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Elaidic and *trans*-vaccenic acids in plasma phospholipids as indicators of dietary intake of 18:1 *trans*-fatty acids

Tuulikki Seppänen-Laakso[a,*], Into Laakso[a], Peter Backlund[b], Hannu Vanhanen[c], Jorma Viikari[d]

[a]*Department of Pharmacy, P.O. Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland*
[b]*Department of Organic Chemistry, Åbo Akademi University, Turku, Finland*
[c]*Second Department of Medicine, University of Helsinki, Finland*
[d]*Department of Medicine, University of Turku, Turku, Finland*

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Abstract

Octadecenoic (18:1) *trans*-fatty acid fractions from margarine, butter and plasma phospholipids (PL) were isolated by silver ion TLC, and nine positional isomers (*n*-11–*n*-3) were identified by GC–MS based on their ozonolysis products. The GC analysis of the isolated fractions gave similar peak profiles and separated seven *trans*-isomers (*n*-11–*n*-6 and *n*-3). Without a preceding isolation step, the reproducibility of the GC method for plasma PL elaidic (18:1 *n*-9 *trans*) and *trans*-vaccenic acids (*n*-7) was 3.4 and 2.7% (R.S.D.), respectively. These *trans*-isomers were rapidly incorporated and cleared in plasma PL and they closely reflected both increased and decreased intake of 18:1 *trans*-fatty acids during moderate fat substitutions. Significant associations between high-density lipoprotein cholesterol (HDL-C) and PL elaidic and *trans*-vaccenic acids appeared in habitual margarine users only.

Keywords: Dietary indicators; Elaidic acid; *trans*-Vaccenic acid; Phospholipids; Fatty acids

1. Introduction

Dietary fat usually contains varying amounts of *trans*-fatty acids, especially octadecenoic acid (18:1) isomers, which originate from partially hydrogenated vegetable oils, i.e. margarines and shortenings, but also from milk and milk fat products [1–4]. *Trans*-fatty acids are well absorbed by the body, and long-term intake is reflected in the fatty acid composition of adipose tissue [1]. Recent studies indicate that also human milk can contain considerable amounts of *trans*-isomers [4]. *Trans*-fatty acids may have an adverse impact on essential fatty acid metabolism [5,6] and on serum lipid levels by increasing the low-density (LDL-C) or decreasing the high-density lipoprotein cholesterol (HDL-C) concentrations [7–10], although these effects have not been consistently found [11]. In addition, the possible link between *trans*-fatty acid intake and the risk of coronary heart diseases is still equivocal [12,13].

The analysis of 18:1 *trans*-isomers requires their isolation by silver ion TLC ($AgNO_3$-TLC) or HPLC [3,14–17]. The double-bond distribution is then determined after ozonolysis [3,17,18] by identifying the reaction products by GC and MS. Without a

*Corresponding author.

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preceding isolation of 18:1 *trans*-fraction only a part of the isomers can be separated by a single GC run due to the overlapping by 18:1 *cis*-isomers [1,14,19,20]. Usually, long capillary columns with highly polar phases and varying oven temperature conditions are used [1,3,14,17,19,20].

Fatty-acid composition of plasma phospholipids (PL) offers several advantages over other lipid fractions when monitoring changes during replacement of saturated fat with vegetable oils. It reflects not only changes in the essential linoleic and α-linolenic acids and their long-chain metabolites but also interactions between unsaturated fatty acid families and relationships with serum lipid levels [21,22]. When evaluating the effects of *trans*-fatty acids in relation to other fatty acids their determination in plasma PL becomes especially important.

In this study, the 18:1 *trans*-isomers from margarine, butter and plasma PL were isolated and identified. The GC conditions were optimized for the separation of single *trans*-isomers. A direct GC method, without prior isolation of the *trans*-fraction, was applied in the determination of PL elaidic (18:1 *n*-9 *trans*) and *trans*-vaccenic acid (*n*-7) levels during moderate fat substitutions including an increase or a decrease in 18:1 *trans*-fatty acid intake.

