Influence of Moderate Amounts of *trans* Fatty Acids on the Formation of Polyunsaturated Fatty Acids

Anette Bysted*, **Gunhild Hølmer, and Pia Lund**

Department of Biochemistry and Nutrition, The Technical University of Denmark, DK-2800 Lyngby, Copenhagen, Denmark

ABSTRACT: The effect of *trans* fatty acids from partially hydrogenated soybean oil and butterfat on the formation of polyunsaturated fatty acids was investigated. Five groups of rats were fed diets that contained 20 wt% fat. The content of linoleic acid was adjusted to 10 wt% of the dietary fats in all diets, whereas the amount of *trans* fatty acids from partially hydrogenated soybean oil (PHSBO) was varied from 4.5 to 15 wt% in three of the five diets. The fourth group received *trans* fatty acids from butterfat (BF), while the control group was fed palm oil without *trans* fatty acids. *Trans* fatty acids in the diet were proportionally reflected in rat liver and heart phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol, and phosphatidylserine. Incorporation in the *sn*-1 position was compensated by a decrease in saturated fatty acids. *Trans* fatty acids were not detected in diphosphatidylglycerol. Compared to the presence in the dietary fats, 8*t*- and 10*t*-18:1 were discriminated against in the incorporation in PE and PC from liver and heart, whereas 9*t*- and 12*t*-18:1 were preferred. The formation of 20:4n-6 was not influenced by 4.5 wt% *trans* fatty acids (from PHSBO) but apparently was by 10 wt% in liver. In contrast, even a content of 2.5 wt% *trans* fatty acids from BF reduced the formation of 20:4n-6. The inhibitory effect of *trans* isomers on linoleic acid conversion was reflected less in heart than in liver and less for PE than for PC. Groups with *trans* fatty acids showed increased 22:6n-3 and 22:5n-3 deposition in liver and heart PE and PC.

JAOCS 75, 225–234 (1998).

KEY WORDS: Desaturation, discrimination, docosahexaenoic acid, elongation, incorporation, phosphoacylglycerols, polyunsaturated fatty acids, positional isomers, rats, *trans* fatty acids.

Trans fatty acids are formed industrially during partial hydrogenation of polyunsaturated fatty acids (PUFA) from vegetable and marine oils. Additionally, *trans* fatty acids are formed during biohydrogenation in ruminants. Consequently, dairy products, such as butter and cheese, and fat from these animals contain *trans* isomers; in addition *trans* fatty acids occur in margarines (1–3).

The safety of dietary *trans* fatty acids has been under vigorous discussion during recent years, mainly owing to the results from an epidemiological study reported by Willett *et al.* (4). They found a positive correlation between intake of *trans* fatty acids from partially hydrogenated vegetable oils and risk of coronary heart disease, whereas no correlation to butter consumption was seen in spite of the presence of *trans* isomers in dairy products. The interpretation and the statistical evaluation used in the study have been questioned (5–8). However, other studies (9–14) have also indicated that *trans* fatty acids (like saturated fatty acids) have an unfavorable effect on blood lipids and therefore on the development of coronary heart disease. Because these experiments used relatively high amounts of *trans* fatty acids in the diets, the effect of a moderate content of *trans* fatty acids on the development of coronary heart disease is still unknown.

Intake of *trans* fatty acids during human pregnancy is thought to impair essential fatty acid metabolism and thereby influence the growth of the fetus (15–17). Feeding of diets that contain *cis*- and *trans*-octadecenoic acids leads to incorporation of the isomers into various tissues of the rat (18,19), and consumption of diets rich in *trans* fatty acids (more than 33 wt% of total fatty acids) was shown to have a negative effect on the formation of PUFA when dietary 18:2n-6 was less than 14 wt% (20–22). *Trans* fatty acids from partially hydrogenated vegetable oils were shown to inhibit the Δ6-desaturase, which catalyzes the initial rate-limiting desaturation step, and those from partially hydrogenated marine oils inhibit both the Δ5- and the Δ6-desaturase (23). The decreasing effect of *trans* fatty acids on the amount of arachidonic acid was diminished by higher linoleic acid levels in the diets, as shown by Zevenbergen *et al.* (21) for partially hydrogenated soybean oil (PHSBO) and in our laboratory (20) for partially hydrogenated marine oil.

The aim of the present study was to investigate whether even moderate amounts of *trans* fatty acids in the diet influence the formation and deposition of PUFA in rat liver and heart in the presence of sufficient amounts of linoleic acid. The content of linoleic acid in the diets was held constant at about 10 wt%, whereas the amount of *trans* fatty acids from PHSBO ranged from 4.5 to 15 wt% of the dietary fats with a balanced decrease in oleic acid. A diet that included butterfat (BF) was given to a group of rats to compare the effect of isomers present in dairy products with the isomers from PHSBO. The rats in the control group received palm oil (PO). The sum of *trans* fatty acids and the three major saturated fatty acids—

*To whom correspondence should be addressed at Department of Biochemistry and Nutrition, The Technical University of Denmark, Building 224, DK-2800 Lyngby, Copenhagen, Denmark. E-mail: bysted@mimer.be.dtu.dk.

Copyright © 1998 by AOCS Press

14:0, 16:0, and 18:0—present in the 15 wt% *trans* PHSBO and BF diets was comparable with their content in the PO diet. This is of interest because decreased amounts of *trans* fatty acids in margarines are often balanced with saturated fatty acids to keep the same spreadability and melting characteristics.

EXPERIMENTAL PROCEDURES

Animal experiment. Fifty weanling male Wistar rats (4 wk, specific pathogen-free; Møllegaard Laboratory, Ll. Skensved, Denmark) were divided into five groups, each with 10 rats of similar average weights (90 ± 11 g). The rats were fed a semisynthetic diet that contained (wt%) maizestarch (Maizena-Companiet A/S, Copenhagen, Denmark) 40, fat 20, casein (MD Foods, Viby, Denmark) 20, sucrose 10, salt mixture (including trace elements) 5, cellulose powder (Frisenette & Sons, Egsmark, Denmark) 4, vitamin mixture 0.5, and choline chloride 0.5. The composition of the salt and vitamin mixtures was as previously described (24). The dietary fats were PHSBO, soybean oil, PO (all from Aarhus Oliefabrik A/S, Aarhus, Denmark), butter (MD Foods, Rødkjærsbro, Denmark), olive oil (Cavanna, Svansø Import, Denmark), grapeseed oil (Prouvénço, Marseille, France), and lard (Raffinol, Copenhagen, Denmark). The fats were mixed to give the same linoleic acid content and comparable levels of saturated fatty acids. The rats were caged three or four together under conditions of 21°C mean temperature and 50% relative humidity. Diet and water were supplied *ad libitum*. The rats were examined and weighed weekly during the experimental period. After 8 wk, the rats were killed by decapitation (after an overnight fast), and liver and heart were immediately excised, weighed, frozen in liquid nitrogen, and kept at –80°C until analysis.

Analysis of lipids. Total lipids were extracted from rat liver and heart with chloroform/methanol 2:1 (vol/vol) according to Folch *et al.* (25). The five major phosphoacylglycerols (PL)—phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), and diphosphatidylglycerol (CL)—were separated by thin-layer chromatography on silica plates (DC-Fertigplatten Kieselgel 60, Merck, Darmstadt, Germany) with chloroform/methanol/hexane/glacial acetic acid/boric acid (40:20:30:10:1.8, vol/vol/vol/vol/wt) as eluent. The lipids were visualized with 2′,7′-dichlorofluorescein in 96% ethanol, scraped off, saponified with 0.5 M NaOH in methanol, and subsequently methylated with 14% (wt/vol) BF_3 in methanol in the presence of 0.02% (wt/vol) hydroquinone. The fatty acid methyl esters were extracted with heptane and analyzed by gas–liquid chromatography (GLC) on a Hewlett-Packard 5880A instrument (Palo Alto, CA) with flame-ionization detector (FID) and an SP2380 capillary column (30 m × 0.32 mm i.d., 0.2 µm film; Supelco, Bellefonte, PA). The initial oven temperature was 140°C. The temperature was raised to 160°C at 2°C/min, then held constant for 2 min, and finally raised 3°C/min to 200°C, where it was unchanged for 5 min. The inlet pressure of the carrier gas (helium) was 76 kPa. The injector (split mode) and the detector were maintained at 250 and 260°C, respectively.

