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Home use of margarine is an important determinant of plasma *trans* fatty acid status: a biomarker study

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The contribution of the home use of margarines, made with partially hydrogenated vegetables oils, to total *trans* fatty acid intake is difficult to determine using dietary assessment because food composition databases are incomplete for *trans* fatty acids; moreover, hidden fats in manufactured foods may be the predominant sources of *trans* fatty acids. The objective of our study was to determine, using plasma phospholipid *trans* fatty acid composition as a surrogate measure of exposure, whether the home use of margarine or butter is an important determinant of *trans* fatty acid status. We conducted a community-based (Dunedin, New Zealand), cross-sectional survey of people who consumed either margarine (*n* 65) or butter (*n* 64) but not both for home use. The levels of the 18 : 1 *trans* isomers commonly found in partially hydrogenated vegetable oils were all significantly higher in the plasma phospholipids of margarine compared with butter consumers, with the exception of 18 : 1*n*-7t, which did not differ. Among margarine consumers, the percentage of total fat from margarine was significantly correlated with levels of phospholipid 18 : 1*n*-6t, 18 : 1*n*-8t and 18 : 1*n*-12/9t isomers (r 0·57–0·63, $P<0·001$) but only weakly with 18 : 1*n*-7t (r 0·30, $P=0·016$). The intake of fat from fast foods, bakery products or meat and meat products was not associated with plasma phospholipid *trans* isomeric composition. The home use of margarine, made with partially hydrogenated vegetable oils, is an important determinant of *trans* fatty acid exposure in New Zealand.

***Trans* fatty acids: Dietary fats: Margarine: Plasma phospholipids: Biological marker**

A high intake of *trans* fatty acids from partially hydrogenated plant oils has been associated with an increased risk of cardiovascular morbidity and mortality (Ascherio *et al.* 1996; Hu *et al.* 1997; Pietinen *et al.* 1997). The association is biologically plausible because *trans* unsaturated fatty acids increase plasma total and LDL-cholesterol and decrease HDL-lipoprotein cholesterol concentrations in comparison with *cis* unsaturated fatty acids (Mensink & Katan, 1990; Lichtenstein *et al.* 1999).

Dietary sources of *trans* fatty acids are foods containing ruminant fat and partially hydrogenated vegetable or fish oils. The *trans* fatty acid content of foods containing partially hydrogenated oils tends to be higher than in ruminant fats, although levels in the former vary considerably (Firestone & Sheppard, 1992). The two sources also differ in positional isomer composition: *trans* fatty acids from ruminant fats are characterised by the predominance of *trans* vaccenic acid (C18 : 1*n*-7t; Parodi, 1976; Precht & Molkentin, 1995; Wolff, 1995), whereas 18 : 1*n*-9t, 18 : 1*n*-8t and 18 : 1*n*-7t are the major *trans* isomers in partially hydrogenated oils (Marchand, 1982; Slover *et al.* 1985; Molkentin & Precht, 1996).

Assessing individual intakes of *trans* fatty acids by traditional methods of dietary assessment is not possible in most countries because few national food composition databases include a full dataset of the *trans* fatty acid content of foods. Even if the relevant database included *trans* fatty acids, obtaining accurate estimates of intake would be difficult because the food that an individual consumes may not have been prepared with the same type of fat as the matching food in the database. This 'hidden' fat is derived predominantly from manufactured foods and foods prepared outside the home (Elias & Innis, 2002), it represents a substantial proportion of total fat intake and its contribution to *trans* fatty acid intake is difficult to estimate (Innis *et al.* 1999). The other major food source of *trans* fatty acids in the diet is the home consumption of margarine, made with partially hydrogenated plant oils, or butter. To what extent this choice, in the context of unknown and potentially much larger amounts of *trans* fat from manufactured foods, affects *trans* fatty acid exposure is not known.

Biological markers of fat intake offer an alternative to assessing *trans* fatty acid exposure because tissue fatty acid composition reflects actual rather than reported intake, thus avoiding the particular problems of quantifying 'hidden' fats or the well-established underreporting of fat intake (Beaton *et al.* 1979; Bingham, 1987; Bingham *et al.* 1994). Biomarkers of *trans* fatty acid intake are particularly good (Seppanen-Laakso *et al.* 1996; Vidgren *et al.* 1998; Lichtenstein *et al.* 2003) because there is negligible endogenous synthesis of *trans* fatty acids.