2. Experimental

2.1. Subjects and substitutions

The subjects were Finnish men and women of working age, who were butter ($n=23$) or margarine users ($n=23$). The groups were similar as regards age (mean 45 yr), gender, energy and fat intake and serum lipid levels (Table 1). During a 6-week substitution period, butter on bread was replaced by margarine containing 22% 18:1 *trans*-fatty acids (Table 2). The other group replaced margarine with olive oil (no *trans*-fatty acids). During the post-experimental period (6 weeks) the subjects returned to using butter or margarine on bread. No other changes were made in the diet. The control group ($n=11$) continued their habitual diet during the 12-week trial. Plasma samples were taken at the baseline, after 3 and 6 weeks substitution and at the end of the study. Substitution was also followed by dietary questionnaires, 3 day diaries and total plasma fatty-acid analyses [21,22].

Table 1
Daily fat consumption, serum lipid levels and PL fatty acid composition at the baseline

	Butter users 11M/12F[a]	Margarine users 13M/10F	Controls 5M/6F
Energy intake (MJ)	8.5	9.0	8.4
Fat intake (E-%)[b]	37.6	38.5	36.4
Fat on bread (E-%)	7.3	9.2	9.7
% of fat	19.4	23.2	26.4
Serum lipids (mmol/l)[c]			
TC	6.2	6.3	6.1
HDL-C	1.5	1.6	1.6
TG	0.9	1.0	0.9
PL fatty acids (%)[d]			
SaFA	45.3	44.4 ↓↓↓	44.4
MUFA	14.4	14.1	14.1
18:1	13.7	13.4	13.3
PUFA	40.4	41.6 ↑↑	41.6

t-test: ↓↓↓, $p<0.001$; ↑↑, $p<0.01$ compared to butter users.
[a] M=male; F=female.
[b] E-%=proportion of total energy intake.
[c] TC=total cholesterol, TG=serum triacylglycerols.
[d] SaFA: 14:0, 16:0, 18:0; MUFA: 16:1 *n*-7, 18:1 *n*-9.
PUFA: 18:2 *n*-6, 18:3 *n*-6, 20:4 *n*-6, 18:3 *n*-3, 20:5 *n*-3, 22:5 *n*-3, 22:6 *n*-3.

2.2. Isolation and identification of 18:1 trans-fatty acids

Substitute margarine, butter and pooled plasma PL samples were esterified with sodium methoxide [23] and applied on $AgNO_3$-impregnated (10%) Kieselgel 60 TLC-plates (Merck, Darmstadt, Germany). The 18:1 *trans*-band was scraped off and extracted with hexane–chloroform (1:1, v/v) [14]. The purity and isomeric profiles of the fractions were determined by GC on an SP-2340 column (Fig. 1A).

In order to determine the double-bond positions of 18:1 *trans*-fatty acids, the isolated fractions were ozonised by bubbling [18] the samples for 2 min in 1.2% ozone (4.8 mg/sample) developed by an ozonizator (Gebr. Herrman, Köln, Germany). The products were then methylated with diazomethane and analyzed by GC–MS using a Hewlett-Packard (HP) 5890 GC equipped with an NB-54 fused-silica capillary column (15 m×0.20 mm I.D.) (Nordion, Helsinki, Finland) and interfaced with an HP 5970A

Table 2
Fatty acid compositions of butter, common margarines and the substitute fats

Fatty acid (%)	Fat on bread before substitution		Substitute fats	
	Butter[a]	Margarines[b,c]	Olive oil[b]	Margarine[a]
Saturated				
Σ 10:0–24:0	69.7	24.1	13.9	20.6
Monounsaturated				
Σ 16:1, 20:1–24:1	1.9	0.9	1.1	1.0
Σ 18:1 *cis*	22.6	31.1	74.6	25.3
n-9	22.2	28.9	73.1	22.5
n-7	0.4	1.9	1.5	1.9
n-6	tr	0.3	–	0.9
Σ 18:1 *trans*	3.6	8.1	–	22.2
n-11	tr	tr	–	tr
n-10	0.1	1.6	–	3.2
n-9	0.3	2.4	–	4.7
n-8	0.3	2.1	–	4.6
n-7	1.2	2.0	–	3.9
n-6	0.3	-[d]	–	2.8
n-5+*n*-4	1.1	-[d]	–	2.6
n-3	0.3	-[e]	–	0.4
Polyunsaturated				
Σ 18:2, 18:3, 20:2	2.2	35.8	10.3	30.9

[a] Percentages of 18:1 *trans*-fatty acids are based on GC analysis of fatty acid methyl esters (FAME) on SP-2340 column after isolation by $AgNO_3$-TLC (cf. Fig. 1).
[b] Direct GC analysis of FAMEs.
[c] Mean of five different margarines.
[d] Co-eluted with 18:1 *n*-9 *cis*.
[e] Co-eluted with 18:1 *n*-7 *cis*.