The different *trans* isomers were identified and quantitated by GLC on a Fison 8160 instrument (Manchester, United Kingdom), equipped with FID and a CP-Sil 88 capillary column (100 m × 0.25 mm i.d., 0.2 µm film; Chrompack, Middelburg, The Netherlands). The initial oven temperature of 170°C was held for 50 min and then raised to 225°C at 4°C/min. The inlet pressure of the carrier gas (helium) was 210 kPa. The injector (split mode) and the detector were maintained at 275°C.

The distribution of *trans* 18:1 isomers between the *sn*-1 and *sn*-2 positions in pooled PE and PC samples was determined by using phospholipase A_2. The procedure of Christie (26) was used after slight modifications.

Statistical analysis. Results are expressed as the means ± standard deviations. Differences in the fatty acid composition of rat liver and heart PL between the groups were assessed by one-way analysis of variance (one-way ANOVA). A *P*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Dietary fats. The dietary fats were mixed into five different diets (Table 1). In three of the five groups, the total level of *trans* fatty acids (originating from PHSBO) was varied from 4.5 to 15 wt% of the dietary fatty acids (5-10-15 PHSBO). Additional fats and oils were added as described below. The notation 5-10-15 PHSBO thus refers to the percentages of *trans* fatty acids in the dietary fats. The BF group received

TABLE 1
Fatty Acid Composition (wt%) of Dietary Fats

	Dietary fats[a]				
	15 PHSBO	10 PHSBO	5 PHSBO	BF	PO
Fatty acid	(wt%)	(wt%)	(wt%)	(wt%)	(wt%)
8:0	—[b]	—	—	0.3	—
10:0	—	—	—	1.7	—
12:0	—	—	—	2.9	0.7
14:0	1.1	1.1	1.0	9.5	1.3
16:0	20.9	21.5	21.8	29.8	44.5
16:1n-7	1.5	1.7	1.9	1.8	—
18:0	13.4	14.0	12.6	11.2	4.7
Σ 18:1 *trans*[c]	15.1	9.9	4.5	2.5	—
18:1n-9	30.5	35.3	42.0	22.2	37.3
18:1n-7	3.0	2.9	2.8	0.8	0.7
18:2n-6	9.9	10.0	10.4	11.1	10.2
18:3n-3	0.5	0.6	0.6	0.4	0.2
20:0	0.3	0.3	0.4	0.3	0.4
20:1n-9	0.4	0.5	0.5	0.7	—
20:2n-6	0.2	0.2	0.2	—	—
Σ SFA[d]	35.7	36.9	35.8	55.7	51.6

[a]PHSBO, partially hydrogenated soybean oil; BF, butterfat; PO, palm oil (control group). The notation 5-10-15 PHSBO refers to the percentages of *trans* fatty acids in the PHSBO. Note that additional fats and oils were added.
[b]— denotes undetectable quantities.
[c]Σ 18:1 *trans* = the sum of *trans* 18:1 isomers.
[d]Σ SFA = the sum of saturated fatty acids.

trans fatty acids from BF. The control group (PO) was fed a PO diet with 52 wt% saturated fatty acids, but without *trans* fatty acids. Different vegetable oils were included in the dietary fats to obtain equal amounts of linoleic acid in the five diets, about 10 wt% as in a typical Danish diet. Lard was added to the PHSBO groups to keep the levels of saturated fatty acids constant. The only difference in these three groups was therefore the change in the 18:1 *trans-cis* ratios. The content of saturated fat in the PO group is comparable to that of butter and to the sum of saturated and *trans* fatty acids in 15 PHSBO.

Animal experiment. No notable differences in growth and general performance were found during the 8-wk feeding period. Average weights for all groups of rats at the end of the study were 318 ± 30 g with no significant deviations between groups ($P < 0.05$). The food intake was also similar for all five groups. In addition, no significant differences were observed in the weights of liver and heart, when related to body weights of the rats.

Incorporation of total 18:1 trans *in the PL.* Figure 1 shows the incorporation of 18:1 *trans* from the PHSBO diets in rat liver and heart PL. The curves are similar for the two organs. However, in rat liver, the amounts of *trans* fatty acids found in PE, PC, and PI were of the same magnitude, whereas in rat heart, the largest incorporation was observed in PE and PI, followed by PC. The amounts incorporated were always less than 7 wt%. There was a positive correlation (r = 0.981–0.997) between the amount of *trans* fatty acids in the diet and in PE, PC, PI, and PS from both rat liver and heart. No 18:1 *trans* was detected in CL of either organ. The content of saturated fatty acids in PE, PC, PI, and PS from liver and heart (Tables 2 and 3) decreased proportionally with increasing amounts of *trans* fatty acids in the diet (r = 0.970–0.996) in spite of the same amounts of total saturated fatty acids in all PHSBO diets (about 36 wt%). The reason for this decrease is the incorporation of *trans* fatty acids in the *sn*-1 position of the PL instead of saturated fatty acids, as will be discussed later. This substitution of *trans* fatty acids for saturated fatty acids in the PL, due to their spatial similarity, is in agreement with previous experiments (5,18,27–29). The

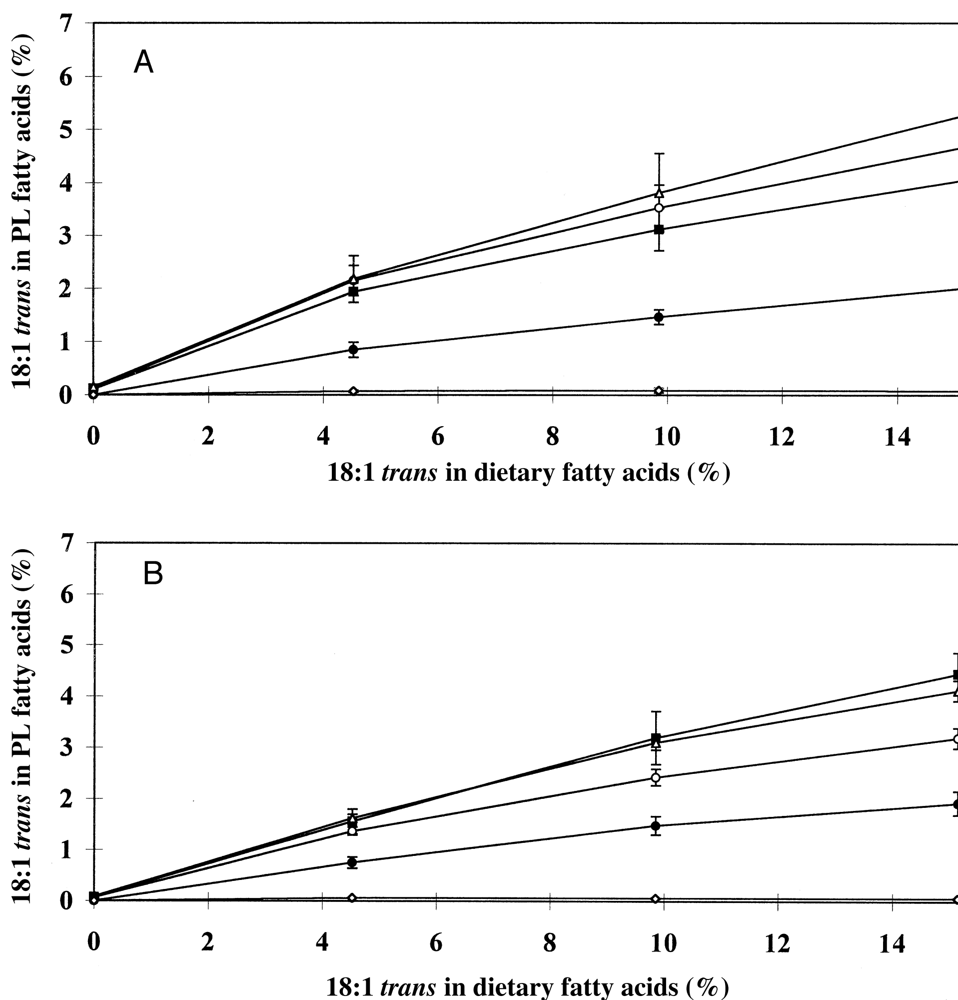

FIG. 1. Incorporation of total *trans* 18:1 from partially hydrogenated soybean oil (PHSBO) diets in the phosphoacylglycerols (PL) of (A) rat liver and (B) heart (mean ± standard deviation, n = 10). ■, 18:1 *trans* in phosphatidylethanolamine (PE); ●, 18:1 *trans* in phosphatidylserine; ○, 18:1 *trans* in phosphatidylcholine; △, 18:1 *trans* in phosphatidylinositol; ◇, 18:1 *trans* in diphosphatidylglycerol.

