat, followed by 5 weeks on a diet high (8.7% of energy, en%) in* trans *fatty acids from partially hydrogenated vegetable oil (40 subjects) or a similar diet high in stearic acid (9.3 en%, 40 subjects). All diets contained equal amounts of fat and* cis-*monounsaturated and* cis-*polyunsaturated fatty acids. The fatty acid compositions of the pooled diets and fasting serum samples drawn at the end of the diet periods were analyzed by gas chromatography, and CLA was identified by comparison with a standard of C18:2 conjugated dienes. The proportions of CLA in the dairy fat,* trans *fatty acid, and stearic acid diets were 0.37, 0.04, and 0.10% of total methylated fatty acids, respectively. The corresponding mean (SD) proportions in serum were 0.33 (0.07)% after the dairy fat diet, higher, 0.43 (0.12)%, $P < 0.001$, after the* trans *fatty acid diet, and lower, 0.17 (0.06)%, $P < 0.001$, after the stearic acid diet. The difference between dairy fat and stearic acid diets was explained by different dietary intakes but increased amounts of CLA not present in the diet were incorporated into serum lipids during the* trans *fatty acid diet. CLA in human tissues is partly derived from the diet but part of it may be formed by conversion from dietary* trans *fatty acids.* (J. Nutr. Biochem. 9:93–98, 1998) © *Elsevier Science Inc. 1998*

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Introduction

In ruminant animals polyunsaturated fatty acids are affected by the enzymatic activity of micro-organisms in the rumen, leading to disruption of the methylene-interrupted sequence of *cis*-double bonds and to *trans*-isomerization.[1] In this process linoleic acid may be transformed into *trans*-monoenoic fatty acids or into *cis/trans*-isomers, some of which contain conjugated double bonds at positions 9 and 11 or 10 and 12.[2] These fatty acids are commonly referred to as conjugated linoleic acid. CLA has recently received attention as an anticarcinogenic agent because it has been shown to inhibit DMBA-induced mouse skin cancer[3] and chemically induced mouse forestomach neoplasia[3,4] when applied topically and, given in the diet at levels of 1.5% or less, DMBA-induced mammary carcinogenesis in rats[5,6] and phorbol ester skin tumor promotion in mice.[7]

CLA is found particularly in foods of animal origin. Highest levels have been measured in dairy products, lamb meat and beef.[8–11] The *cis*-9, *trans*-11 CLA is thought to be the biologically active isomer, because it is incorporated into tissue phospholipids of animals.[4,5] It is also the main isomer found in lamb fat[12] and dairy products.[2] CLA has been identified in human tissues including blood, bile, adipose tissue and milk.[8,13–16] The *cis*-9, *trans*-11 isomer has been reported to represent more than 95% of the conjugated dienes in human tissues.[15] The origin is thought to be dietary, and the consumption of CLA-containing foods

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Table 1 Characteristics of the subjects before the study

Characteristic	Stearic acid group (n = 40)		*Trans* fatty acid group (n = 40)	
	Mean (SD)	Range	Mean (SD)	Range
Age (yr)	29.0 (9.5)	20–52	28.7 (7.8)	20–50
BMI (kg/m^2)	22.7 (2.6)	15.2–27.7	23.0 (3.4)	18.8–32.8
Serum cholesterol (mmol/L)	4.72 (0.87)	3.48–6.64	4.79 (0.92)	3.11–6.82
Serum HDL cholesterol (mmol/L)	1.52 (0.35)	0.93–2.75	1.40 (0.32)	0.39–2.34
Serum triglycerides (mmol/L)	1.01 (0.55)	0.50–3.53	1.10 (0.81)	0.37–5.52

like cheese has been shown to increase plasma CLA levels.[2,11,17] In rats CLA may be produced by biohydrogenation of free linoleic acid by the intestinal flora[18] or possibly by Δ9-desaturation of *trans*-vaccenic acid (*trans*-11 C18:1) in liver microsomes.[19] It has been hypothesized that CLA might be produced by a similar mechanism in the human organism[8] but there has not been direct evidence for this so far.