MS detector operating in electron impact mode (70 eV). Pure elaidic acid (Sigma, St. Louis, MO, USA) was also ozonised to confirm the elution order and spectra of the four major products: nonanal, nonanoic acid, nonanoic acid aldehyde and nonanoic diacid.

Among the reaction products in the isolated and ozonised *trans*-fractions, the homologous series of dicarboxylic acid methyl esters (Table 3) was chosen to describe the double-bond distributions of positional 18:1 *trans*-isomers in the substitute margarine, butter and plasma PL samples (Fig. 1B).

2.3. GC separation of PL 18:1 isomers

Plasma PL fatty-acid compositions (other than *trans*-fatty acids) have been described earlier [21,22]. For this study, the samples were reanalysed on an SP-2340 column, and the percentages of the 18:1 isomers (Table 4 and Table 5) were calculated from the proportions of 18:1 fatty acids in the total PL fatty-acid analyses. The total phospholipids had been separated from EDTA-plasma samples, 0.5 ml of each, on Kieselgel $60F_{254}$ HPTLC plates. PL fractions (without TLC-isolation of the *trans*-isomers) were esterified [23] and 2-μl samples were taken for GC analyses (Fig. 2).

The GC runs were performed on an SP-2340 fused-silica capillary column (60 m×0.25 mm I.D., Supelco, Gland, Switzerland). A DANI 3865 GC equipped with a programmed temperature vaporizing injector (PTV) was used in solvent split sampling mode (split ratio 50:1). This technique, which allows elimination of the major part of the solvent through the split valve before injector heating, is used for dilute samples and when the boiling points of the solvent and solute differ clearly from each other [24]. After manual injection (PTV at 70°C) the split

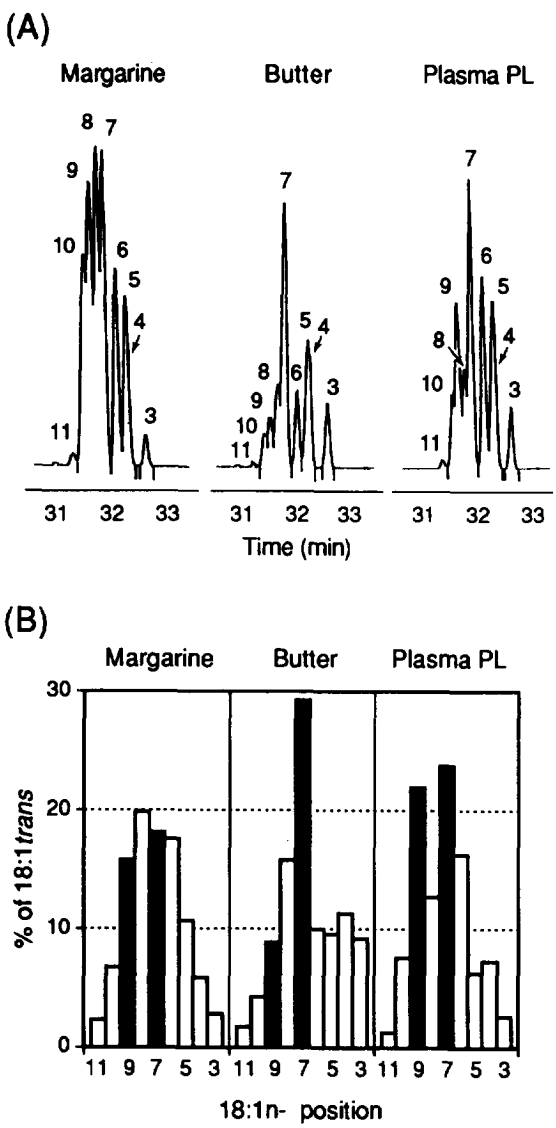

Fig. 1. (A) GC analysis of $AgNO_3$-TLC isolated 18:1 *trans*-fractions from margarine, butter and pooled plasma PL samples on an SP-2340 capillary column. Other GC conditions were as described in the Experimental section. Peak numbers refer to the double-bond positions from *n*-11 to *n*-3. (B) Double-bond distributions obtained after ozonolysis of the same samples. The 18:1 *n*-9 and *n*-7 *trans*-isomers are indicated by black bars.

