Absorption of very-long-chain saturated fatty acids in totally hydrogenated fish oil

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Partially hydrogenated fish oil (PHFO) contains a high amount of *trans* fatty acids (TFA). Total hydrogenation results in a minimal amount of TFA, but a high content of very-long-chain saturated fatty acids (VLCSFA). Absorption and metabolism of VLCSFA from totally hydrogenated fish oil (THFO) were studied in rats. Groups of eight rats were fed one of four diets containing 40 g soyabean oil (SBO)/kg (low-fat diet), 150 g SBO/kg (SBO diet), 40 g SBO + 110 g PHFO/kg (PHFO diet) or 40 g SBO + 110 g THFO/kg (THFO diet) for 4 weeks. A lower absorption coefficient of the fat content was found in the THFO group (61 %) compared with the other groups (PHFO 95 %, SBO 99 %, low fat 98 %; $P < 0{\cdot}05$), which was mainly due to reduced absorption of VLCSFA. A reduced weight gain was found for the THFO group compared with the other groups, but this was only significant when compared with the SBO group ($P < 0{\cdot}05$). Faecal fat excretion (dry weight) was markedly increased in the THFO group (47 %), which was 2·4, 4·8 and 8·3 times higher compared with the groups fed PHFO, SBO and low-fat diets ($P < 0{\cdot}05$), respectively. Serum total cholesterol was reduced for the PHFO and THFO groups ($P < 0{\cdot}05$), whereas serum triacylglycerol was increased for the PHFO group compared with the other groups ($P < 0{\cdot}05$). Animals fed THFO diet had an increased content of 20:0 and 22:0 in the serum triacylglycerol fraction ($P < 0{\cdot}05$), whereas only 20:0 was increased in the serum phospholipid fraction ($P < 0{\cdot}05$). The low absorption coefficient of THFO must be considered if this fat is to be used for consumption by animals or man.

Dietary fat: Hydrogenated fish oil: Bioavailability

Appreciable quantities of fish oil are available for food production, but because of the stability, taste and flavour of this oil, processing, in practice partial hydrogenation, has been necessary before introducing it to, for example, margarine. Partial hydrogenation will, in addition to conversion of unsaturated fatty acids to saturated fatty acids, result in *cis–trans* isomerism, with formation of relatively high amounts of *trans* fatty acids (TFA). Intake of TFA is associated with increased risk of CHD (Ascherio *et al.* 1994). One alternative method for processing is total hydrogenation, which results in fat containing high amounts of long-chain saturated fatty acids (LCSFA) (stearic acid (18:0)) and very-long-chain saturated fatty acids (VLCSFA) (arachidic acid (20:0), behenic acid (22:0) and tetracosanoic acid (24:0)). Only minor amounts of TFA are formed during total hydrogenation of fish oil.

Kaplan & Greenwood (1998) showed a low absorption of totally hydrogenated soyabean oil (SBO) compared with medium-chain triacylglycerols, hydrogenated coconut oil and SBO. They related the low bioavailability of totally hydrogenated SBO to the high content of LCSFA. Totally hydrogenated SBO is especially rich in stearic acid (18:0) (800 g/kg total fat content). High content of stearic acid (18:0) leads to an increased portion present as tristearin, which is shown to reduce stearic acid absorption (Mattson, 1959; Bergstedt *et al.* 1990). The relationship between stearic acid (18:0) and tristearin may also apply to fatty acids with longer chain length (Mattson, 1959), but not for mono- and polyunsaturated fatty acids with the same chain length (Bergstedt *et al.* 1990). No previous studies have been published regarding the absorption of VLCSFA from totally hydrogenated fish oil (THFO). Our hypothesis, based upon the results from Mattson (1959) and Kaplan & Greenwood (1998), would be that reduced bioavailability

Abbreviations: LCSFA, long-chain saturated fatty acids; PHFO, partially hydrogenated fish oil; SBO, soyabean oil; TFA, *trans* fatty acids; THFO, totally hydrogenated fish oil; VLCSFA, very-long-chain saturated fatty acids.

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of THFO compared with partially hydrogenated fish oil (PHFO) is due to the increased content of VLCSFA. The purpose of the present study was to determine the absorption and metabolic effects of VLCSFA in THFO in comparison with SBO and PHFO.