We studied the dietary and serum CLA levels in a carefully controlled dietary intervention trial in healthy subjects comparing similar amounts of *trans* monoenoic fatty acids and stearic acid against a dairy fat-based background diet and found evidence suggesting bioconversion of *trans* fatty acids into CLA in man.

Methods and materials

Subjects

The participants were 80 healthy volunteers (49 women and 31 men) with a mean age of 29 years (range, 20–52 years). Subjects with hypercholesterolemia, hypertension, anemia, glycosuria, and proteinuria were excluded. The subjects were allocated into two groups pairwise in the order of their serum cholesterol values. The baseline characteristics of the subjects in the two groups are shown in *Table 1*. The participants were asked to maintain their smoking habits, alcohol consumption and physical activity unchanged. There was only one regular smoker. All women except one were premenopausal, and 19 used oral contraceptives. Apart from free food, they received no payment.

Study design

The study included two successive 5-week periods. During the first 5 weeks all subjects consumed a diet high in saturated fatty acids, mainly from dairy fat. For the second 5 weeks 40 subjects consumed a high *trans* fatty acid diet and 40 a diet high in stearic acid. On weekdays the subjects had their lunch at the Division of Nutrition. When leaving they received takeaway food for the evening and the following morning, on Fridays for the whole weekend. All food was weighed for each participant. In addition the subjects were allowed to choose 10% of their energy intake from fat-free foods. The participants recorded in diaries the freely chosen foods and leftover food. They were weighed twice weekly and the energy intake was adjusted to avoid weight changes. A fasting blood sample was drawn during the last week of the study periods. The study protocol was approved by the ethics committees of the University of Helsinki and the National Public Health Institute. Results of the biochemical determinations were not available to the subjects before the end of the study. Both the participants and the laboratory personnel were blinded to the grouping of the subjects.

Diets

The three diets contained similar proportions of energy from fat, carbohydrate and protein, similar proportions of cis monounsaturated and polyunsaturated fatty acids and similar amounts of cholesterol (*Table 2*). They only differed in their composition of saturated and *trans* fatty acids. Part of the saturated fatty acids of the dairy fat diet were replaced with C18:1 *trans* fatty acids in the *trans* fatty acid diet, and a similar part by stearic acid in the stearic acid diet. Duplicate portions of each diet were collected daily and pooled separately for each period. The pooled diets were analyzed for energy, protein, cholesterol and fiber at the Agricultural Research Centre, Jokioinen, Finland and for fatty acid composition at the National Public Health Institute. The final composition of the diets (*Table 2*) was calculated by combining the analyzed values and those calculated from the free-choice and leftover diaries on the basis of food composition tables.

The fat of the dairy fat diet was mainly composed of dairy fat plus some meat fat and coconut oil. Butter and a butter-vegetable oil mixture (85% butter, 15% canola rapeseed oil) were used on bread, in baking, and cooking. The amounts of *trans* fatty acids

Table 2 Mean daily intake of energy and lipids according to duplicate-portion analysis plus calculated contribution of freely selected items

Nutrient	Dairy fat diet	*Trans* fatty acid diet	Stearic acid diet
Energy (MJ)	10.8	10.8	11.3
Fat, g/day (% of energy)	92.4 (32.2)	97.3 (33.9)	99.9 (33.4)
Fatty acids, g/day (% of fatty acids)			
Saturated	39.6 (46.8)	20.4 (22.7)	44.9 (48.9)
Stearic acid	10.3 (12.4)	7.5 (8.34)	27.9 (30.5)
Monounsaturated	35.0 (41.4)	60.9 (67.9)	36.6 (39.9)
Oleic acid	31.3 (37.7)	33.6 (37.1)	33.9 (37.3)
C18:1 *trans* fatty acids	2.3 (2.35)	24.9 (28.6)	1.2 (1.12)
Polyunsaturated	9.8 (11.7)	8.3 (9.25)	10.5 (11.3)
Linoleic acid	8.3 (9.60)	7.7 (8.48)	9.3 (9.96)
Conjugated linoleic acid (CLA)	0.31 (0.37)	0.04 (0.04)	0.09 (0.10)