In our study, we compared the *trans* fatty acid composition of plasma phospholipid in people who used either butter or partially hydrogenated table spreads, but not both, to examine the extent to which this food choice is a determinant of total *trans* fatty acid exposure.

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Methods

Participants were recruited from the Dunedin community by advertisements in local newspapers and posters around the city. Inclusion criteria for the study were: 18 years of age or older, and a user of either butter or margarine – but not both – as a spread. The type of fat used in cooking or baking was not a criterion for selection. For the sake of simplicity, vegetable oil-based margarines and table spreads are referred to as margarines throughout the manuscript. The study was outlined to the participants, both verbally and via a written information sheet, and signed informed consent was obtained. The Human Ethics Committee of the University of Otago approved the study protocol.

Single measurements of weight (SECA digital scales; SECA, Hamburg, Germany) and height (custom made stadiometer) were taken at the Human Nutrition Department Research Clinic. Medication use was self-reported. Volunteers were excluded from participation if they were using medications known to affect blood lipids. Smoking status was ascertained by questionnaire.

Participants completed a weighed and estimated 4 d diet record, encompassing three weekdays and one weekend day, over a 1 week period. Group instruction sessions on keeping a diet record were given. The energy and nutrient composition of the diets was calculated with reference to the New Zealand Food Composition (Burlingame *et al.* 1993). The likelihood that reported dietary intake represented actual intake was determined using the cut-offs developed by Goldberg *et al.* (1991). Participants with a ratio of energy intake:BMR of less than 0·88 were excluded from the analysis.

A blood sample drawn from an arm vein was collected from participants who had fasted for 10–12 h overnight. All samples were obtained no later than 1 week after completing the diet record. Blood was collected into a Vacutainer (Becton, Dickenson and Company, Franklin Lakes, NJ, USA) with EDTA as anticoagulant, stored on ice and centrifuged within 2 h at 2500 ***g*** for 15 min at −4°C. Plasma was removed, and several aliquots were stored at −80°C.

An internal standard, di-heptadecanoylphosphatidylcholine (C17 : 0 PC; Sigma, St Louis, MO, USA) was added to plasma samples prior to lipid extraction. Lipids from 1 ml plasma were extracted, the phospholipids separated and fatty acid methyl esters prepared as previously described (Holub & Skeaff, 1987). A sample of water, as a blank, was extracted with every eight plasma samples.

Fatty acid methyl esters were auto-injected and separated using a BPX-70 capillary column, 100 m × 0·22 mm internal diameter, 0·25 μm film (SGE, Melbourne, Australia) on a HP6890 gas chromatograph (Agilent, Avondale, PA, USA). The oven was held at an initial temperature of 165°C for 52 min, then increased at a rate of 5°C/min to a final temperature of 210°C, which was held for 59 min. Total run time was 120 min. Both the injector and the flame ionisation detector ports were at 250°C. Carrier gas flow (He) was maintained at 1·0 ml/min (linear gas velocity 20 cm/s) throughout the temperature programme, with an inlet split ratio of 30:1.

A composite standard made from commercially available methyl esters (NuCheck Prep, Elysian, MN, USA; Sigma) was run every eight samples to ensure a correct estimation of retention times. The 18 : 1 and 18 : 2 positional and geometrical isomers were identified by retention time matching with commercial standards, and comparison with published separations on similar highly polar cyanopropyl polysiloxane stationary phases (Ratnayake & Beare-Rogers, 1990; Hudgins *et al.* 1991; Ratnayake & Pelletier, 1992; Wolff, 1995; Molkentin & Precht, 1996). The *n*-12 to *n*-9 *trans* isomers of C18 : 1 co-eluted and are described in the paper as 18 : 1*n*-12/9t. The position of the double bonds in the 18 : 2 cis/*trans* isomers was not established, and these isomers are arbitrarily identified by sequential number.

