The proportion of *trans* monounsaturated fatty acids in serum triacylglycerols or platelet phospholipids as an objective indicator of their short-term intake in healthy men

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(*Received 21 March 1994 – Revised 27 June 1994 – Accepted 24 August 1994*)

Unfavourable effects of *trans* monounsaturated fatty acid (*trans*-C18:1) isomers on health variables have been reported. Reports on their actual intake, however, are scarce, because of the absence in many nutrient databases of values for *trans*-C18:1, and the wide variation in the level of *trans* fatty acids between different brands of the same product. We therefore examined whether the intake of *trans*-C18:1 is reflected by *trans*-C18:1 concentrations in serum triacylglycerols or platelet phospholipids. Thirty-eight men received two diets in random order. During the first experimental period twenty men consumed a Western-type control diet for six weeks, and eighteen men consumed a modified diet in which 70% of the fat was replaced by palm oil. After a wash-out period of 3 weeks, regimens were crossed over (second experimental period). The proportion of total fatty acids from *trans*-C18:1 in the diet decreased from 4·7 (SEM 0·27) during the control to 2·1 (SEM 0·16) on the modified diet ($P < 0·001$). *Trans*-C18:1 in serum triacylglycerols decreased from 3·5 (SEM 0·13) to 2·8 (SEM 0·11)% ($P < 0·001$), and in platelet phospholipids from 1·0 (SEM 0·06) to 0·7 (SEM 0·04)% ($P < 0·001$). After the first experimental period *trans*-C18:1 in the diet correlated with *trans*-C18:1 in serum triacylglycerols (r 0·41; $P = 0·014$), and platelet phospholipids (r 0·52; $P = 0·001$). Also, differences in the intake between the two periods correlated with changes in the proportion of *trans*-C18:1 in serum triacylglycerols (r 0·56; $P = 0·001$) and platelet phospholipids (r 0·58; $P < 0·001$). These results suggest that analyses of blood lipid fractions can be used to estimate the intake, and to monitor changes in the intake, of *trans*-C18:1.

Dietary intake: *Trans* fatty acids: Serum triacylglycerols: Platelet phospholipids

A wide variety of *trans* isomeric fatty acids are found to various extents in many food items. These *trans* fatty acids originate primarily from commercially hydrogenated vegetable oils, but a small part is also derived from ruminant fats (British Nutrition Foundation's Task Force on Trans Fatty Acids, 1987). Most *trans* fatty acids have eighteen C atoms and one double bond (*trans*-C18:1), but isomeric fatty acids with fewer C atoms or more double bonds are also present in the human diet (Sommerfeld, 1983; Van den Reek *et al.* 1986). It has been reported that *trans*-C18:1, relative to *cis*-C18:1 (oleic acid), increases the concentration of the atherogenic lipoproteins, and decreases that of anti-atherogenic lipoproteins (Mensink & Katan, 1990; Mensink *et al.* 1992). In addition, an inverse correlation of *trans*-C18:1 in plasma cholesteryl esters of the mother with birthweight in prematurely born neonates has been reported (Koletzko, 1991). Finally, a negative association has been observed between *trans* fatty acids and the essential fatty acid status (Koletzko, 1991; Siguel & Lerman, 1993). Thus, these and other studies (Willett *et al.* 1993) suggest that *trans*-C18:1 may have potential adverse effects on health.

The average intake of *trans*-C18:1 amounts to 7–8 g/d (Gurr, 1986), but estimates of more than 10 g have also been published (Enig *et al.* 1990). The absence in most nutrient databanks of figures for the *trans* fatty acid content of foods makes it difficult to assess the

actual intake for individuals. Theoretically, the proportion of isomeric fatty acids in human lipids should reflect intake: *trans* fatty acids are taken up and metabolized (Emken, 1984), and probably originate solely from dietary sources.