TABLE 2
Major Fatty Acids (wt% of total) in Phosphatidylethanolamines (PE) and Phosphatidylcholines (PC) from Rat Liver

	Dietary fats[a]				
Fatty acid	15 PHSBO (wt%)[b]	10 PHSBO (wt%)	5 PHSBO (wt%)	BF (wt%)	PO (wt%)
Liver PE					
16 ald.	0.6 ± 0.1[e]	0.6 ± 0.1[e]	0.6 ± 0.1[e]	0.7 ± 0.1[e]	0.9 ± 0.1
16:0	12.6 ± 0.6[e]	13.1 ± 0.7[e]	13.1 ± 0.4[e]	14.7 ± 1.0	15.2 ± 0.8
16:1n-7	0.2 ± 0.1	0.2 ± 0.1[e]	0.2 ± 0.1	0.6 ± 0.3	0.3 ± 0.2
18 ald.	0.9 ± 0.3	0.9 ± 0.4	1.1 ± 0.2[e]	1.2 ± 0.1[e]	0.9 ± 0.1
18:0	18.2 ± 1.4[e]	20.0 ± 1.0[e]	21.8 ± 1.2	20.8 ± 2.3[e]	22.8 ± 2.0
Σ18:1 *trans*[c]	4.1 ± 0.4[e]	3.1 ± 0.4[e]	1.9 ± 0.2[e]	1.0 ± 0.1[e]	0.1 ± 0.0
18:1n-9	7.8 ± 0.5[e]	7.0 ± 0.3[e]	5.9 ± 0.6[e]	4.2 ± 0.5	4.5 ± 0.8
18:1n-7	1.8 ± 0.5	1.6 ± 0.3	1.9 ± 0.4	2.4 ± 0.9	2.1 ± 0.9
18:2n-6	5.6 ± 0.7[e]	5.0 ± 0.9[e]	4.1 ± 0.6	4.8 ± 1.0	3.9 ± 0.9
20:3n-6	0.7 ± 0.1	0.7 ± 0.3	0.5 ± 0.2	1.0 ± 0.2[e]	0.7 ± 0.2
20:4n-6	29.2 ± 0.9[e]	29.3 ± 0.7[e]	30.9 ± 0.8[e]	31.8 ± 1.1[e]	33.9 ± 1.2
22:4n-6	0.5 ± 0.0[e]	0.5 ± 0.1[e]	0.5 ± 0.1[e]	0.6 ± 0.1[e]	1.0 ± 0.2
22:5n-6	0.8 ± 0.2[e]	0.9 ± 0.3[e]	0.8 ± 0.3[e]	1.3 ± 0.6[e]	4.2 ± 1.7
22:5n-3	0.9 ± 0.2[e]	1.1 ± 0.2[e]	0.9 ± 0.2[e]	1.0 ± 0.2[e]	0.5 ± 0.1
22:6n-3	13.4 ± 0.9[e]	13.6 ± 1.4[e]	13.8 ± 1.0[e]	10.9 ± 1.4[e]	6.9 ± 0.7
Ratio					
18:2n-6/20:4n-6	0.19 ± 0.03[e]	0.17 ± 0.03[e]	0.13 ± 0.02	0.15 ± 0.03[e]	0.12 ± 0.03
Σ SFA[d]	31.2 ± 1.4[e]	33.5 ± 1.0[e]	35.3 ± 1.0[e]	36.2 ± 1.6[e]	38.4 ± 1.7
Liver PC					
16 ald.	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
16:0	15.5 ± 1.0[e]	16.0 ± 1.0[e]	16.2 ± 0.8[e]	18.2 ± 0.7[e]	19.8 ± 0.8
16:1n-7	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.1	1.2 ± 0.7[e]	0.6 ± 0.3
18 ald.	0.1 ± 0.0[e]	0.1 ± 0.0[e]	0.2 ± 0.0	0.3 ± 0.1[e]	0.2 ± 0.0
18:0	17.1 ± 1.5[e]	19.2 ± 1.1[e]	21.0 ± 1.7	18.9 ± 2.3[e]	21.3 ± 1.8
Σ 18:1 *trans*[c]	4.7 ± 0.5[e]	3.5 ± 0.4[e]	2.2 ± 0.3[e]	1.1 ± 0.1[e]	0.1 ± 0.0
18:1n-9	10.4 ± 0.8[e]	9.5 ± 0.7[e]	8.6 ± 0.9[e]	7.0 ± 0.7	7.3 ± 0.8
18:1n-7	2.2 ± 0.6	2.2 ± 0.4	2.4 ± 0.4	3.1 ± 1.3	2.4 ± 0.9
18:2n-6	11.4 ± 1.7[e]	10.3 ± 2.3[e]	8.2 ± 1.0	10.1 ± 2.5[e]	7.4 ± 2.0
20:3n-6	1.4 ± 0.3	1.3 ± 0.6	1.0 ± 0.3	2.1 ± 0.7[e]	1.3 ± 0.7
20:4n-6	26.1 ± 2.5[e]	27.6 ± 3.1[e]	30.5 ± 1.6	28.5 ± 3.2[e]	31.6 ± 3.2
22:4n-6	0.2 ± 0.0[e]	0.2 ± 0.1[e]	0.2 ± 0.1[e]	0.2 ± 0.0[e]	0.3 ± 0.1
22:5n-6	0.5 ± 0.2[e]	0.6 ± 0.2[e]	0.5 ± 0.2[e]	0.7 ± 0.3[e]	2.2 ± 0.8
22:5n-3	0.3 ± 0.1[e]	0.4 ± 0.1[e]	0.3 ± 0.1[e]	0.4 ± 0.1[e]	0.2 ± 0.0
22:6n-3	5.6 ± 0.6[e]	5.5 ± 0.7[e]	5.7 ± 0.5[e]	4.3 ± 0.6[e]	2.7 ± 0.3
Ratio					
18:2n-6/20:4n-6	0.44 ± 0.11[e]	0.37 ± 0.14[e]	0.27 ± 0.05	0.37 ± 0.13[e]	0.24 ± 0.10
Σ SFA[d]	33.0 ± 1.9[e]	35.7 ± 1.2[e]	37.7 ± 1.2[e]	38.0 ± 2.1[e]	41.5 ± 1.9

[a]For other abbreviations and additional notes see Table 1.
[b]The weight percentage represents the mean ± standard deviation, $n = 10$.
[c]Σ 18:1 *trans* = the sum of *trans* 18:1 isomers.
[d]Σ SFA = the sum of saturated fatty acids.
[e]Significant difference from the PO group ($P < 0.05$); ald., aldehyde.

content of saturated fatty acids in CL from the five groups, in which no *trans* fatty acids were detected, was less than 4 wt% in rat liver and less than 2 wt% in rat heart.

The *trans* fatty acids from BF were also incorporated in PE, PC, PI, and PS from rat liver and heart. The order of incorporation of *trans* fatty acids in these PL was as found in the rats fed the PHSBO diets (data not shown for PI and PS). By assuming a linear relationship between 0 and 4.5 wt% *trans* fatty acids from the PHSBO diets (Fig. 1), the amount of *trans* fatty acids incorporated in the PL from rats in the BF group corresponded to the level from rats fed PHSBO and seemed to be independent of the distribution of the individual *trans* isomers present in the dietary fats. The short- and medium-chain saturated fatty acids (8:0–14:0) present in the BF diet were not incorporated in the PL of either rat liver or heart.