and stearic acid were chosen to represent the highest amounts that could be incorporated into the diets without increasing total fat content and without too much compromizing palatability. For the *trans* fatty acid and stearic acid diets specially composed margarines were provided by the Unilever Research Laboratorium, Vlaardingen, the Netherlands. These were used on bread, for shortening, desserts and in baking and cooking. The margarine high in *trans* fatty acids was composed of 65% partially hydrogenated high-oleic-acid sunflower oil and 35% of the unaltered oil.[20] The *trans* monoenoic fatty acids included 35% Δ6–8 isomers, 22% Δ9 (elaidic acid), 19% Δ10, 12% Δ11 (*trans*-vaccenic acid), 7% Δ12, 5% Δ13, and <1% Δ14, as analyzed by the manufacturer. For the margarine high in stearic acid a mixture of fully hydrogenated sunflower oil (49%), high-oleic acid (43%) and high-linoleic acid (8%) sunflower oils was interesterified.[20]

Analysis of fatty acids

Serum was separated within 1 hour of venipuncture. Samples for fatty acid determinations were stored at −70°C for 5 to 7 months before analysis. The total fatty acid composition of serum was determined from a 0.5-mL sample. Lipids were extracted with dichloromethane-methanol (2 + 1 vol/vol) and *trans*-esterified with acidic methanol. Free cholesterol was removed with NH2 columns (Bond Elut NH2, Varian, Harbor City, California USA). The samples were analyzed with a gas chromatograph (HRCG 412, HNU-Nordion Oy, Helsinki, Finland) equipped with a 60 m SP 2380 column and split injection and with helium as carrier gas, in a temperature program. Chromatographic data was processed with an SC-WorkStation (Sunicom OY, Helsinki, Finland). The fatty acid composition was adjusted to 100%. The mean interassay coefficient of variation was between 1% and 5% for the major fatty acid peaks, 5–10% for the minor ones, and over 10% for the smallest peaks of less than 1% (14% for CLA, $n = 11$). The same method was used to analyze the fatty acid composition of the pooled diets.

For the identification of the CLA-peak, a standard mixture of conjugated C18:2 dienes (NuChek-Prep. Inc., Elysian, MN USA) was used. This mixture contains two major isomers, identified as Δ9,11 (*cis/trans* plus *trans/cis*) (41%) and *trans*-10, *cis*-12 (44%) by Ha *et al.*[21] plus smaller amounts of *cis*-10, *cis*-12, and other isomers. The position of *cis*-9, *trans*-11/*trans*-9, *cis*-11 CLA in the chromatogram of the mixture was concluded comparing the order of the peaks to previous identifications[21] and from the declared composition of the mixture. The suspected CLA and the Δ9,11 CLA standard peak matched with four different GC columns ranging from nonpolar to highly polar (HP 5, NB-351, SP-380, and SP-2560, respectively). In a qualitative high performance liquid chromatography (HPLC) analysis both the CLA standard and the sample methyl esters had the same retention time and gave an UV-absorption at 234 nm. A mass spectrum of the GC peak at the retention time of the CLA methyl ester with the HP 5-column gave a molecular ion of 294 in accordance with that of Iversen *et al.*[14] On an argentation thin-layer plate at −20°C with toluene as solvent[22] the suspected CLA-peak migrated to the top of the *cis*-monoene spot as described by Christie.[23] No fatty acid peaks matching with the Δ10,12 CLA standard peak were detected in the samples.

To separate between C18:1 *trans* monoenes and oleic acid in the pooled diets, additional argentation chromatography on silver impregnated thin-layer plates[24] was done. Cholesterol and triglycerides of fresh whole serum and ultracentrifugally separated lipoproteins were analyzed as described elsewhere.[25]

Statistical methods

The baseline characteristics and the differences between the groups were analyzed with the unpaired *t*-test and the differences between the dairy fat diet and the experimental diets with the paired *t*-test. The results are expressed as means and SD.