Recently, we have carried out a double-blind crossover trial to evaluate the effect of replacing a major part of the habitual fat content in a Dutch diet by palm oil on risk factors for coronary heart disease (Sundram *et al.* 1992). This change in fat type was accompanied by a decrease in the intake of *trans* fatty acids of more than 50%. During this study, subjects collected duplicate portions of their total diets, while the fatty acid composition of serum triacylglycerols and platelet phospholipids was determined. In this way it was possible to examine whether analysis of *trans*-C18:1 in blood lipids can be used to estimate the intake of *trans*-C18:1 by free-living individuals.

METHODS

Subjects

Forty non-obese men, aged between 19 and 45 years, entered this dietary study. All were in good health, as judged before the start of the experiment by the absence of glucose and protein in urine, systolic blood pressure values between 100 and 140 mmHg and diastolic blood pressure levels between 40 and 90 mmHg, fasting serum total cholesterol values below 6·8 mmol/l and triacylglycerols less than 2·0 mmol/l. None of the men received any medication or smoked more than seven cigarettes/week. One man dropped out of the study because of job commitments, and another volunteer was excluded because he was prescribed medication for treatment of gout. Thus, data for thirty-eight men were processed. They were between 19 and 45 years of age (mean 36 years), and their body mass indices ranged from 20·1 to 26·4 kg/m^2 (mean 23·8 kg/m^2).

The research protocol was approved by the Medical Ethical Committee of the University of Limburg, The Netherlands. Written informed consent was obtained from all participants.

Diets and design

The main purpose of the present study was to test the effects of a control Western-type diet *v.* a palm-oil enriched diet on risk factors for coronary heart disease. Details of this study and part of the results have been previously published (Hornstra *et al.* 1991; Sundram *et al.* 1992).

The experiment was designed as a double-blind crossover trial. During a run-in period of 3 weeks subjects received control products, containing usual fats and oils. At the end of this period, subjects were randomly divided into two groups, each with an identical number of subjects. One half of the subjects continued on the control diet for another 6 weeks, while the other half of the men consumed comparable products in which the habitual fat was largely replaced by palm oil (first experimental period). These products included margarines, frying and bakery fats, snack foods (hamburgers, chicken nuggets, meat croquettes), dairy products (cheese, chocolate milk, ice cream, dairy products), bakery products and chocolate spread (Sundram *et al.* 1990). During a wash-out period of 3 weeks all volunteers consumed the control diet, and for the last 6 weeks of the study (second experimental period) the dietary regimens were crossed over. Maximal substitution of the habitual fat was achieved by preparing a series of palm-oil based products, in which the normal fat component was replaced. All food products were identically packed to prevent identification of the two different diets by both the volunteers and the investigators.

The food products were displayed in a special shop and volunteers could choose from these items freely. Each product was bar-coded, and a computer program was designed to decode the information. Passage was only allowed if an item from the correct dietary group

was chosen. All items were free of charge for both the participants and their family members so as to optimize compliance.

Measurements

During the fourth week of each experimental period volunteers collected, over a 48 h period, a duplicate portion of all foods and liquids consumed. The duplicate portions were brought to the laboratory on the following day, weighed, homogenized with a food mixer and approximately 750 g was collected in lidded tins and stored at −20° until analysis. After thawing, approximately 500 g of this material was freeze-dried. Of the freeze-dried material, 20 g was used for quantification of the fat content by Soxhlet-extraction with petroleum ether (boiling range: 40–60°). Fasting blood samples were taken at 3-week intervals. Triacylglycerols from the serum lipid fraction and phospholipids from the platelets were isolated (Rand *et al.* 1986; Sundram *et al.* 1992) in the samples obtained at the end of each experimental period. After saponification and methylation, the proportion of individual fatty acids in the diet, serum triacylglycerols and platelet phospholipids were determined with a Hewlett-Packard 5840A gas chromatograph fitted with a 50 m CP Sil 88 capillary column with an inner diameter of 0·25 mm and 0·20 μm film thickness (Chrompack, Middelburg, The Netherlands). Helium was used as a carrier gas. The oven temperature was programmed to rise from 125 to 245° at a rate of 5·5°/min and then kept constant. The temperatures of the injector and the flame ionization detector were set at 250°. A standard mixture was used to identify the fatty acid methyl esters by means of the retention times. Results were expressed as a proportion of total fatty acids. To estimate the reproducibility of the methods, one diet and one serum sample were analysed ten times. For these samples the mean analysed levels of *trans*-C18:1 were respectively 4·4 and 0·4 g/100 g fatty acids, while the inter-assay coefficients of variation amounted to 6·0 and 3·9%.