To estimate the precision of the analytical method, one pooled plasma sample was analysed for every eight study samples. The CV for the major phospholipid fatty acids contributing more than 5 mol % ranged from 1·0 % to 3·6 %, whereas for fatty acids contributing 1–5 mol %, the CV ranged from 1·2 % to 6·3 %. The CV for the 18 : 1 *trans* positional isomers *n*-12/9t, *n*-8t, *n*-7t and *n*-6t were 5·6, 9·0, 1·3 and 2·3, respectively.

Statistical analyses were performed using SPSS v.4.0 for the Macintosh (SPSS Inc, Chicago, IL, USA). Multiple regression, with adjustment for sex, age and BMI where appropriate, was used to estimate the mean difference (95 % CI) in dietary constituents or plasma fatty acids. When necessary, variables were log-transformed to improve the normality and/or equality of variance prior to analysis. Pearson correlation coefficients were calculated to assess the relationship between dietary intake and plasma phospholipid fatty acid composition. Correlations were performed within the butter and margarine users separately because an analysis including all participants tended to cluster butter users around the zero margarine intake and margarine users around the zero butter intake. The resultant highly skewed distributions were not appropriate for correlation analysis.

The study sample size was calculated to detect a minimum difference between butter and table spread consumers of 0·01 weight % in total *trans* isomers in human plasma phospholipids; the assumed population standard deviation was 0·02 (van de Vijver *et al.* 1996). Sixty-three participants were needed in each group to detect a difference greater than or equal to 0·01 weight % with 80 % power and an α level of 0·05. Recruitment took place during April and May of 1996, and by mid June 133 participants (sixty-six butter and sixty-seven margarine users) had completed the study.

Results

Four participants (two butter and two margarine users) reported energy intakes less than 88 % of their BMR and were excluded because they were considered to have underreported their food intake; data from 129 participants were included in the analyses.

Margarine users were, on average, 5 years older, 2·3 kg heavier and 1·2 cm shorter, and had a higher BMI (1·1 kg/m^2), than those who used butter (Table 1). There were more men and fewer women in the margarine group. The mean BMI of male and female participants was 26·1 kg/m^2 (*n* 58) and 25·6 kg/m^2 (*n* 71), respectively.

Butter users consumed more butter per d (32 g) as a spread or in baking and cooking than margarine users did table spread (20 g; Table 2). The energy intake of butter users was 12 % higher (P=0·002) than the intake of margarine users. Protein

Table 1. Participant characteristics
(Mean values and standard deviations)

	Margarine (*n* 65)		Butter (*n* 64)	
Characteristic	Mean	SD	Mean	SD
Women (*n*)	33		38	
Men (*n*)	32		26	
Smokers (*n*)	3		6	
Age (years)	51	16	46	18
Weight (kg)	74·7	15·8	72·4	18·1
Height (cm)	168·0	8·1	169·2	8·9
BMI (kg/m^2)	26·4	4·8	25·3	6·5

For details of subjects and procedures, see p. 378 of proofs.

provided 15 % energy in both butter and margarine users; however, butter users obtained less energy from carbohydrate (4 %kJ, $P=0·001$) and more from total fat (4 %kJ, $P<0·001$). Saturated fat intake was higher (6 %kJ, $P<0·0001$) in butter users, whereas that of polyunsaturated fat was lower (2 %kJ, $P<0·001$) than in participants who used margarine. Monounsaturated fat intake contributed an equivalent percentage energy (11) in butter and margarine users. Similar differences were seen when dietary fat was expressed as a percentage of total fat intake. Accordingly, the dietary polyunsaturated: saturated fat ratio of butter users was less than half the ratio of the diets of margarine users (0·2 *v.* 0·5, $P<0·001$). The amount or proportion of dietary fat from all the food groups except dairy products (including butter) – higher in butter consumers – and fats and oils (includes margarine) – higher in margarine consumers – was similar in butter and margarine users (Table 3).

The mean proportion of total fatty acids as 14 : 0 and 15 : 0 in the plasma phospholipids of butter users was significantly higher ($P\leq0·001$) than that in margarine users (Table 4). There was no difference in 16 : 0; however, butter users showed a lower 18 : 0 level ($P=0·029$). Levels of the MUFA 16 : 1*n*-7 and 18 : 1*n*-9 were significantly lower in the plasma phospholipid of margarine consumers ($P\leq0·027$). Plasma phospholipid linoleate (18 : 2*n*-6) and arachidonate (20 : 4*n*-6) concentrations were higher in margarine than butter consumers ($P\leq0·001$) whereas those of the *n*-3 PUFA 18 : 3*n*-3, 20 : 4*n*-3, 20 : 5*n*-3 and 22 : 5*n*-3 were lower.