valve was open for 6 s, then closed (and PTV rapidly heated to 235°C) and opened again after 60 s. A two-step oven temperature programme from 70°C (at 7°C/min) to 150°C and then to 180°C (at 1°C/min) was used. The carrier gas (hydrogen) flow was 0.6 ml/min and the detector (FID) temperature was 235°C.

3. Results

3.1. Identification of 18:1 trans-isomers

The GC analyses of the $AgNO_3$-TLC isolated *trans*-fractions from margarine, butter and plasma PL gave eight peaks (Fig. 1A) while GC–MS analysis of dicarboxylic acid methyl esters resulted in nine positional isomers after ozonolysis (*n*-11–*n*-3) (Fig. 1B). The homologous series of these esters was best separated from the other products, and characteristic fragments were found at higher mass range also ($m/z>150$) (Table 3). Instead of a molecular ion [M^+], fragments corresponding to losses of methoxy [M^+-31] and hydroxyl groups [M^+-17] were detected through the whole series.

The isomeric profiles obtained by GC and ozonolysis indicated that 18:1 *n*-5 and *n*-4 *trans*-isomers were coeluted in the GC analysis (Fig. 1). The isomeric pattern in margarine resembled the normal distribution, whereas in butter *trans*-vaccenic acid (*n*-7) was predominant. Among the *n*-11–*n*-7 *trans*-isomers, elaidic (*n*-9) and *trans*-vaccenic acids were most abundant in plasma PL.

The relative amounts of all 18:1 *trans*-isomers accounted for 22.2% of total fatty acids in substitute margarine and 3.6% in butter (Table 2). The proportion of *n*-11–*n*-7 isomers obtained by GC without prior $AgNO_3$-TLC isolation was 16.4 and 1.9%, respectively. Common Finnish margarines ($n=5$) contained 8% of *n*-11–*n*-7 *trans*-isomers, on the average.

3.2. GC analysis of PL 18:1 isomers

Direct analyses of plasma PL samples from a dietary study were performed without $AgNO_3$-TLC isolation of the *trans*-fraction. Typical GC chromatograms of the PL samples of a margarine (A) and a butter user (B) are shown in Fig. 2. Elaidic and *trans*-vaccenic acids were separated from *n*-10 and *n*-8 *trans*- and from all *cis*-isomers. The major compound, oleic acid (*n*-9 *cis*), overlapped the *n*-6–*n*-4 *trans*-isomers. *Cis*-vaccenic acid (*n*-7; 1.67%)

Table 3
Characteristic GC–MS fragments (*m/z*) of dicarboxylic acid methyl esters

Chain length[a]	Double-bond position in 18:1 *trans*-fatty acid	Base peak	$[M^+ - OCH_3]$	$[M^+ - OH]$	Other fragments
15	*n*-3	98	269	283	55, 69, 112, 241
14	*n*-4	98	255	269	55, 69, 112, 227
13	*n*-5	98	241	255	55, 112, 149, 167, 213
12	*n*-6	98	227	241	55, 153, 199
11	*n*-7	98	213	227	55, 139, 69
10	*n*-8	55	199	213	125, 98
9	*n*-9	55	185	199	152, 83, 111
8	*n*-10	55	171	185	138, 83, 111
7	*n*-11	55	157	171	125, 83

[a] Number of carbon atoms.

was completely resolved from oleic acid but it coeluted with the *n*-3 *trans*-isomer (<0.1%). About two thirds of the total amount of 18:1 *trans*-isomers in plasma PL could be determined by the direct GC analysis.