During the fifth week of each experimental period all participants were subjected to a dietary history interview by a dietitian. Distinction was made between weekdays and weekend days. Portion sizes were estimated using common household units and converted into weight (g). Finally, foods were coded and the nutrient composition was calculated using the Netherlands Nutrient Data Base to which the composition of the experimental products was added.

Statistical analysis

For each subject, differences in dietary intake for variables of interest were calculated as the change between the values obtained after the first and second experimental periods or as the difference between the levels on the palm-oil diet and the control diet. The same calculations were performed for the proportion of *trans*-C18:1 in serum triacylglycerols or platelet phospholipids. Differences were tested with Student's paired t test. To examine whether the proportion of *trans*-C18:1 in the diet can be estimated by analyses of blood lipids, Pearson correlation coefficients (Snedecor & Cochran, 1980) were calculated using data from the first experimental period. At that time half the men consumed the control diet and the other half consumed the palm-oil-enriched diet. To test whether the proportion of *trans*-C18:1 in blood lipids changes if the intake changes, correlation coefficients between differences in these variables were also calculated. For all analyses a two-tailed P value of less than 0·05 was considered to be statistically significant. All analyses were carried out with the Statistical Analysis System (SAS) (1985).

RESULTS

Twenty men first consumed the control diet and eighteen men the palm-oil diet. The calculated proportion of energy derived from fat was 41·1 (SEM 0·69)% on the control diet

Table 1. *Proportion of* trans *monounsaturated fatty acids* (trans-*C18:1*) *in the diet, serum triacylglycerols and platelet phospholipids of men consuming a control diet or a diet in which the habitual fat was largely replaced by palm oil*†

(Mean values with their standard errors for thirty-five to thirty-eight determinations)

	g/100 g fatty acids					
	Diet		Serum triacylglycerols		Platelet phospholipids	
	Mean	SE	Mean	SE	Mean	SE
Control diet	4·7	0·27	3·5	0·13	1·0	0·06
Range	1·4–8·9		1·6–5·7		0·5–1·7	
Palm-oil diet	2·1	0·16	2·8	0·11	0·7	0·04
Range	1·1–4·8		1·5–4·6		0·2–1·3	
Change‡	2·5***	0·31	0·8***	0·13	0·3***	0·06
Range	−0·7–7·7		−1·4–2·6		−0·5–1·1	

*** Mean change was statistically significant, $P < 0·001$.

† For details of subjects and procedures, see pp. 606–607.

‡ Calculated as the difference between the level on the control diet and the level on the palm-oil diet for each subject.

and 41·0 (SEM 0·78) % on the palm-oil diet. On average, 70 % of the habitual fat was replaced by palm oil. According to duplicate portion analysis, the contributions to total fat intake of palmitic acid (28·6 (SEM 0·67) *v.* 20·8 (SEM 0·64) %; $P < 0·001$), oleic acid (36·7 (SEM 0·37) *v.* 32·5 (SEM 0·67) %; $P < 0·001$) and linoleic acid (14·3 (SEM 0·59) *v.* 12·6 (SEM 0·60) %; $P < 0·001$) were higher, and those of myristic acid (2·4 (SEM 0·11) *v.* 4·7 (SEM 0·25) %; $P < 0·001$) and stearic acid (6·9 (SEM 0·17) *v.* 8·6 (SEM 0·20) %; $P < 0·001$) were lower on the palm-oil diet (Hornstra *et al.* 1991). The proportion of *trans*-C18:1 decreased from 4·7 (SEM 0·27) % on the control diet to 2·1 (SEM 0·16) % on the palm-oil diet (Table 1).