Plasma phospholipid 15 : 0 and 14 : 0 composition was significantly correlated with dairy fat as well as saturated fat intake (Table 5) but not with ruminant meat fat ($r-0·11$, $P=0·117$). The 15 : 0 correlations were strongest when fat intake – for example, saturated fat – was expressed as a percentage of total fat ($r0·59$, $P<0·001$) or of total energy ($r0·55$, $P<0·001$) rather than in g/d ($r0·42$, $P<0·001$). The same was true for 14 : 0. The correlation coefficients were identical whether plasma phospholipid fatty acid composition was expressed as weight % or mol %.

Significant differences in the *trans* isomer composition of plasma phospholipids were detected between butter and margarine consumers (Table 6). Only an aggregate mol % composition of the 18 : 1 *trans* positional isomers with the double bond located at the *n*-12, *n*-11, n-10 and *n*-9 positions (18 : 1*n*-12/9t) could be obtained as these isomers co-eluted. *Trans* vaccenic acid (18 : 1*n*-7t) comprised an equivalent proportion of plasma phospholipid fatty acids in both butter and margarine users (0·46 mol %), whereas all the other 18 : 1 *trans* positional isomers – 18 : 1*n*-12/*n*-9t, 18 : 1*n*-8t and 18 : 1*n*-6t – were higher in the plasma phospholipids of margarine compared with butter users ($P<0·001$). Consequently, the total 18 : 1 *trans* fatty acid content was 0·306 mol % ($P<0·001$) higher in margarine than butter users.

18 : 1 *trans* isomers accounted for 68 % and 74 % of the total *trans* fatty acid content of phospholipids in butter and margarine users, respectively. *Trans* vaccenic acid (18 : 1*n*-7t) was the

Table 2. Energy and nutrient composition of diets
(Mean values and standard deviations)

	Margarine (*n* 65)		Butter (*n* 64)		Difference†	
Diet component	Mean	SD	Mean	SD	Mean	95 % CI
			10			
Energy (kJ)	9 227	2 008	486	2 862	1 110*	425, 1 796
Protein (%kJ)	15	3	15	2	0	−1, 1
Carbohydrate (%kJ)	50	7	46	6	−4*	−6, −2
Total Fat (%kJ)	32	5	36	6	4*	2, 7
SAFA (%kJ)	12	3	18	4	6*	5, 7
MUFA (%kJ)	11	2	11	2	0	−1, 1
PUFA (%kJ)	6	1	4	1	−2*	−3, −2
SAFA (%TF)	37	5	49	5	12*	10, 14
MUFA (%TF)	33	3	29	3	−4*	−5, −3
PUFA (%TF)	19	4	11	4	−8*	−10, −7
P:S ratio	0·5	0·2	0·2	0·1	−0·3*	−0·4, −0·2
Alcohol‡ (%kJ)	4	5	4	5	0	0, 0
Cholesterol (mg/d)	237	93	348	133	119*	80, 157
Butter (g/d)	3	3	32	23	30*	25, 36
Margarine (g/d)	20	11	1	2	19*	17, 22

SAFA, saturated fat; TF, total fat; P:S, polyunsaturated:saturated.
Mean values were significantly different; *$P < 0·05$.
† Mean difference by regression analysis, adjusted for sex and age.
‡ Data were transformed for regression analysis; arithmetic mean presented.
For details of subjects and procedures, see p. 378 of proofs.

Table 3. Percentage contribution of food groups to total dietary fat intake

(Mean values and standard deviations)

	Margarine (*n* 65)		Butter (*n* 64)	
Food group	Mean	SD	Mean	SD
Bakery products	13·8	8·6	10·7	8
Dairy	16·5	8·7	43·5***	15·1
Butter	2·8	3·3	25·8***	12·9
Other	13·7	8·3	17·7*	10
Eggs	1·9	2	2·4	3·2
Fast foods	4·7	7·6	5·8	8·5
Fats and oils	25·2	10·7	5·2***	5·9
Margarine	20·4	10·5	0·5***	1·6
Other	4·8	5·3	4·7	5·7
Meat and meat products	19·6	10·9	15·0*	8·7
Nuts	4·1	6·2	4·4	6

Mean values were significantly different from margarine group, adjusted for age and sex; *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.