Trans isomers of PUFA represented less than 0.1 wt% in the PL.

Discrimination of individual trans *18:1 isomers.* The distribution of *trans* 18:1 isomers in PHSBO, BF and PE from rat liver and heart of the groups fed 15 PHSBO and BF, respectively, is shown in Figure 2. The *trans* 18:1 isomers pre-

TABLE 3
Major Fatty Acids (wt% of total) in PE and PC from Rat Heart

Fatty acid	Dietary fats[a] 15 PHSBO (wt%)[b]	10 PHSBO (wt%)	5 PHSBO (wt%)	BF (wt%)	PO (wt%)
Heart PE					
16 ald.	3.2 ± 0.6[e]	3.4 ± 0.5[e]	3.5 ± 0.5[e]	4.4 ± 0.6[e]	5.7 ± 0.9
16:0	5.3 ± 0.2[e]	5.5 ± 0.4[e]	5.5 ± 0.3[e]	6.2 ± 0.3[e]	6.8 ± 0.4
16:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0[e]	0.1 ± 0.0
18 ald.	5.2 ± 1.8	5.1 ± 1.5	5.4 ± 1.8	4.8 ± 1.1	4.2 ± 1.2
18:0	17.4 ± 0.8[e]	18.6 ± 0.9[e]	19.8 ± 0.9	20.2 ± 0.9	20.6 ± 0.9
Σ 18:1 *trans*[c]	4.4 ± 0.4[e]	3.2 ± 0.5[e]	1.6 ± 0.2[e]	0.9 ± 0.1[e]	0.1 ± 0.0
18:1n-9	6.1 ± 0.4[e]	6.0 ± 0.6[e]	5.7 ± 1.0	4.3 ± 0.7	4.6 ± 0.7
18:1n-7	2.6 ± 0.2[e]	2.7 ± 0.2[e]	2.5 ± 0.2[e]	2.1 ± 0.2	2.1 ± 0.2
18:2n-6	5.4 ± 1.1	5.5 ± 1.1	5.4 ± 1.3	6.3 ± 1.1	5.8 ± 1.4
20:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1[e]	0.2 ± 0.0
20:4n-6	21.8 ± 1.0	21.9 ± 1.6	21.7 ± 1.3	21.8 ± 1.8	21.6 ± 0.8
22:4n-6	1.5 ± 0.1[e]	1.5 ± 0.2[e]	1.7 ± 0.2[e]	1.7 ± 0.2[e]	2.5 ± 0.2
22:5n-6	2.6 ± 0.4[e]	2.6 ± 0.6[e]	3.2 ± 0.6[e]	4.5 ± 0.8[e]	12.6 ± 2.3
22:5n-3	2.6 ± 0.3[e]	2.5 ± 0.3[e]	2.3 ± 0.1[e]	2.5 ± 0.5[e]	0.9 ± 0.1
22:6n-3	19.8 ± 1.4[e]	19.6 ± 1.6[e]	19.9 ± 1.4[e]	17.4 ± 1.7[e]	10.7 ± 1.4
Ratio					
18:2n-6/20:4n-6	0.25 ± 0.06	0.25 ± 0.05	0.25 ± 0.07	0.29 ± 0.05	0.27 ± 0.07
Σ SFA[d]	23.1 ± 0.9[e]	24.5 ± 0.8[e]	25.7 ± 0.8[e]	27.0 ± 0.9	27.8 ± 1.0
Heart PC					
16 ald.	0.7 ± 0.1[e]	0.7 ± 0.1[e]	0.7 ± 0.2[e]	0.8 ± 0.2[e]	1.2 ± 0.4
16:0	13.8 ± 0.7[e]	14.2 ± 0.7[e]	13.6 ± 0.3[e]	14.6 ± 0.7[e]	15.9 ± 0.7
16:1n-7	0.2 ± 0.0[e]	0.2 ± 0.0[e]	0.2 ± 0.0[e]	0.4 ± 0.0[e]	0.1 ± 0.0
18 ald.	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2
18:0	22.8 ± 0.7[e]	23.6 ± 0.4[e]	25.8 ± 0.4	25.3 ± 0.4[e]	25.9 ± 0.6
Σ 18:1 *trans*[c]	3.2 ± 0.2[e]	2.4 ± 0.2[e]	1.4 ± 0.1[e]	0.7 ± 0.0[e]	0.1 ± 0.0
18:1n-9	6.9 ± 0.3[e]	6.9 ± 0.4[e]	6.4 ± 0.5[e]	5.3 ± 0.3	5.6 ± 0.6
18:1n-7	3.9 ± 0.2[e]	4.2 ± 0.3[e]	4.2 ± 0.2[e]	3.5 ± 0.2	3.4 ± 0.2
18:2n-6	8.6 ± 1.1	8.8 ± 1.9	7.1 ± 1.4	10.1 ± 1.5[e]	8.3 ± 2.3
20:3n-6	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.2[e]	0.4 ± 0.1
20:4n-6	29.1 ± 1.1[e]	28.9 ± 2.3[e]	31.2 ± 1.4	29.5 ± 1.6[e]	31.7 ± 2.5
22:4n-6	0.6 ± 0.1[e]	0.6 ± 0.1[e]	0.7 ± 0.1[e]	0.6 ± 0.1[e]	0.9 ± 0.1
22:5n-6	0.6 ± 0.1[e]	0.6 ± 0.2[e]	0.6 ± 0.1[e]	0.8 ± 0.2[e]	2.1 ± 0.4
22:5n-3	1.5 ± 0.2[e]	1.4 ± 0.2[e]	1.3 ± 0.2[e]	1.3 ± 0.4[e]	0.4 ± 0.0
22:6n-3	4.6 ± 0.6[e]	4.3 ± 0.5[e]	4.3 ± 0.5[e]	3.6 ± 0.5[e]	2.2 ± 0.4
Ratio					
18:2n-6/20:4n-6	0.30 ± 0.05	0.31 ± 0.09	0.23 ± 0.06	0.35 ± 0.06[e]	0.27 ± 0.10
Σ SFA[d]	37.1 ± 0.4[e]	38.3 ± 0.5[e]	39.8 ± 0.4[e]	40.6 ± 0.5[e]	42.1 ± 0.6

[a]For abbreviations and additional notes see Tables 1 and 2.
[b]The weight percentage represents the mean ± standard deviation, $n = 10$.
[c]Σ 18:1 *trans* = the sum of *trans* 18:1 isomers.
[d]Σ SFA = the sum of saturated fatty acids.
[e]Significant difference from the PO group ($P < 0.05$).

sent in PHSBO are equally distributed. In contrast, more than half of the *trans* fatty acids in BF is vaccenic acid (11*t*-18:1). The rest is distributed almost equally between 8*t*-, 9*t*-, 10*t*-, and 12*t*-18:1 (the 8*t*-18:1 peak may contain minor amounts of the 6*t*- and 7*t*-18:1 isomers). These results are in accordance with previous reports (1–3,30).