Results

Diets

There were no dropouts during the study, and the free-choice food diaries indicated good compliance to the diets; the fat intake from the free-choice foods was on average only 1.2 grams per day. Analysis of the pooled diets revealed that the study plan had been well realized and the diets were comparable with respect to their energy and fat content and the amounts of *cis*-monounsaturated and PUFAs (*Table 2*). Separation of *cis*- and *trans*-monoenoic fatty acids by argentation chromatography revealed that two peaks of *trans* isomers, amounting to 12% of total C18:1 *trans* fatty acids, were overlapped by oleic acid in the original chromatogram. This has been corrected for in the values given in *Table 2*. The *trans* fatty acid diet contained 8.7% of energy as *trans* fatty acids and these were replaced with stearic acid (9.3% of energy) in the stearic acid diet. The dairy fat and the stearic acid diets contained less than 1% of energy as *trans* fatty acids. The diets contained only small amounts of CLA, the dairy fat diet more than the *trans* fatty acid and stearic acid diets. Expressed as % of dietary fat, the dairy fat diet included 0.37%, the *trans* fatty acid diet 0.04%, and the stearic acid diet 0.10% CLA.

Serum fatty acids

During the *trans* fatty acid diet the proportion of CLA was significantly increased, by 30% compared with the dairy fat diet but during the stearic acid diet it was reduced to about one-half of that found at the end of the dairy fat diet (*Table 3*, *Figure 1*). The range of individual serum CLA values was 0.20 to 0.70% during the *trans* fatty acid diet and 0.06 to 0.31% during the stearic acid diet.

Stearic acid in serum lipids was significantly increased during the stearic acid diet and reduced during the *trans* fatty acid diet whereas *trans* fatty acids were increased during the *trans* fatty acid diet but showed no significant change during the stearic acid diet. The proportions of palmitic acid were significantly reduced during both the *trans* fatty acid and the stearic acid diets from those of the dairy fat diet. Linoleic acid values remained unchanged but the values of oleic acid were some what increased during the *trans* fatty acid diet (*Table 3*). As in the diets, a minor part of C18:1 *trans* fatty acids was evidently overlapped by the large oleic acid peak and falsely included into oleic acid. This error that has not been corrected for in the serum fatty acid data, explains the higher proportion of oleic acid on the *trans* fatty acid diet. Results of the serum and lipoprotein lipid determinations are published elsewhere.[25]

Discussion

The diets of this intervention study were carefully designed to provide similar amounts of *cis*-monounsaturated and PUFAs and to allow direct comparison between the effects of C18:1 *trans* fatty acids and stearic acid. The common background diet also contained similar proportions of major fatty acid classes but the composition of saturated and *trans*

Table 3 Mean (SD) percentual fatty acid composition of total serum during the dairy fat, *trans* fatty acid and stearic acid diets

Fatty acid	*Trans* fatty acid group (n = 40)		Stearic acid group (n = 40)	
	Dairy fat diet	*Trans* fatty acid diet	Dairy fat diet	Stearic acid diet
Palmitic acid	23.74 (1.27)	20.31 (1.60)*‡	23.66 (1.39)	21.42 (1.38)*
Stearic acid	7.47 (0.64)	6.68 (0.89)*†	7.53 (0.67)	10.30 (1.40)*
C18:1 *trans* fatty acids	0.59 (0.17)	4.47 (1.37)*†	0.57 (0.17)	0.49 (0.26)
Oleic acid	23.39 (2.03)	24.39 (1.66)*‡	23.06 (1.72)	23.11 (1.78)
Linoleic acid	30.03 (2.79)	29.73 (2.22)	29.39 (2.57)	29.78 (1.87)
Conjugated linoleic acid (CLA)	0.32 (0.06)	0.43 (0.12)*†	0.34 (0.08)	0.17 (0.06)*

*$P < 0.001$ for comparison with the dairy fat diet, paired t-test.
†$P < 0.001$ for comparison with the stearic acid diet, unpaired t-test.
‡$P < 0.01$ for comparison with the stearic acid diet, unpaired t-test.

fatty acids was derived from dairy fat and meat fat. About 90% of dietary energy intake and more than 98% of dietary fat intake were provided for the participants during the 10-week study, and the composition of the diets was analyzed biochemically from pooled daily diets of each study period.