The proportion of *trans*-C18:1 in serum triacylglycerols and platelet phospholipids also decreased during consumption of the palm-oil diet (Table 1). Levels on the palm-oil diet were consistently lower, although overlap existed between control and palm-oil values. During the first experimental period the ratio of *trans*-C18:1 in serum triacylglycerols to dietary *trans*-C18:1 was 1·15 (SEM 0·11; 95 % confidence interval 0·94 to 1·36). The platelet phospholipid:dietary *trans*-C18:1 ratio was 4·00 (SEM 0·35; 95 % confidence interval 3·28 to 4·72). During the second experimental period, these ratios were 1·04 (SEM 0·10; 95 % confidence interval 0·85 to 1·24) and 4·25 (SEM 0·38; 95 % confidence interval 3·49 to 5·01) respectively.

After the first experimental period when half of the men consumed the control diet and the other half the palm-oil diet, the correlation coefficient between the proportion of *trans*-C18:1 in the diet and in serum triacylglycerols was 0·41 ($P = 0·014$, n 36). The differences in proportions between the second and first experimental period were also highly correlated (Fig. 1; r 0·58, $P = 0·001$, n 33). For platelet phospholipids the correlation coefficients with dietary *trans*-C18:1 were 0·52 ($P = 0·001$, n 35) and 0·58 (Fig. 2; $P < 0·001$, n 31) respectively. The correlation coefficient between the proportion of *trans*-C18:1 in serum triacylglycerols and platelet phospholipids was 0·59 ($P < 0·001$, n 35), while the changes in the proportion of *trans*-C18:1 in these two lipid fractions were also highly correlated (Fig. 3; r 0·68; $P < 0·001$, n 34).

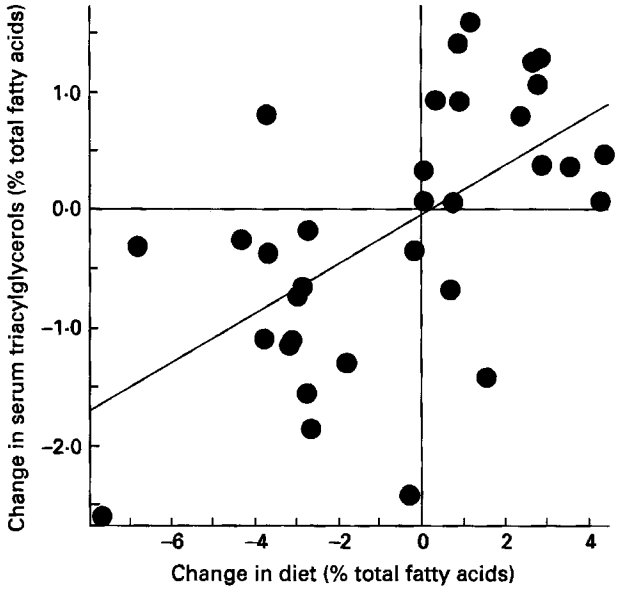

Fig. 1. Relationship between changes in the level of *trans*-C18:1 fatty acids (as a percentage of total fatty acids) in the diet and in serum triacylglycerols of men consuming a control diet and a diet rich in palm oil in a crossover design, (r 0·58).