For details of subjects and procedures, see p. 378 of proofs.

predominant 18 : 1 *trans* isomer in plasma phospholipid, accounting for 50 % of the total 18 : 1 *trans* fatty acid content in butter consumers and 37 % in margarine consumers. Overall, the total *trans* fatty acid content (16 : 1, 18 : 1 and 18 : 2 *trans* isomers) of plasma phospholipids in butter users was 0·318 mol % lower than in margarine users ($P<0·001$ for difference), which was almost entirely explained by the difference in the 18 : 1 *trans* isomer content (0·306 mol %).

Three 18 : 2 isomers containing a *trans* double bond were identified in plasma phospholipids. Concentrations of both the 18 : 2ct3 and 18 : 2ct4 isomers were higher in the plasma phospholipids of margarine than butter users, although the magnitude of the difference was small (adjusted mean differences 0·010 mol %, $P<0·001$ and 0·021 mol %, $P=0·003$; respectively). In contrast, the 18 : 2ct2 isomer level was higher in the phospholipids of butter compared with margarine users (0·033 mol % *v.* 0·023 mol %, $P<0·001$). The sum of all 18 : 2ct isomers was no different between the two groups.

The relationship between plasma phospholipid 18 : 1 and 18 : 2 *trans* isomers, and the percentages of total fat from butter (in butter users only) and margarine (in margarine users only) are shown in Fig. 1. Levels of all 18 : 1 *trans* positional isomers in plasma phospholipid were positively related to the percentage of dietary fat from margarine in margarine users. Margarine intake was more strongly associated with the phospholipids 18 : 1*n*-6t, 18 : 1*n*-8t and 18 : 1*n*-12/*n*-9t isomers (r 0·57–0·63, $P<0·001$) than with 18 : 1*n*-7t (r 0·30, $P=0·016$). There was no correlation in butter users between the percentage of fat from butter and the phospholipid 18 : 1 *trans* positional isomers (r 0·13–0·24, $P=0·290$–0·058). Phospholipid 18 : 2ct2 was positively associated with the percentage of dietary fat from butter in butter users (r 0·41, $P=0·001$) but unrelated to the percentage of dietary fat from margarine in margarine users (r 0·05, $P=0·714$).

Aside from butter and margarine, fat from ruminant meat, meat and meat products, bakery products, vegetable oils (some of which could be 'brush hydrogenated'), fast foods and dairy products other than butter are potential dietary sources of *trans* fatty acids. The percentage of fat from these food groups was not significantly related to 18 : 1 or 18 : 2 *trans* isomers in the plasma phospholipids of margarine or butter users after controlling for margarine and butter intake, respectively (data not shown).

Discussion

Home use of margarines made with partially hydrogenated vegetables oils is a source of *trans* fatty acids, but it is difficult to determine the relative importance of this food choice in determining total intake because, in the absence of a food composition database containing *trans* fatty acid data, the amount of *trans* fatty acid from other food sources, such as manufactured and institutional foods, is difficult to quantify.

Table 4. Plasma phospholipid *cis* fatty acid composition in butter and margarine users

(Values in mol% and standard deviations)

	Margarine (*n* 65)		Butter (*n* 64)		Difference†	
Fatty acid	Mean	SD	Mean	SD	Mean	95 % CI
14 : 0	0·487	0·116	0·623	0·118	0·144*	0·103, 0·184
15 : 0	0·262	0·063	0·348	0·061	0·088*	0·066, 0·110
16 : 0	30·644	1·607	31·105	1·36	0·484	−0·048, 1·016
16 : 1*n*-7	0·646	0·191	0·761	0·272	0·115*	0·033, 0·198
18 : 0	13·114	1·216	12·630	1·132	−0·442*	−0·863, −0·021
18 : 1*n*-9	7·812	1·116	8·912	1·334	1·144*	0·704, 1·584
18 : 2*n*-6	19·282	2·533	17·933	2·609	−1·502*	−2·403, −0·600
18 : 3*n*-3	0·185	0·078	0·276	0·103	0·095*	0·063, 0·128
20 : 3*n*-6	2·616	0·512	2·531	0·418	−0·117	−0·282, 0·048
20 : 4*n*-6	7·438	1·318	6·562	1·295	−0·966*	−1·429, −0·504
20 : 5*n*-3	0·859	0·425	1·244	0·361	0·407*	0·275, 0·538
22 : 0	1·556	0·239	1·455	0·252	−0·098*	−0·185, −0·011
22 : 5*n*-3	0·846	0·180	0·973	0·175	0·149*	0·095, 0·203
22 : 6*n*-3	2·338	0·628	2·359	0·571	0·038	−0·173, 0·249
24 : 0	1·338	0·191	1·301	0·204	−0·034	−0·102, 0·033
24 : 1*n*-9	1·629	0·321	1·765	0·268	0·120*	0·018, 0·222