Dairy fat and meat fat of the background diet were partly replaced by fats rich in *trans* fatty acids and stearic acid in

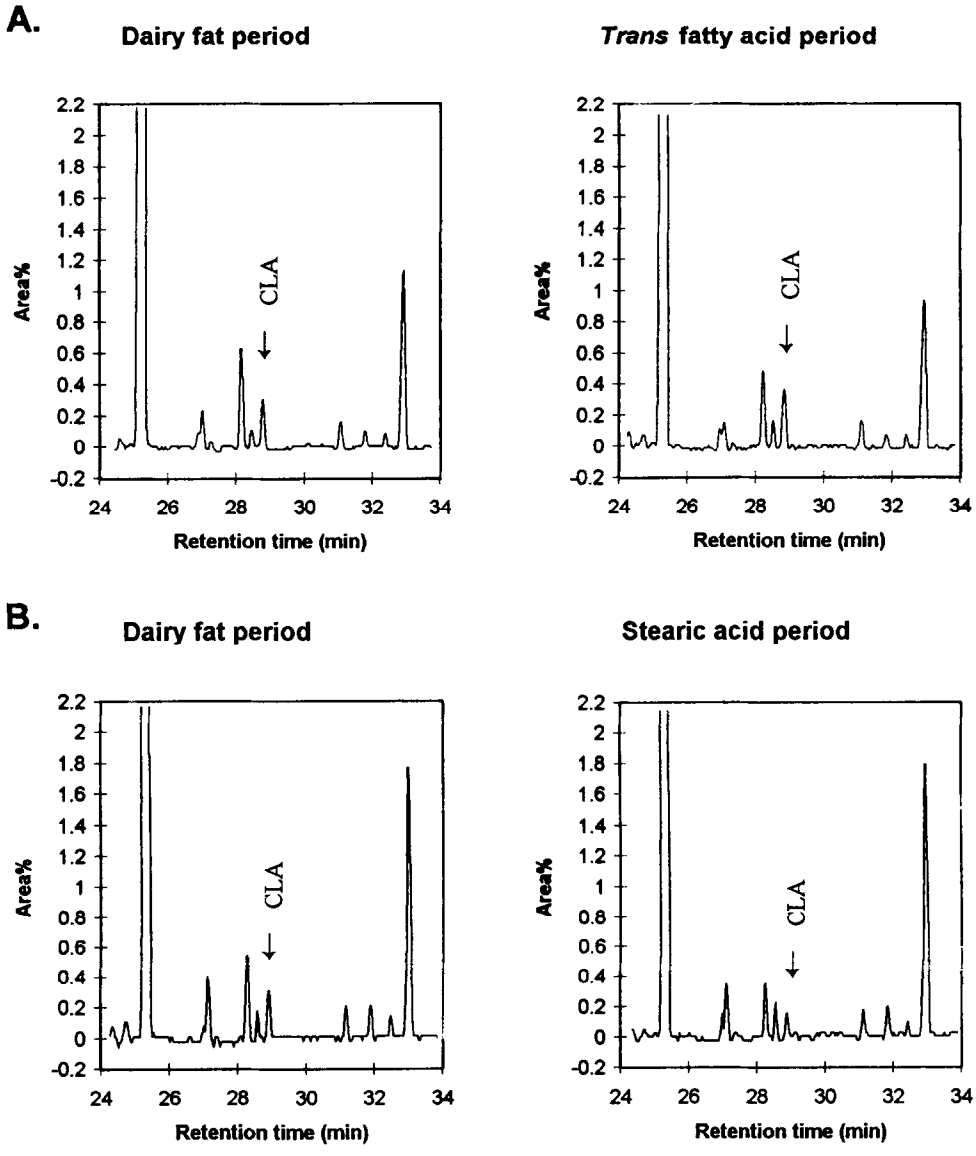

Figure 1 Gas chromatograms of total serum fatty acids of two representative subjects at the end of the baseline dairy fat diet and the *trans* fatty acid diet (subject A) and the stearic acid diet (subject B).