Reproducibility of the method including lipid extraction, PL isolation by TLC, esterification and GC run is given in Table 4. Among the 18:1 *trans*-isomers, R.S.D. for elaidic (*n*-9) and *trans*-vaccenic acids (*n*-7) was 3.4 and 2.7%, respectively. For these isomers, the proportion of methodological variation relative to total variation in a group of 57 subjects was 1.5 and 1.1%, respectively (Table 4).

3.3. Fat substitution and PL 18:1 isomers

Before dietary experiments, margarine users had higher proportions of elaidic acid and 18:1 *n*-6 *cis*-isomer in plasma PL compared to butter users ($p<0.01$ and $p<0.001$, respectively) (Table 5). The levels of *trans*- and *cis*-vaccenic acids did not differ between these groups. In margarine users, but not in butter users, the baseline levels of PL elaidic and *trans*-vaccenic acids were inversely associated with those of HDL-C ($r=-0.46$, $p<0.05$; $r=-0.62$, $p<0.01$, respectively) and HDL-C/TC ratio ($r=-0.57$, $p<0.01$; $r=-0.62$, $p<0.01$, respectively).

During replacement of butter by margarine the average intake of 18:1 *n*-11–*n*-7 *trans*-isomers derived from substitute margarine was 3.7 g/day, accounting for about 4.3% of total fat and 1.7% of total energy intake. The proportions of elaidic and *trans*-vaccenic acids and the *n*-6 *cis*-isomer in PL increased significantly during the first 3 weeks and remained stable for the next 3 weeks (Table 5). The levels returned to the baseline during the post-experimental period. No associations between the changes in these isomers and serum lipids were found.

When common margarines (Table 2) were re-

Table 4
Reproducibility of the method for minor 18:1 fatty acid isomers in plasma PL

Fatty acid	Repeated analyses ($n=6$)		Subjects ($n=57$)			Methodological variation/ total variation (%)[a]
	Mean (%)	R.S.D. (%)	Range (%)	Mean (%)	R.S.D. (%)	
18:1 *n*-10 *trans*	0.21	8.7	–	–	–	–
18:1 *n*-9 *trans*	0.51	3.4	0.09–0.66	0.29	49.1	1.5
18:1 *n*-8 *trans*	0.19	6.8	–	–	–	–
18:1 *n*-7 *trans*	0.54	2.7	0.15–0.77	0.40	34.0	1.1
18:1 *n*-7 *cis*	1.48	0.3	1.33–2.28	1.67	12.5	0.1
18:1 *n*-6 *cis*	0.23	3.0	0.06–0.61	0.23	53.8	4×10^{-3}

[a] Based on variances for corresponding isomers in repeated and population analyses.

Table 5
Proportions of PL elaidic, *trans*-vaccenic, oleic and *cis*-vaccenic acids and 18:1 *n*-6 *cis*-isomer in the substitution and control groups

Fatty acid (mean ± S.D.)	Baseline	Substitution period		
		3 weeks	6 weeks	12 weeks
Butter→margarine, $n=23$				
18:1 *n*-9 *trans*	0.22±0.11	0.35±0.12↑↑↑	0.35±0.11↑↑	0.22±0.10
18:1 *n*-7 *trans*	0.38±0.11	0.47±0.09↑↑	0.47±0.13↑	0.37±0.12
18:1 *n*-9 *cis*	11.36±1.01	10.63±0.96↓↓	10.36±1.52↓↓	11.21±1.75
18:1 *n*-7 *cis*	1.57±0.18	1.63±0.14↑↑	1.54±0.15	1.50±0.19↓
18:1 *n*-6 *cis*	0.14±0.06	0.19±0.07↑↑	0.19±0.06↑	0.15±0.07
Margarine→olive oil, $n=23$				
18:1 *n*-9 *trans*	0.35±0.17[a]	0.25±0.11↓↓	0.28±0.11↓	0.40±0.16↑
18:1 *n*-7 *trans*	0.41±0.16[b]	0.31±0.10↓	0.33±0.11↓	0.44±0.11
18:1 *n*-9 *cis*	10.70±1.13[c]	11.92±1.06↑↑↑	12.06±1.10↑↑↑	10.31±1.19
18:1 *n*-7 *cis*	1.69±0.20	1.65±0.23	1.66±0.22	1.52±0.21↓↓
18:1 *n*-6 *cis*	0.28±0.12[d]	0.15±0.08↓↓↓	0.20±0.11↓	0.18±0.07↓↓↓
Control group, $n=11$				
18:1 *n*-9 *trans*	0.31±0.08	0.33±0.12	0.37±0.13	0.34±0.10
18:1 *n*-7 *trans*	0.41±0.13	0.39±0.10	0.40±0.12	0.46±0.11
18:1 *n*-9 *cis*	10.62±1.10	10.99±1.45	10.08±1.02	12.01±1.54↑↑
18:1 *n*-7 *cis*	1.81±0.21	1.77±0.18	1.68±0.21	1.88±0.33
18:1 *n*-6 *cis*	0.19±0.06	0.20±0.06	0.18±0.02	0.22±0.11