Mean values were significantly different; *$P<0·05$.

† Mean difference by regression analysis, adjusted for sex and age.

For details of subjects and procedures, see p. 378 of proofs.

Table 5. Correlation coefficients for the association between plasma phospholipid fatty acids and fat intake

Type of fat	Unit of fat	14 : 0†	15 : 0†	16 : 0
Saturated fat	g/d	0·34	0·42	−0·06 ($P<0·476$)
	% energy	0·45	0·55	−0·03 ($P<0·718$)
	% total fat	0·56	0·59	0·15 ($P<0·100$)
Dairy fat	g/d	0·42	0·52	−0·03 ($P<0·725$)
	% energy	0·49	0·60	0·02 ($P<0·842$)
	% total fat	0·54	0·62	0·09 ($P<0·287$)

† All correlation coefficients for 14 : 0 and 15 : 0 with fat type are $P<0·001$.
For details of subjects and procedures, see p. 378 of proofs.

We have shown, using plasma phospholipid *trans* fatty acid composition as a biomarker of dietary intake, that the consumption of margarine made with partially hydrogenated vegetable oil is an important determinant of *trans* fatty exposure in New Zealand. The diets of participants in the present study were similar in fat content to those reported in the National Nutrition Survey (Skeaff *et al.* 2001), suggesting that our results are applicable generally to adult New Zealanders.

The position of the carbon–carbon double bond in the 18 : 1 *trans* isomers of partially hydrogenated vegetable oils tends to follow a normal distribution along the molecule, with a predominance around the centre (*n*-6 to *n*-12; Marchand, 1982; Molkentin & Precht, 1996), whereas in butter there is a distinct predominance of the 18 : 1*n*-7 *trans* isomer (Precht & Molkentin, 1995; Wolff, 1995). The higher proportion of 18 : 1*n*-12/9t, 18 : 1*n*-8t and 18 : 1*n*-6t isomers in the plasma phospholipid of margarine consumers is consistent with this difference in the *trans* composition of margarine. Furthermore, margarine consumption explained almost 40 % of the variation in the plasma phospholipid composition of these isomers, whereas butter consumption was not associated with these isomers. Had the majority of partially hydrogenated oils in the New Zealand diet come from food sources other than margarine, we would have expected to find no or only weak associations between margarine intake and *trans* fatty acid status. When the study was conducted, the average *trans* fatty content of New Zealand margarines was between 5·9 and 16·0 weight % of total fatty acids (Ball *et al.* 1993; Lake *et al.* 1996); hard or stick margarines with much higher *trans* composition as well as 'zero *trans*' spreads were not available to consumers. It is possible that in countries where the use of partially hydrogenated vegetable oils in manufactured and institutional foods is higher, the consumption of margarines containing partially hydrogenated fats will be a less important determinant of *trans* fatty acid status.