the respective experimental diets. Because dairy fat contains more CLA than vegetable fat-based margarines, the amounts of CLA in the *trans* fatty acid and stearic acid diets were smaller than in the background dairy fat diet. In accordance with this, the mean proportion of CLA in serum lipids was reduced significantly during the stearic acid diet to about one-half of that found during the preceding dairy fat diet. In contrast to this, in the subjects who consumed the *trans* fatty acid diet the proportion of CLA in serum lipids increased from the level of the dairy fat diet. At the end of the 5-week experimental period, the proportion of CLA in serum was 2.5-fold compared with the stearic acid group with similar dietary CLA intake. Evidently, CLA was formed during the consumption of the diet rich in C18:1 *trans* fatty acids and incorporated into serum lipids. We could identify only one CLA peak that corresponded to the Δ9,11 CLA standard whereas Δ10,12 CLA was evidently below the detection limit of our method. By the method that we used, it is impossible to separate the different *cis/trans* isomers of Δ9,11 CLA. However, previous findings have suggested that the *cis*-9, *trans*-11 CLA is practically the only isomer that is found in human tissues.[15] It thus seems probable that the peak that we identified was composed mainly of *cis*-9, *trans*-11 CLA.

It has been hypothesized that CLA might be synthesized from linoleic acid in the human organism through anaerobic microbial activity in the large bowel[8] or by free radical-induced isomerization,[13] or from *trans*-vaccenic acid by Δ9-desaturation as found in rat liver microsomal preparations.[19] Our results do not favor the concept of production of CLA from linoleic acid, because significantly different levels of CLA were found in serum lipids during the three dietary periods although the diets contained similar amounts of linoleic acid. Desaturation of *trans*-vaccenic acid by the Δ9-desaturase remains a possible explanation for the increase of CLA in serum lipids during the *trans* fatty acid diet. Another possibility would be the formation of CLA from *trans* vaccenic acid by the action of intestinal bacteria. This does not seem probable, however, because CLA formed in the colon would be poorly absorbed. In the *trans* fatty acid margarine 12% of *trans* fatty acids were composed of the *trans*-11 isomer. Because the diet contained a very high amount of *trans* fatty acids, the mean daily intake of *trans*-vaccenic acid was more than 2 g, an amount that exceeds the customary total *trans* fatty acid intake from dairy products in the European countries.[26] *Trans*-vaccenic acid is the predominant C18 *trans* isomer found in dairy fat,[26–28] whereas in hydrogenated vegetable oils the distribution between the Δ9, 10, and 11 isomers is generally more equal.[27,28]

The amounts of *trans* fatty acids fed to the *trans* fatty acid group in our study exceeded the habitual intakes of different populations by three- to tenfold. The impact of smaller amounts of *trans* fatty acids on the production of CLA remains to be established. However, taking into account the fairly small amounts of CLA found in the diets of most people, it is possible that even the smaller amounts of dietary *trans* fatty acids in the habitual diets of people may contribute significantly to the amount of CLA in the human organism. The health effects of CLA in the human organism are unknown. However, in a Finnish cohort study, intake of dairy products showed an inverse association with risk of breast cancer, and the authors considered CLA as one possible factor that might explain the finding.[29] According to our results, vaccenic acid present in dairy products might make a further contribution in addition to the effect of preformed CLA.

The mode of the anticarcinogenic effect of CLA is not known. Antioxidation[4,5] is one postulated mechanism, but its importance has been questioned recently.[30] It does not seem probable, either, that CLA would compete with the metabolism of linoleic acid, because its effects in animals are independent of the fatty acid composition of the diet.[31] Only limited information is available on the effects of *trans* fatty acids on carcinogenesis or risk of cancer. In a recent review article[32] it was concluded that there is little evidence that *trans* fatty acids are related to the risk of cancer at any of the major sites. None of the available studies has addressed the effects of different *trans* isomers separately.

C18:1 *trans* fatty acids from partially hydrogenated vegetable oils increase serum LDL-cholesterol and tend to decrease HDL-cholesterol levels,[33] and a similar effect was also confirmed in the present study.[25] These changes are unfavorable in terms of coronary heart disease risk. However, different C18:1 *trans* isomers may have different effects on lipids and risk of diseases. There are observations suggesting that *trans*-vaccenic acid may affect risk of coronary heart disease in a different way compared with elaidic acid.[34,35] Further studies on the associations between *trans* fatty acid intake and risk of chronic diseases should focus on the separate effects on risk factors and potential protective elements of different isomeric fatty acids. Particularly the effects of *trans* vaccenic acid warrant further attention.

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