Paired *t*-test: ↑↑↑, ↓↓↓ $p<0.001$; ↑↑, ↓↓, $p<0.01$; ↑, ↓, $p<0.05$ compared to the baseline.
[a] *t*-test: $p<0.01$, compared to butter users; correlation with HDL-C concentration: $r=-0.46$, $p<0.05$.
[b] Correlation with HDL-C concentration: $r=-0.62$, $p<0.01$.
[c] *t*-test: $p<0.05$, compared to butter users.
[d] *t*-test: $p<0.001$, compared to butter users.

placed by olive oil free from *trans*-fatty acids, the proportions of PL elaidic and *trans*-vaccenic acids decreased during the first 3 weeks (Table 5), remained low until the end of substitution and increased when the subjects started to use margarine in their home diet. The level of 18:1 *n*-6 *cis*-isomer followed the same trend during substitution but was further decreased at the end of the study. In this group also the changes in these isomers were not associated with those in serum lipids. In both substitution groups, *cis*-vaccenic acid showed temporary changes only.

The control group resembled more margarine than butter users in respect to the PL elaidic acid level but no significant changes occurred during the study (Table 5).

4. Discussion

Determination of *trans*-fatty acids in edible fats and biological tissues is important due to their possible adverse effects on serum lipid levels [7–10]. GC is a suitable and reproducible technique in the follow-up of fatty acid compositions of plasma lipid fractions. However, when *cis*-isomers are present, the separation of 18:1 *trans*-isomers is difficult, and, therefore, they are often presented as an unresolved group only. In this study, optimized GC conditions were used in the separation of single 18:1 *trans*-isomers. The isomeric patterns of 18:1 *trans*-fractions obtained with or without ozonolysis were compared, and a direct GC method was applied in the determination of elaidic and *trans*-vaccenic acid levels in plasma PL during fat substitution.

As in classical GC techniques [19], a slow oven temperature rise of 1°C/min from 150°C was most suitable for the SP-2340 column. Improved separation of the isomers was most likely due to the injection at 70°C followed by programmed temperature vaporization, since the effects of split or solvent split samplings were slight only. GC analyses of the TLC-isolated 18:1 *trans*-fractions gave isomeric profiles which were very similar to those calculated

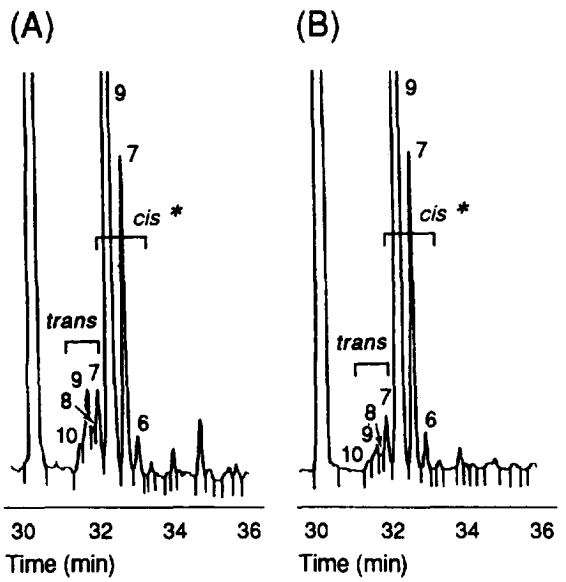

Fig. 2. Direct GC analysis of 18:1 isomers from plasma PL of a margarine (A) and a butter user (B) on an SP-2340 capillary column. Other GC conditions were as described in the Experimental section. Peak numbers show the double-bond positions of the 18:1 *trans*- and *cis*-isomers. * – 18:1 *n*-9 *cis* contains also *n*-5 and *n*-4 *trans*-isomers, and 18:1 *n*-7 *cis* traces of *n*-3 *trans*-isomer.