Comparison of the composition of *trans* fatty acids in the dietary fats with that in rat liver and heart PE indicates that 8*t*- and 10*t*-18:1 are discriminated against in the incorporation; 10*t*-18:1 is nearly absent, whereas 12*t*-18:1, with a double-bond position characteristic of essential fatty acids, is strongly preferred. The proportion of 9*t*-18:1 is also enhanced. This is in agreement with the data reported by Høy and Hølmer (19) for liver mitochondrial membranes from rats fed diets that contained partially hydrogenated peanut oil. They demonstrated a preferential incorporation particularly of the 12*t*-18:1 isomer, but also the 9*t*-18:1 isomer, whereas 10*t*-18:1 and, to a lesser extent, the 8*t*- and 11*t*-18:1 isomers were discriminated against. Likewise, Lawson *et al.* (22) found a discrimination against incorporation of 8*t*- and 10*t*-18:1 in rat liver PC and a preferential incorporation of the 12*t*-18:1 isomer after feeding PHSBO. Wood (18) investi-

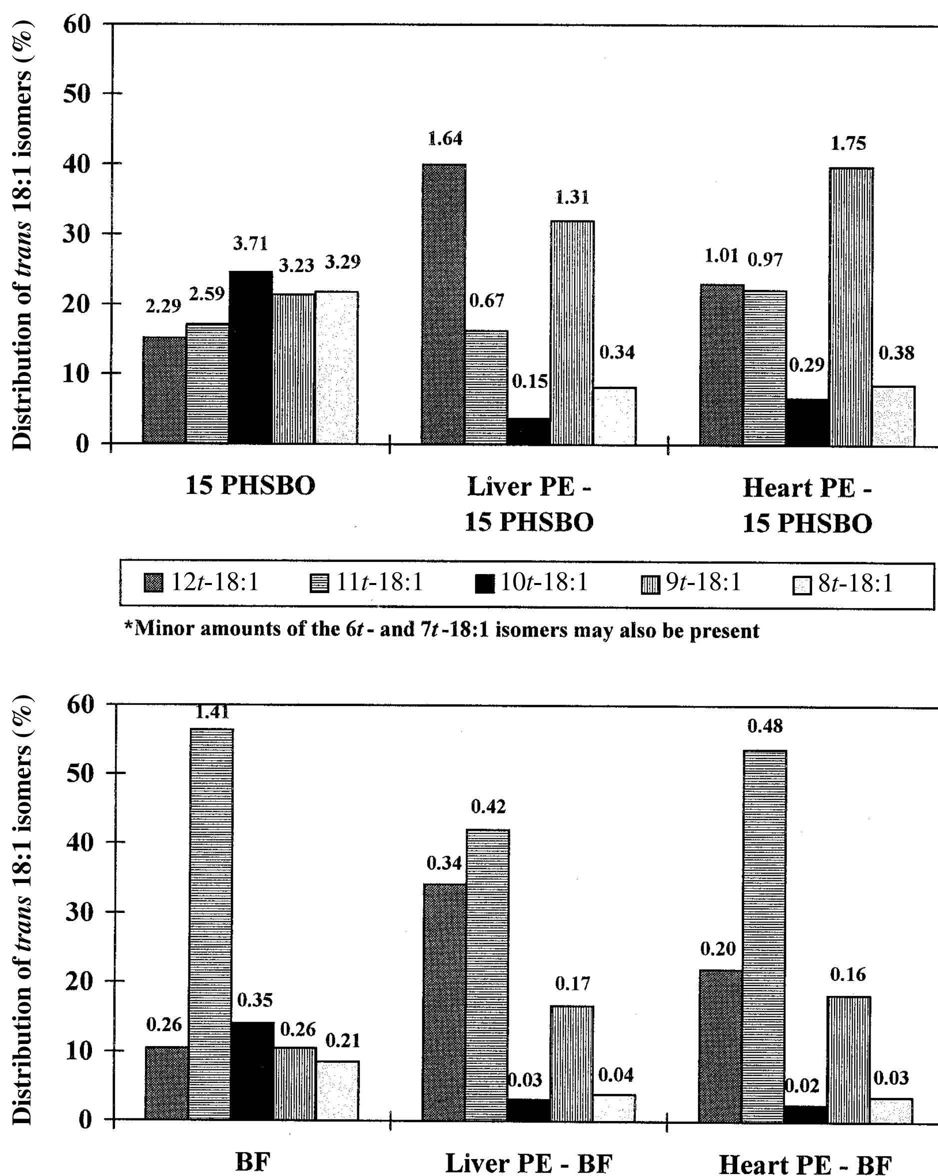

FIG. 2. Distribution of individual *trans* 18:1 isomers in PHSBO, butterfat (BF), and liver and heart phosphatidylethanolamines (PE) from rats fed 15 PHSBO (meaning 15% *trans* fatty acids in PHSBO) and BF, respectively. The actual percentage values for the *trans* 18:1 isomers are included above the bars. Note that additional fats and oils were added; for composition, see Table 1.

gated the incorporation of *cis*- and *trans*-octadecenoic acids (from partially hydrogenated safflower oil) in PE and PC from different rat tissues (liver, heart, brain, kidney, lung, muscle, spleen, and adipose tissues). He found that the 10*t*-18:1 isomer was discriminated against in all tissues analyzed but particularly in liver and heart. Contrary to the other results mentioned here, a higher percentage of 8*t*-18:1 was found in the PL than in the diets. Discrimination against the 8*t*- and 10*t*-18:1 isomers is also in accordance with human studies performed by Emken *et al.* (31,32).

The pattern of the individual *trans* 18:1 isomers in rat liver PE differed from that of rat heart PE in the 15 PHSBO and BF groups, respectively (Fig. 2). In rat liver PE from the 15 PHSBO group, 1.64 wt% 12*t*-18:1 was present, compared to only 1.01 wt% in rat heart PE. However, the percentages of 9*t*- and 11*t*-18:1 were lower in rat liver than in rat heart (0.67/0.97 and 1.31/1.75 wt%, respectively). The 8*t*- and 10*t*-18:1 isomers, which were strongly discriminated against compared to the dietary contents, represented only 0.34 and 0.15 wt% in rat liver PE and 0.38 and 0.29 wt% in rat heart PE after PHSBO feeding.

For the BF group, the same tendencies were seen, and even lower values of the 8*t*- and 10*t*-18:1 isomers (0.04/0.03 and 0.03/0.02 wt%, respectively) were deposited as a consequence of the lower contents in butter. The major 18:1 isomer in both liver and heart PE from the rats fed BF was, as would be expected, 11*t*-18:1, which constitutes nearly 50% of the total isomer fraction in the dietary fat.

The same trends in the distribution of the individual *trans* 18:1 isomers were found for PC (data not shown).

Formation of PUFA in rat liver and heart. In rat liver PE and PC, the amount of linoleic acid increased with increasing content of *trans* fatty acids from the PHSBO diets, whereas the level of arachidonic acid decreased (Tables 2 and 3). These changes could be consequences of either inhibition of the conversion or competition for acylation of the *sn*-2 position. In comparison to the PO group, the 18:2n-6 content was increased, and the 20:4n-6 level was decreased for all PHSBO groups. In contrast to rat liver PE and PC, no significant differences ($P < 0.05$) were seen in the levels of the two n-6 fatty acids in rat heart PE. In rat heart PC, the contents of 18:2n-6 in the three PHSBO groups and the PO group were of the same magnitude, whereas the amount of 20:4n-6 decreased significantly ($P < 0.05$) in the two groups fed 10 and 15 wt% *trans* fatty acids from PHSBO. The BF diets influenced the contents of 18:2n-6 and 20:4n-6 in rat liver PE and PC and rat heart PC in the same direction as the PHSBO diets that contained 10 and 15 wt% *trans* fatty acids, whereas the contents were hardly affected in rat heart PE.

Because the levels of 20:3n-6 are small compared to the amounts of 20:4n-6, the precursor/product ratios 18:2n-6/20:4n-6 (Tables 2 and 3) are used to illustrate the effect of *trans* fatty acids on the conversion of linoleic acid. The ratios for rat liver PE and PC from group 5 PHSBO did not differ significantly from the PO control group, in contrast to the 10 and 15 PHSBO groups. Therefore, the formation of 20:4n-6 in rat liver was not influenced by 4.5 wt% *trans* fatty acids from PHSBO but apparently was by 10 wt%. In rat heart, the conversion of linoleic acid was not significantly affected by any of the three PHSBO diets. The group fed BF showed significantly higher ratios in PE and PC from rat liver and in PC from rat heart, compared to the PO group. This means that even a content of 2.5 wt% *trans* fatty acids from BF influenced the formation of arachidonic acid. The results reported by Willett *et al.* (4) from the Nurses' Health Study showed an association between high intake of *trans* fatty acids and risk of coronary heart disease after adjustment for age and total energy intake. This positive correlation was entirely ascribed to the intake of *trans* fatty acids from partially hydrogenated vegetable oils rather than from ruminant sources. The present data indicate, on the contrary, that *trans* fatty acids from butter are at least as inhibitory as other positional isomers.