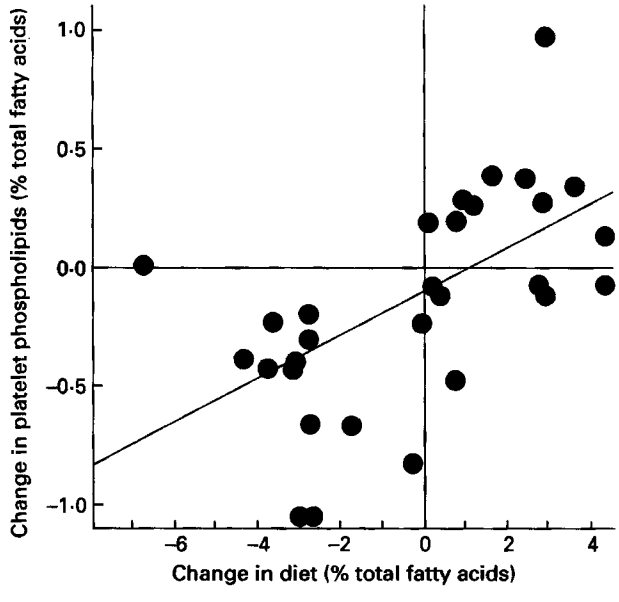

Fig. 2. Relationship between changes in the level of *trans*-C18:1 fatty acids (as a percentage of total fatty acids) in the diet and in platelet phospholipids of men consuming a control diet and a diet rich in palm oil in a crossover design, (r 0·58).

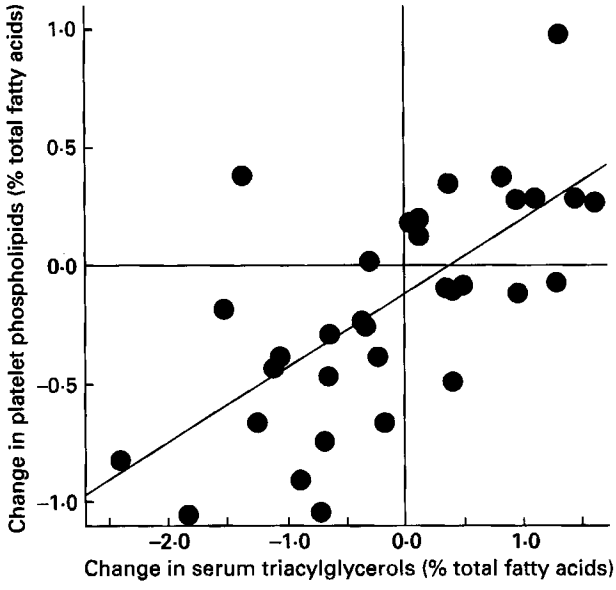

Fig. 3. Relationship between changes in the level of *trans*-C18:1 fatty acids (as a percentage of total fatty acids) in serum triacylglycerols and platelet phospholipids of men consuming a control diet and a diet rich in palm oil in a crossover design, (r 0·68).

DISCUSSION

Reliable estimates for the intake of *trans* isomeric fatty acids for individuals are difficult to obtain. In many countries data are lacking, while the level of isomeric fatty acids varies widely between different brands of the same product (Enig *et al.* 1983; Slover *et al.* 1985). We have now shown that the proportion of *trans*-C18:1 in the diet, which accounts for more than 80% of all dietary isomeric fatty acids (Van den Reek *et al.* 1986; London *et al.* 1991), can be estimated from the *trans*-C18:1 concentration in serum triacylglycerols or platelet phospholipids. Duplicate portions were collected for 48 h. Due to day-to-day variation in intake, the correlation coefficients may even have been underestimated (Liu *et al.* 1979). Relationships of comparable strength have been found between the intake of linoleic acid or *n*-3 fatty acids of marine origin with their respective levels in blood lipids or adipose tissue (Van Houwelingen *et al.* 1989; London *et al.* 1991). Like *trans*-C18:1, these fatty acids are also derived mainly, if not exclusively, from the diet and are not endogenously synthesized.