from their ozonolysis products (Fig. 1). Thus, most of the isomers can be reliably determined from these fractions without ozonolysis. From the nine identified compounds *n*-5 and *n*-4 isomers were coeluted only. The isomeric distribution in plasma PL showed that the *n*-10–*n*-7 *trans*-isomers from margarine are not reflected in PL as such (Fig. 1A, Fig. 2A). The lower levels of *n*-10 and *n*-8 isomers in PL have been explained by discrimination occurring during incorporation of *trans*-fatty acids into tissue PL [25–27]. In PL analysis, the GC separation of elaidic and *trans*-vaccenic acids is apparently less affected by these interfering isomers than in the analysis of neutral lipid fractions.

Repeated analyses showed that elaidic and *trans*-vaccenic acids can be reliably determined in plasma PL without preceding isolation of 18:1 *trans*-fraction (mean R.S.D. 3.4 and 2.7%, respectively). Although the relative amounts of these isomers are very low (<0.5%), the proportion of methodological variation out of population variation ranged from 1.0 to 1.5% only (Table 4). This is even less than the corresponding value for α-linolenic acid (3.2%), the dietary precursor of *n*-3 fatty acids, appearing in similar proportions (0.5%) in plasma PL [21–23]. The direct GC method is also reproducible for the determination of *cis*-vaccenic acid (R.S.D. 0.3%). This fatty acid, which is usually accompanied with oleic acid, is common in natural lipids, but its role is largely unknown [1]. In this study, *cis*-vaccenic acid did not give any response at plasma PL level during olive oil substitution (Table 5), while oleic acid well reflected the use of oil [22]. This suggests that clear differences exist in the incorporation of these 18:1 *cis*-isomers in plasma PL.

Fat substitutions showed that elaidic and *trans*-vaccenic acids were effectively and rapidly incorporated and cleared in plasma PL. A significantly higher proportion of elaidic acid was characteristic to habitual margarine users (Table 5). However, a 3-week moderate margarine substitution in butter users was sufficient to increase the relative amount of PL elaidic acid to the same level. Despite the relatively high proportion of *trans*-fatty acids in the substitute margarine used in this study, the total and LDL-C levels decreased significantly [21]. Our earlier data also showed that a decrease in PL stearic acid and an increase in linoleic acid levels were related to the decrease in serum cholesterol levels. The results of this group suggest that the effects of 18:1 *trans*-fatty acids derived from moderate margarine intake remain clearly minor when compared to those of major dietary fatty acids. It can be expected that the importance of margarines as sources of *trans*-fatty acids will be further decreased due to the use of interesterification in margarine manufacturing.

When margarine was replaced by olive oil, the levels of PL elaidic and *trans*-vaccenic acids closely followed the dietary change (Table 5) showing that these isomers are also good markers when the intake of *trans*-fatty acids is reduced. That no significant relationships between the fall in 18:1 *trans*-fatty acids and serum lipids existed, was most probably due to the low degree of fat substitution. However, additional sources of *trans*-fatty acids in habitual diets of margarine users may contribute to the inverse correlation between HDL-C and PL elaidic ($p<0.05$) and *trans*-vaccenic acids ($p<0.01$) found at the baseline (Table 5). A similar relationship has also been reported between serum HDL-C concentration and the dietary intake of *trans*-fatty acids [9].

The results demonstrate that GC analysis of individual 18:1 *trans*-isomers from TLC isolated

18:1 *trans*-fractions of dietary fats and plasma PL gives corresponding isomeric profiles as obtained by ozonolysis. The direct GC method is applicable for the determination of elaidic and *trans*-vaccenic acid levels in plasma PL during fat substitutions. PL elaidic and *trans*-vaccenic acids proved to be reliable indicators for increased or decreased intake of 18:1 *trans*-fatty acids. They can be well used for controlling the reduction of *trans*-fatty acids in the diet in order to avoid possible HDL-C lowering effects.