The decreased amount of arachidonic acid found in both rat liver PE and PC and heart PC might be due to competition from the *trans* fatty acids present, especially the 12*t*-18:1 isomer, for incorporation in the *sn*-2 position. Determination of fatty acid distributions in the *sn*-1 and *sn*-2 positions, however, revealed that, even in the 15 PHSBO group, *trans* 18:1 isomers comprised only 0.3–0.4 wt% of the total fatty acids in the *sn*-2 position of rat liver and heart PE, compared to 51 and 37 wt% of 20:4n-6 in rat liver and heart PE, respectively (Table 4). In both organs total PUFA represented more than 85 wt% of the fatty acids in the *sn*-2 position. On the contrary, the *sn*-1 position of rat liver and heart PE from the rats fed the 15 PHSBO diet contained more than 8 wt% *trans* 18:1 isomers and less than 3 wt% 20:4n-6 (data not shown). In the BF group, *trans* 18:1 isomers were also particularly incorporated in the *sn*-1 position of rat liver and heart PE, and 20:4n-6 in the *sn*-2 position. The same trends as observed for PE were seen for PC (data not shown). Our findings are in line with data reported by Reichwald-Hacker *et al.* (33) who fed rats diets with 20 wt% PHSBO with 41 wt% *trans* 18:1 isomers and found 2 wt% *trans* 18:1 in the *sn*-2 position of liver PC, compared to 20 wt% in the *sn*-1 position.

According to the fatty acid composition in the *sn*-1 and *sn*-2 positions of rat liver and heart PE and PC, competition from the *trans* 18:1 isomers for incorporation in the *sn*-2 position may therefore be negligible.

TABLE 4
Major Fatty Acids (wt% of total) in the *sn*-2 Position of PE from Rat Liver and Heart

	Dietary fats (wt%)[a]				
Fatty acid	15 PHSBO	10 PHSBO	5 PHSBO	BF	PO
sn-2 position of liver PE					
16 ald.	0.3	0.4	0.3	0.4	0.4
16:0	1.3	0.8	0.8	1.9	1.6
16:1n-7	0.2	0.2	0.2	0.5	0.5
18 ald.	0.1	0.1	0.1	0.2	0.2
18:0	1.0	0.6	0.4	1.3	1.1
Σ 18:1 *trans*[c]	0.3	0.2	0.1	0.2	0.1
18:1n-9	2.8	2.9	3.5	4.2	4.4
18:1n-7	0.9	1.2	1.0	1.2	1.6
18:2n-6	10.7	9.2	8.7	11.3	8.9
20:3n-6	1.3	1.2	1.1	1.9	1.4
20:4n-6	51.4	52.6	55.2	52.6	56.5
22:4n-6	0.5	0.6	0.6	0.6	1.2
22:5n-6	1.1	1.6	1.3	1.8	6.0
22:5n-3	1.6	1.8	1.4	1.5	1.0
22:6n-3	20.9	22.7	22.2	15.5	10.1
Σ PUFA[d]	87.5	89.7	90.5	85.2	85.1
sn-2 position of heart PE					
16 ald.	0.4	0.2	0.3	0.3	0.3
16:0	1.8	1.2	1.4	1.6	1.4
16:1n-7	0.1	0.1	0.1	0.1	0.1
18 ald.	0.6	0.1	0.1	0.6	0.3
18:0	0.9	0.5	0.5	0.8	0.7
Σ 18:1 *trans*[c]	0.4	0.2	0.1	0.1	0.1
18:1n-9	2.5	2.4	2.4	2.2	2.7
18:1n-7	4.2	3.1	2.4	3.5	4.2
18:2n-6	6.0	5.8	4.4	7.1	5.7
20:3n-6	0.4	0.4	0.3	0.5	0.3
20:4n-6	36.7	40.4	39.8	37.2	35.5
22:4n-6	2.1	2.3	2.4	2.5	3.6
22:5n-6	5.0	4.7	6.0	8.2	23.9
22:5n-3	3.5	3.8	3.4	3.7	1.2
22:6n-3	32.2	32.6	34.4	28.7	18.0
Σ PUFA[d]	85.9	90.0	90.7	87.9	88.2

[a]For abbreviations and additional notes see Tables 1 and 2.
[b]The weight percentage represents results from pooled samples.
[c]Σ 18:1 *trans* = the sum of *trans* 18:1 isomers.
[d]Σ PUFA = the sum of polyunsaturated fatty acids.

Previous rat experiments showed suppressed levels of 20:4n-6 and increased levels of 18:2n-6 in rat liver PL after feeding partially hydrogenated vegetable oils (20,22,34,35). Reduced conversion of linoleic acid in rat liver and heart has also been reported in rats fed partially hydrogenated marine oil with a content of 33 wt% *trans* fatty acids, and this could not be overcome by raising the dietary linoleic acid content up to 14 wt% (20). Studies with rat liver microsomes have shown that this was a result of a decrease in Δ6-desaturase activity for rats fed partially hydrogenated vegetable oils and in both Δ6- and Δ5-desaturase activities after partially hydrogenated marine oil feeding (23).

The distribution of n-6 and n-3 C_{22}-PUFA in rat heart PE (Fig. 3) from the groups fed *trans* fatty acids, either as PHSBO or BF, differed considerably from the PO control group. All groups were fed the same content of linoleic acid, and the diets contained approximately the same amounts of 18:3n-3, ranging from 0.2 to 0.6 wt%. The long-chain n-3 fatty acids, mainly 22:6, were deposited instead of 22:5n-6 and, to a lesser extent, 22:4n-6 in all groups that received dietary *trans* fatty acids. For rat liver PE, the same trend was observed as for heart PE. Also for the PC fractions of liver and heart, the intake of *trans* fatty acids led to increased contents of 22:6n-3 and 22:5n-3 and correspondingly lower levels of 22:5n-6 and 22:4n-6. This is in agreement with the findings of Lawson *et al.* (22), who observed increased content of 22:6n-3 in liver PE from rats fed 36% *trans* fatty acids in PHSBO, whereas Koga *et al.* (34) found unchanged amounts of 22:6n-3 and decreased levels of 22:5n-6 in rat liver PE and PC after feeding diets with 39% *trans* fatty acids from partially hydrogenated corn oils.

The metabolism of linoleic and linolenic acid to the very long-chain PUFA through desaturations and elongations has recently been reinvestigated (36,37). Voss *et al.* (38) showed that, in rat hepatocytes, the metabolism of 22:5n-3 to 22:6n-3 is independent of a Δ4-desaturase. Instead, 22:5n-3 is sequentially chain-elongated to 24:5n-3 and subsequently desaturated to 24:6n-3. Finally, 24:6n-3 moves from the endoplasmic reticulum to a site for β-oxidation (presumably the peroxisomes), where it is chain-shortened to 22:6n-3. Mohammed *et al.* (39) reported an analogous pathway for the conversion of 22:4n-6 to 22:5n-6. In human cells, the same pathway exists for the synthesis of 22:6n-3 from 20:5n-3 (40,41). Marzo *et al.* (41) propose the existence of two distinct but related Δ6-desaturases. The one acting on 18:2n-6 and 18:3n-3 is different from the other, which catalyzes the desaturation of 24:4n-6 and 24:5n-3 to 24:5n-6 and 24:6n-3, respectively.

The increased deposition of 22:6n-3 and also, to a lesser extent, 22:5n-3 in PE and PC, caused by *trans* fatty acids as observed in our experiment, may indicate a different influence on the second Δ6-desaturation of n-6 and n-3 fatty acids, favoring the n-3 species. Although there are only small differences in the amounts of linolenic acid (0.4–0.6 wt%) present in the PHSBO and BF diets, compared to the PO diet (0.2 wt%), we cannot exclude that the PO group was on the borderline of n-3 deficiency, resulting in decreased amounts of long-chain n-3 fatty acids.