There was no difference between the butter and margarine groups in the 18 : 1*n*-7t composition of plasma phospholipid, nor was there an association between margarine or butter intake and this isomer in plasma phospholipids. This suggests that the intake of 18 : 1*n*-7t was similar in butter and margarine consumers, and that home use of butter or margarine was not a major source of this isomer relative to other foods. 18 : 1n-7t was the *trans* isomer present in the highest proportion in plasma phospholipid, but we found no correlation between it and the intake of fat from any of the food groups. The most likely explanation is that dairy and other ruminant fats are commonly used in most manufactured foods and intake is evenly spread across the food groups. There is some evidence that 18 : 1*n*-7t (Δ11) can be desaturated in essential fatty acid-deficient animals to $18:2\Delta^{9\text{-}cis}\Delta^{11\text{-}trans}$ (Mahfouz *et al.* 1980; Pollard *et al.* 1980); if this occurred in man, it would attenuate the association between the dietary intake and plasma composition of this fatty acid; however, using ^{2}H-labelled 18 : 1i-7t, Emkem *et al.* (1986) found no evidence of bioconversion.

Consistent with results reported by others (Wolk *et al.* 1998; Smedman *et al.* 1999) we confirm that plasma phospholipid 15 : 0 and 14 : 0 are good biomarkers of dairy fat intake, with 15 : 0 appearing to be marginally better.

Butter was not a determinant of *trans* MUFA but was correlated weakly with 18 : 2ct2 in plasma phospholipid. This isomer is most probably one of the group of isomers comprising conjugated linoleic acid. Conjugated linoleic acid is consumed in small quantities, but its higher content in butter (Jensen, 2002) relative to other foods is consistent with the association we found.

Table 6. Positional and geometrical fatty acid isomers of plasma phospholipids
(Values in mol% and standard deviations)

	Margarine (*n* 65)		Butter (*n* 64)		Difference†	
Fatty acid	Mean	SD	Mean	SD	Mean	95 % CI
16 : 1*n*-7t	0·063	0·031	0·069	0·023	0·007	−0·003, 0·017
18 : 1*n*-12/9t	0·374	0·120	0·230	0·036	−0·138	−0·169, −0·106
18 : 1*n*-8t	0·145	0·043	0·091	0·015	−0·052*	−0·063, −0·040
18 : 1*n*-7t	0·455	0·129	0·456	0·085	0·003	−0·036, 0·043
18 : 1*n*-6t	0·266	0·102	0·140	0·033	−0·120*	−0·148, −0·093
Sum 18 : 1t isomers	1·240	0·344	0·918	0·130	−0·306*	−0·399, −0·213
18 : 2ct1§	0·210	0·029	0·209	0·024	0·001	−0·008, 0·011
18 : 2ct2§	0·023	0·009	0·033	0·014	0·011*	0·007, 0·015
18 : 2ct3§	0·038	0·015	0·028	0·009	−0·010*	−0·015, −0·006
18 : 2ct4§	0·110	0·042	0·088	0·031	−0·021*	−0·034, −0·007
Sum C18 : 2t isomers	0·381	0·065	0·358	0·053	−0·019	−0·040, 0·003
Sum *trans* isomers	1·684	0·387	1·345	0·161	−0·318*	−0·423, −0·212

Mean values were significantly different; *$P < 0·05$.
† Mean difference by regression analysis, adjusted for sex and age.
‡ May include the 18 : 1*n*-12 to *n*-12 to *n*-10 cis isomers.
§ Position of double bonds unknown.