References

[1] L.C. Hudgins, J. Hirsch and E.A. Emken, Am. J. Clin. Nutr., 53 (1991) 474.
[2] J.E. Hunter and T.H. Applewhite, Am. J. Clin. Nutr., 54 (1991) 363.
[3] J.L. Sebedio, A. Bonpunt, J. Prevost and A. Grandgirard, Fat Sci. Technol., 96 (1994) 235.
[4] Z.Y. Chen, G. Pelletier, R. Hollywood and W.M.N. Ratnayake, Lipids, 30 (1995) 15.
[5] R.T. Holman, in A. Sinclair and R. Gibson (Editors), Essential Fatty Acids and Eicosanoids, American Oil Chemists Society, Champaign, IL, 1992, pp. 3–17.
[6] B. Koletzko, Acta Paediatr., 81 (1992) 302.
[7] R.P. Mensink and M.B. Katan, N. Engl. J. Med., 323 (1990) 439.
[8] P.L. Zock and M.B. Katan, J. Lipid Res., 33 (1992) 399.
[9] R. Troisi, W.C. Willett and S.T. Weiss, Am. J. Clin. Nutr., 56 (1992) 1019.
[10] W.C. Willett, M.J. Stampfer, J.E. Manson, G. A Colditz, F.E. Speizer, B.A. Rosner, L.A. Sampson and C.H. Hennekens, Lancet, 341 (1993) 581.
[11] R. Wood, K. Kubena, B. O'Brien, S. Tseng and G. Martin, J. Lipid Res., 34 (1993) 1.
[12] P.M. Kris-Etherton (Editor), *Trans*-fatty acids and coronary heart disease risk, Supplement to Am. J. Clin. Nutr., 62 (1995) 655S–707S.
[13] A. Aro, A.F.M. Kardinaal, I. Salminen, J.D. Kark, R.A. Riemersma, M. Delgado-Rodriquez, J. Gomez-Aracena, J.K. Huttunen, L. Kohlmeier, B.C. Martin, J.M. Martin-Moreno, V.P. Mazaev, J. Ringstad, M. Thamm, P. van't Veer and F.J. Kok, Lancet, 345 (1995) 273.
[14] W.M.N. Ratnayake and J.L. Beare-Rogers, J. Chromatogr. Sci., 28 (1990) 633.
[15] E. Lanza and H.T. Slover, Lipids, 16 (1981) 260.
[16] B. Nikolova-Damyanova, B.G. Herslof and W.W. Christie, J. Chromatogr., 609 (1992) 133.
[17] B. Fell and W. Schäfer, Fat Sci. Technol., 93 (1991) 329.
[18] R.G. Ackman, Lipids, 12 (1976) 293.
[19] H.T. Slover and E. Lanza, J. Am. Oil Chem. Soc., 56 (1979) 933.
[20] P. Bohov, V. Balaz and J. Hrivnak, J. Chromatogr., 286 (1984) 247.
[21] T. Seppänen-Laakso, H. Vanhanen, I. Laakso, H. Kohtamäki and J. Viikari, Br. J. Nutr., 68 (1992) 639.
[22] T. Seppänen-Laakso, H. Vanhanen, I. Laakso, H. Kohtamäki and J. Viikari, Ann. Nutr. Metab., 37 (1993) 161.
[23] T. Seppänen-Laakso, I. Laakso and R. Hiltunen, Acta Pharm. Fenn., 99 (1990) 109.
[24] F. Poy, S. Visani and F. Terrosi, J. Chromatogr., 217 (1981) 81.
[25] J.B. Ohlrogge, R.M. Gulley and E.A. Emken, Lipids, 17 (1982) 551.
[26] G. Rocquelin, L. Guenot, P.O. Astorg and M. David, Lipids, 24 (1989) 775.
[27] E.A. Emken, R.O. Adlof, W.K. Rohwedder and R.M. Gulley, Lipids, 24 (1989) 61.