The presence of large amounts of 22:6n-3 in heart PE is interesting because Pepe and McLennan (42) and McLennan *et al.* (43) have documented that dietary very long-chain n-3 PUFA from fish oils have an antiarrhythmic effect in rats and monkeys, respectively. Sellmayer *et al.* (44) showed the same effects in man when investigating the effects of dietary n-3 PUFA on the frequency of ventricular premature complexes in 68 patients with frequent ventricular arrhythmias but good ventricular function.

If the increased 22:6n-3 and 22:5n-3 levels in rat liver and

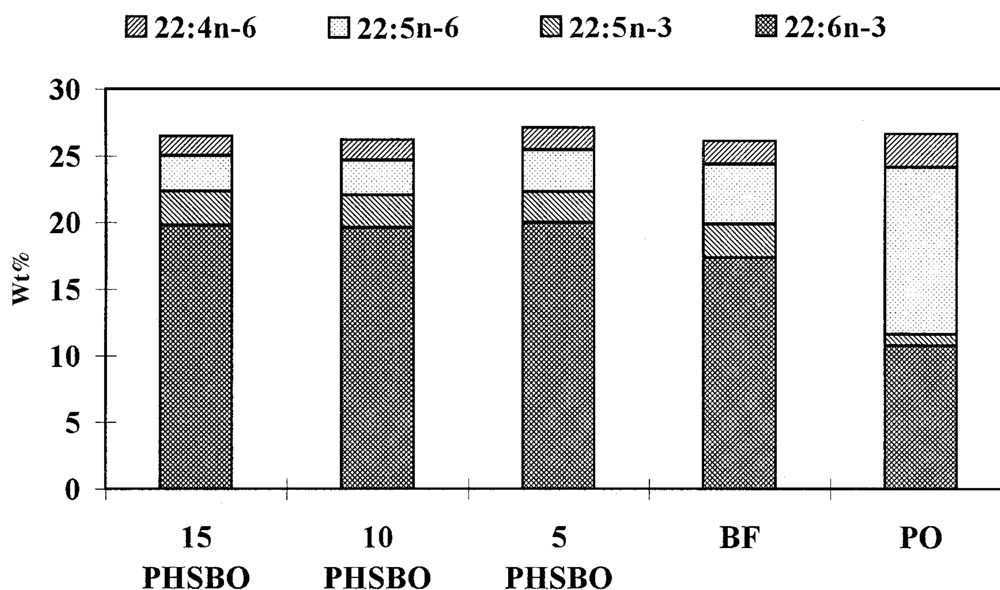

FIG. 3. Content of C_{22}-polyunsaturated fatty acids (wt% of total) in rat heart PE. PO, palm oil; for other abbreviations, see Figures 1 and 2. Note that additional fats and oils were added; for composition, see Table 1.

heart can be related to the presence of *trans* fatty acids in the diets, our results may indicate that, besides their negative effects on blood lipids, *trans* fatty acids may influence 22:6n-3 deposition and thereby create an antiarrhythmic effect. Further studies are required to clarify whether the the levels of 22:6n-3 and 22:5n-3 in the groups fed *trans* fatty acids compared to PO are due to the slightly lower content of dietary 18:3n-3 in this group or a result of the *trans* 18:1 isomers present.

A comparison of the 15 PHSBO, BF, and PO groups, which contained almost equal amounts of *trans* fatty acids plus long-chain saturated fatty acids, is interesting because saturated fatty acids are often substituted for *trans* fatty acids to maintain a desirable texture in margarines. As expected, the PO diet resulted in greater formation of arachidonic acid and simultaneous lower amounts of linoleic acid, compared to the two groups that were fed diets with *trans* fatty acids of either vegetable or animal origin. These groups affected the contents of 18:2n-6 and 20:4n-6 equally in rat liver PC and rat heart PE and PC. In liver PE, however, the differences were more pronounced in the 15 PHSBO group than in the BF group. In considering the accumulation of C_{22}-PUFA in the two PL classes from rat liver and heart, the 15 PHSBO and BF groups changed the composition toward higher amounts of the two n-3 fatty acids at the expense of the n-6 fatty acids. The influence on the contents of 22:4n-6, 22:5n-6, and 22:6n-3 was significantly higher for the 15 PHSBO group than for the BF group, although the content of 18:3n-3 was almost the same (0.5 or 0.4 wt%). The 22:5n-3 level was affected to approximately the same extent in the two groups.

Our results demonstrate that dietary fats affect PE and PC to a different extent in the two organs. The effect of *trans* isomers on linoleic acid conversion is less reflected in heart than in liver and less for PE than for PC. This hierarchy is in line with previous observations (20,21,35).

Further studies are needed to decide whether the extensive deposition of 22:6n-3 seen in tissues from rats that are fed *trans* fatty acids, compared to PO, is due to a specific effect of *trans* 18:1 isomers on the PUFA metabolism or to linolenate deficiency.

REFERENCES

1. Stender, S., J. Dyerberg, G. Hølmer, L. Ovesen, and B. Sandström, The Influence of *trans* Fatty Acids on Health: A Report from the Danish Nutrition Council, *Clin. Sci. 88*:375–392 (1995).
2. Dutton, H.J., Hydrogenation of Fats and Its Significance, in *Geometrical and Positional Fatty Acid Isomers,* edited by E.A. Emken and H.J. Dutton, The American Oil Chemists' Society, Champaign, 1979, pp. 1–16.
3. Fellner, V., F.D. Sauer, and J.K.G. Kramer, Steady-State Rates of Linoleic Acid Biohydrogenation by Ruminal Bacteria in Continuous Culture, *J. Dairy Sci. 78*:1815–1823 (1995).
4. Willett, W.C., M.J. Stampfer, J.E. Manson, G.A. Colditz, F.E. Speizer, B.A. Rosner, L.A. Sampson, and C.H. Hennekens, Intake of *trans* Fatty Acids and Risk of Coronary Heart Disease Among Women, *Lancet 341*:581–585 (1993).
5. Kris-Etherton, P.M., E.A. Emken, D.B. Allison, J.M. Dietschy, R.J. Nicolosi, and M.A. Denke, *Trans* Fatty Acids and Coronary Heart Disease Risk, *Am. J. Clin. Nutr. 62*:655S–707S (1995).
6. Kuller, L.H., *Trans* Fatty Acids and Dieting, *Lancet 341*: 1093–1094 (1993).
7. Salter, A.M., The Influence of *trans* Fatty Acids on Health, *Clin. Sci. 88*:373–374 (1995).
8. The British Nutrition Foundation Task Force, Trans *Fatty Acids,* British Nutrition Foundation, London, 1995, pp. 2–56.
9. Lichtenstein, A.H., L.M. Ausman, W. Carrasco, J.L. Jenner, J.M. Ordovas, and E.J. Schaefer, Hydrogenation Impairs the Hypolipidemic Effect of Corn Oil in Humans, *Arterioscler. Thromb. 13*:154–161 (1993).
10. Mensink, R.P., and M.B. Katan, Effect of Dietary *trans* Fatty Acids on High-Density and Low-Density Lipoprotein Cholesterol Levels in Healthy Subjects, *N. Eng. J. Med. 323*:439–445 (1990).
11. Mensink, R.P., P.L. Zock, M.B. Katan, and G. Hornstra, Effect of Dietary *cis* and *trans* Fatty Acids on Serum Lipoprotein(a) Levels in Humans, *J. Lipid Res. 33*:1493–1501 (1992).
12. Nestel, P., M. Noakes, B. Belling, R. McArthur, P. Clifton, E. Janus, and M. Abbey, Plasma Lipoprotein Lipid and Lp(a) Changes with Substitution of Elaidic Acid for Oleic Acid in the Diet, *Ibid. 33*:1029–1036 (1992).
13. Wood, R., K. Kubena, B. O'Brian, S. Tseng, and G. Martin, Effect of Butter, Mono- and Polyunsaturated Fatty Acid-Enriched Butter, *trans* Fatty Acid Margarine, and Zero *trans* Fatty Acid Margarine on Serum Lipids and Lipoproteins in Healthy Men, *Ibid. 34*:1–11 (1993).
14. Judd, J.T., B.A. Clevidence, R.A. Muesing, J. Wittes, M.E. Sunkin, and J.J. Podczasy, Dietary *trans* Fatty Acids: Effects on Plasma Lipids and Lipoproteins of Healthy Men and Women, *Am. J. Clin. Nutr. 59*:861–868 (1994).
15. Houwelingen, A.C.v., and G. Hornstra, *Trans* Fatty Acids in Early Human Development, in *Fatty Acids and Lipids: Biological Aspects. World Review of Nutrition and Diet,* edited by C. Galli, A.P. Simopoulos, and E. Tremoli, Karger, Basel, 1994, pp. 175–178.
16. Koletzko, B., *Trans* Fatty Acids May Impair Biosynthesis of Long-Chain Polyunsaturates and Growth in Man, *Acta Pædiatr. 81*:302–306 (1992).
17. Decsi, T., and B. Koletzko, Do *Trans* Fatty Acids Impair Linoleic Acid Metabolism in Children? *Ann. Nutr. Metab. 39*:36–41 (1995).
18. Wood, R., Incorporation of Dietary *cis* and *trans* Octadecenoate Isomers in the Lipid Classes of Various Rat Tissues, *Lipids 14*:975–982 (1979).
19. Høy, C.-E., and G. Hølmer, Incorporation of *cis*- and *trans*-Octadecenoic Acids into Membranes of Rat Liver Mitochondria, *Ibid. 14*:727–733 (1979).
20. Høy, C.-E., and G. Hølmer, Dietary Linoleic Acid and the Fatty Acid Profiles in Rats Fed Partially Hydrogenated Marine Oils, *Ibid. 23*:973–980 (1988).
21. Zevenbergen, J.L., U.M.T. Houtsmuller, and J.J. Gottenbos, Linoleic Acid Requirement of Rats Fed *trans* Fatty Acids, *Ibid. 23*:178–186 (1988).
22. Lawson, L.D., E.G. Hill, and R.T. Holman, Dietary Fats Containing Concentrates of *cis* or *trans* Octadecenoates and the Patterns of Polyunsaturated Fatty Acids of Liver Phosphatidylcholine and Phosphatidylethanolamine, *Ibid. 20*:262–267 (1985).
23. Kirstein, D., C.-E. Høy, and G. Hølmer, Effect of Dietary Fats on the Δ6- and Δ5-Desaturation of Fatty Acids in Rat Liver Microsomes, *Br. J. Nutr. 50*:749–756 (1983).
24. Aaes-Jørgensen, E., and G. Hølmer, Essential Fatty Acid-Deficient Rats: I. Growth and Testis Development, *Lipids 4*:501–506 (1969).
25. Folch, J., M. Lees, and G.H.S. Stanley, A Simple Method for