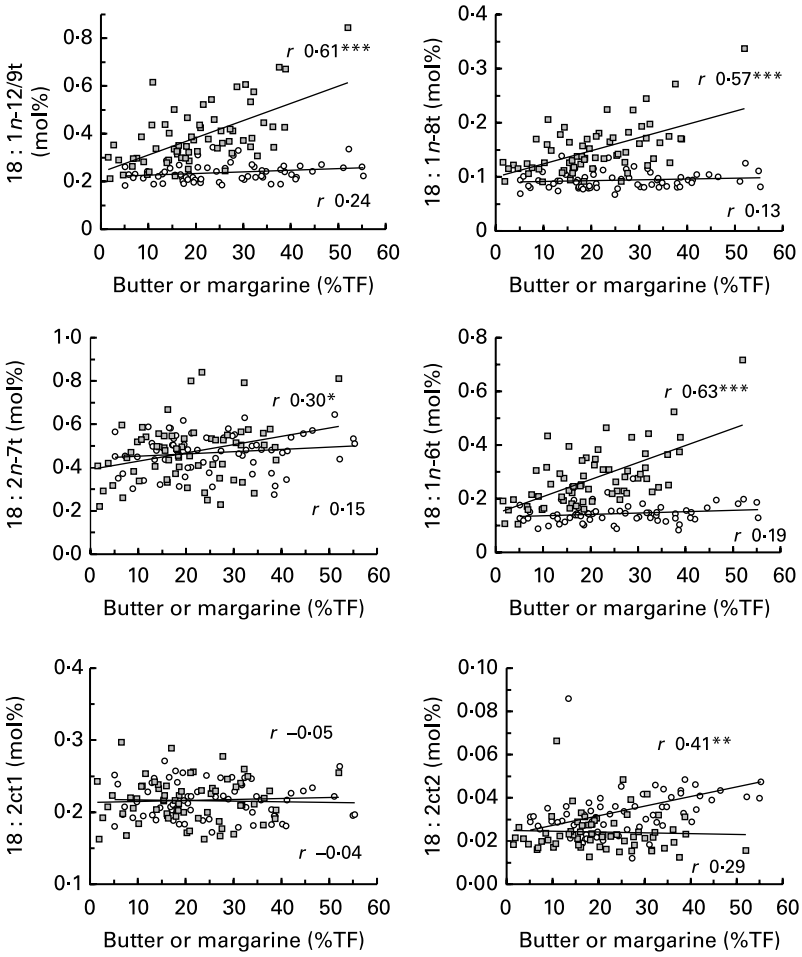

Fig. 1. Relationship between margarine or butter intake and *trans* isomeric composition of plasma phospholipid. ■, margarine consumers; ○, represent butter consumers. %TF, percentage total fat. *$P<0·05$, **$P<0·01$, ***$P<0·001$.

The total *trans* fatty acid content in the plasma phospholipids of butter users in the present study was 1·345 mol %. This is remarkably similar to findings from a controlled feeding trial by Vidgren *et al.* (1998), in which the total *trans* fatty acid content of plasma phospholipids from participants consuming a diet with 1·4 %kJ from *trans* fatty acids was 1·25 mol %; the similarity is likely to be even closer because 16 : 1*n*-7t, the value for which was 0·07 mol % in our study, was not measured by Vidgren *et al*. In that study, the increase in the total *trans* fatty acid composition of plasma phospholipid when changing to a higher *trans* fatty acid diet (5·5 %kJ) was directly proportion to the increase in dietary *trans* intake. Thus, on the basis of the similarity in the *trans* fatty acid content of plasma phospholipids between Vidgren *et al.*'s study and ours, butter users in our study were probably consuming around 1·4 %kJ (4 g/d) as *trans* fatty acids. We estimated from the dietary records that dairy fat (43·5 % total fat intake; Table 3) contributed 2·6 g/d of *trans* fat – assuming a *trans* fatty acid composition of dairy fat of 5·9 weight % (Lake *et al.* 1996). Therefore, in the butter group, the remainder of the diet probably contained no more than 1·4 g/d *trans* fatty acids. The proportion of this *trans* fat derived from animal or plant sources is difficult to estimate.

Obviously, the easiest way to reduce *trans* fatty acid intake is to avoid butter or margarines made with partially hydrogenated plant oils. However, using a fat for spreading and in home baking and cooking is a habit not easily changed in many countries. For this reason, we have previously shown that, in people with a slightly raised plasma cholesterol level, a low-fat diet in which butter is replaced with a polyunsaturated and monounsaturated-rich margarine, despite the latter containing 13 % *trans* fatty acids, lowers plasma LDL-cholesterol (Chisholm *et al.* 1996). The relevance of this finding was that low-*trans* fatty acid margarines, at the time the only ones available in New Zealand, were appropriate for cholesterol-lowering diets despite containing more *trans* fatty acids than butter. However, our current results indicate that if there are untoward effects of *trans* fatty acids, other than on plasma lipoproteins, it would be preferable to use a margarine with no *trans* fatty acids. These margarines are now available, although they were not at the time we conducted the study.

We have used a biological marker of *trans* fatty acid intake to show that the consumption of *trans* fatty acid-containing margarines is a major determinant of *trans* fatty status. This result provides persuasive – albeit indirect – evidence that the use of partially hydrogenated vegetable oils in other manufactured foods at the time of our study was not high in New Zealand. A similar biomarker approach may be useful in other countries where the amount of partially hydrogenated vegetable oils in manufactured foods is unknown.

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