There are only a few other studies that have examined the *trans* fatty acid content of certain blood lipid fractions in relation to diet. Almost 30 years ago, De Iongh *et al.* (1965) published the results of a series of experiments to study the effect of dietary fatty acid composition on serum lipids. Ten different diets were fed for 4 to 6 weeks to seventy-two men. The proportion of *trans* fatty acids ranged from 5 to 50%, which corresponded with 2–17% of total energy intake. From the published group means it can be calculated that a positive relationship existed between *trans* fatty acids in the diet and in three different serum lipid fractions: cholesteryl esters (r 0·79, $P = 0·006$), triacylglycerols (r 0·93, $P < 0·001$) and phospholipids (r 0·76, $P = 0·011$). We have now shown that it is also possible to assess the *trans*-fatty acid intake from blood lipid values for individuals rather than for groups of subjects. Dietary *trans*-C18:1 is incorporated to a different extent into the blood lipid fractions. In the study of De Iongh and co-workers (1965), *trans* fatty acids were found to a greater extent in serum triacylglycerols and phospholipids as compared with

cholesteryl esters, while in the present study the proportion of *trans*-C18:1 was higher in serum triacylglycerols than in platelet phospholipids. The strength of the relationship of both lipid fractions, however, with the estimated *trans*-C18:1 intake was comparable (Figs. 1 and 2). Emken and co-workers (1979) have shown that elaidic acid (*trans*-C18:1*n*-9) and its positional isomers are also incorporated into plasma, erythrocyte and platelet neutral lipids, after consumption of a single dose of these isomers. The purpose of their studies (Emken *et al.* 1979), however, was to compare the metabolic route of these isomers and not to derive a quantitative relationship between dietary levels and blood lipid concentrations.

Apart from blood lipids, *trans* fatty acids are also stored in other tissue lipids. London *et al.* (1991) observed a correlation coefficient of r 0·51 between the *trans*-C18:1 concentration in adipose tissue of 115 post-menopausal American women and their *trans*-C18:1 intake, as calculated from a semi-quantitative food-frequency questionnaire. For 118 American men, however, Hunter *et al.* (1992) reported a much lower correlation coefficient (r 0·29).

The ratio of dietary *trans*-C18:1 to *trans*-C18:1 in serum triacylglycerols was on average 1·15 (SD 0·64). This suggests that for our healthy male subjects this relationship is approximately 1:1, and that the proportion of *trans*-C18:1 in serum triacylglycerols reflects its level per 100 g dietary fat. It is not known whether this relationship is also valid at higher intakes or for other population groups. In addition, hydrogenation of vegetable oils produces many different positional isomers of *trans*-C18:1 (Sampugna *et al.* 1982), which may be incorporated into blood lipids to slightly different extents (Emken *et al.* 1979). We have not differentiated between these various isomers and it cannot be excluded that this relationship varies slightly between the different positional isomers of *trans*-C18:1. From the study of London *et al.* (1991) it can be estimated that the ratio of the proportions of *trans*-C18:1 in adipose tissue to dietary *trans*-C18:1 is also close to unity. For total *trans* fatty acids, however, a ratio of 0·79 was estimated. If this difference is real, these data suggest that incorporation into adipose tissue varies between the different isomeric fatty acids.

In conclusion, fatty acid analysis of serum triacylglycerols or platelet phospholipids can be used to estimate the intake, and to monitor changes in the intake, of *trans*-C18:1. However, the proportion in blood lipids of fatty acids that can only be derived from the diet reflects the habitual dietary intake over a period of only a few days to several weeks, and that of adipose tissue over a period as long as 2–3 years (Dayton *et al.* 1966). For situations to monitor dietary intake and in intervention studies, analysis of *trans*-C18:1 in blood lipids may therefore offer an advantage over adipose-tissue sampling, because changes occur within a relatively short period of time and levels are not confounded by dietary habits in the past. In addition, no extra puncture is needed because blood is usually sampled for other measurements. However, if information is required over a longer period of time and dietary habits have remained stable, it is obvious that measurements in adipose tissue are preferred.

Ronald P. Mensink is a recipient of a fellowship from the Royal Netherlands Academy of Arts and Sciences.

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Printed in Great Britain