the Isolation and Purification of Total Lipides from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1967).
26. Christie, W.W., Structural Analysis of Lipids by Means of Enzymatic Hydrolysis, in *Lipid Analysis,* edited by W.W. Christie, Pergamon Press, Oxford, 1982, pp. 155–166.
27. Wolff, R.L., and B. Entressangles, Steady-State Fluorescence Polarisation Study of Structurally Defined Phospholipids from Liver Mitochondria of Rats Fed Elaidic Acid, *Biochim. Biophys. Acta 1211*:198–206 (1994).
28. Wolff, R.L., Structural Importance of the *cis*-5 Ethylenic Bond in the Endogenous Desaturation Product of Dietary Elaidic Acid, *cis*-5, *trans*-9 18:2 Acid, for the Acylation of Rat Mitochondria Phosphatidylinositol, *Lipids 30*:893–898 (1995).
29. Wood, R., and F. Chumbler, Distribution of Dietary Octadecenoate Isomers at the 1- and 2-Position of Hepatoma and Liver Phospholipids, *Ibid. 13*:75–84 (1978).
30. Parodi, P.W., Distribution of Isomeric Octadecenoic Fatty Acids in Milk Fat, *J. Dairy Sci. 59*:1870–1873 (1976).
31. Emken, E.A., R.O. Adlof, W.K. Rohwedder, and R.M. Gulley, Incorporation of *trans*-8- and *cis*-8-Octadecenoic Acid Isomers in Human Plasma and Lipoprotein Lipids, *Lipids 24*:61–69 (1989).
32. Emken, E.A., W.K. Rohwedder, R.O. Adlof, W.J. DeJarlais, and R.M. Gulley, *In vivo* Distribution and Turnover of *trans*- and *cis*-10-Octadecenoic Acid Isomers in Human Plasma Lipids, *Biochim. Biophys. Acta 836*:233–245 (1985).
33. Reichwald-Hacker, I., S. Grosse-Oetringhaus, I. Kiewitt, and K.D. Mukherjee, Incorporation of Positional Isomers of *cis*- and *trans*-Octadecenoic Acids into Acyl Moieties of Rat Tissue Lipids, *Ibid. 575*:327–334 (1979).
34. Koga, T., T. Yamato, I. Ikeda, and M. Sugano, Effects of Randomization of Partially Hydrogenated Corn Oil on Fatty Acid and Cholesterol Absorption, and Tissue Lipid Levels in Rats, *Lipids 30*:935–940 (1995).
35. Lawson, L.D., E.G. Hill, and R.T. Holman, Suppression of Arachidonic Acid in Lipids of Rat Tissues by Dietary Mixed Isomeric *cis* and *trans* Octadecenoates, *J. Nutr. 113*:1827–1835 (1983).
36. Sprecher, H., D.L. Luthria, B.S. Mohammed, and S.P. Baykousheva, Reevaluation of the Pathways for the Biosynthesis of Polyunsaturated Fatty Acids, *J. Lipid Res. 36*:2471–2477 (1995).
37. Sugano, M., and I. Ikeda, Metabolic Interactions Between Essential and *trans*-Fatty Acids, *Curr. Opin. Lipid 7*:38–42 (1996).
38. Voss, A., M. Reinhart, S. Sankarappa, and H. Sprecher, The Metabolism of 7,10,13,16,19-Docosapentaenoic Acid to 4,7,10,13,16,19-Docosahexaenoic Acid in Rat Liver Is Independent of a 4-Desaturase, *J. Biol. Chem. 266*:19995–20000 (1991).
39. Mohammed, B.S., S. Sankarappa, M. Geiger, and H. Sprecher, Reevaluation of the Pathway for the Metabolism of 7,10,13,16-Docosatetraenoic Acid to 4,7,10,13,16-Docosapentaenoic Acid in Rat Liver, *Arch. Biochem. Biophys. 317*:179–184 (1995).
40. Moore, S.A., E. Hurt, E. Yoder, H. Sprecher, and A.A. Spector, Docosahexaenoic Acid Synthesis in Human Skin Fibroblasts Involves Peroxisomal Retroconversion of Tetracosahexaenoic Acid, *J. Lipid Res. 36*:2433–2443 (1996).
41. Marzo, I., M.A. Alava, A. Piñeiro, and J. Naval, Biosynthesis of Docosahexaenoic Acid in Human Cells: Evidence That Two Different Δ^6-Desaturase Activities May Exist, *Biochim. Biophys. Acta 1301*:263–272 (1996).
42. Pepe, S., and P.L. McLennan, Dietary Fish Oil Confers Direct Antiarrhythmic Properties on the Myocardium of Rats, *J. Nutr. 126*:34–42 (1996).
43. McLennan, P.L., T.M. Bridle, M.Y. Abeywardena, and J.S. Charnock, Comparative Efficacy of n-3 and n-6 Polyunsaturated Fatty Acids in Modulating Ventricular Fibrillation Threshold in Marmoset Monkeys, *Am. J. Clin. Nutr. 58*:666–669 (1993).
44. Sellmayer, A., H. Witzgall, R. Lorenz, and P.C. Weber, Effects of Dietary Fish Oil on Ventricular Premature Complexes, *Am. J. Cardiol. 76*:974–977 (1996).

[Received November 14, 1996; accepted May 19, 1997]