Methods

Animals and diets

Male PVG rats (B&K Universal AB, Sollentuna, Sweden), initially weighing about 120 g, were fed one of four diets *ad libitum* for 4 weeks. The animals were housed individually in stainless-steel wire-bottomed cages specially designed for metabolic experiments (Hansen & Holm, 1971), in a temperature- (22–23°C) and humidity- (40–60 %) controlled environment with a 12 h light–dark cycle. Diets were fed from food cups secured with a spring to minimize spillage. The local responsible veterinarian approved the protocol. The diet composition (Table 1) was as described by Thomassen *et al.* (1979), with small modifications. The diets contained 40 g SBO/kg (low-fat diet), 150 g SBO/kg (SBO diet), 40 g SBO + 110 g PHFO/kg (PHFO diet) and 40 g SBO + 110 g THFO/kg (THFO diet) respectively. The three high-fat diets contained 30 % energy as fat, while the low-fat diet contained 9 % energy from fat. The melting points of PHFO (30–32°C) and THFO (54–56°C) are markedly increased compared with the native oil, due to an increased content of saturated fatty acids after hydrogenation. For this reason PHFO and THFO were heated to 50°C and 65°C respectively, before blending the diet to an homogenous mixture. The fatty acid composition of the diets, determined by GC as described later, is shown in Table 2.

Experimental design

Animals were assigned to one of four dietary treatment groups (*n* 8 per group) by controlled randomization such that mean body weight did not differ among the groups. All the animals consumed one of the four diets for 4 weeks.

Table 1. Composition of the four diets (g/kg)

	Low-fat diet	SBO, PHFO and THFO diets
Cornstarch*	480	370
Casein†	200	200
Sucrose‡	200	200
Mineral mix†	50	50
Vitamin mix†	15	15
Cellulose§	10	10
L-Methionine‖	4	4
SBO¶	40	40
Additional oil¶	0	110

SBO, soyabean oil; PHFO, partially hydrogenated fish oil, THFO, totally hydrogenated fish oil.
* Hydro Chemicals Norge A/S, Etterstad, Norway.
† ICN, Nerlien, Oslo, Norway.
‡ Obtained from a local store.
§ Norsk Medisinaldepot (drugstore).
‖ Sigma Aldrich, Oslo, Norway.
¶ SBO, PHFO (Margarit 30/32) or THFO (HMF 54/56) from Denofa AS Fredrikstad, Norway. The source of the fish oil was a mix of several fish oils.

Table 2. Fatty acid composition (g/100 g) of the four diets fed during the experimental period*

Fatty acid	Low fat	SBO	PHFO	THFO
14:0	0·19	0·11	7·53	4·28
15:0			0·54	0·60
16:0	12·41	12·20	23·31	23·44
16:1*t*			6·11	0·24
16:1	0·10	0·10	2·92	0·19
17:0	0·09	0·09	0·42	0·76
17:1*t*			0·07	0·15
17:1	0·04	0·04		
18:0	3·58	3·51	6·03	20·49
18:1*t*			5·86	0·28
18:1	23·85	23·95	14·01	9·22
19:0			0·27	0·35
18:2*n*-6	53·09	53·31	18·97	20·2
20:0	0·27	0·27	1·56	8·09
20:1*t*			4·17	0·51
20:1+18:3*n*-3	6·07	6·15	5·42	2·27
18:4*n*-6	0·04	0·03	0·04	
21:0			0·10	0·26
22:0	0·26	0·27	1·03	7·48
22:1*t*			2·36	0·20
22:1			1·87	0·08
24:0				0·25

SBO, soyabean oil; PHFO, partially hydrogenated fish oil, THFO, totally hydrogenated fish oil; *t*, *trans*.
* For details of composition of diets see Table 1.

Prior to the start of the experiment there was an adjustment period of 3 d. Food intake and faecal excretion were measured daily, and the animals were weighed every 3 d. The small amount of food wastage was weighed daily and subtracted. Diets and faeces were stored at −20°C. The animals were killed by CO_2 exposure at the end of the experiment. The blood was withdrawn from the abdominal artery and the liver removed for isolation of peroxisomes.

Diet analysis

All diets were analysed for fat, protein (Kjeldahl analysis) and mineral (ash) content. The fat content was extracted using the method of Folch *et al.* (1957). Fatty acid analyses were performed as described later. To calculate the dietary energy available for absorption, fatty acids (the amount ingested and not excreted in faeces) were given the value of 39·4 kJ/g, which is the physical heat of combustion, whereas the Atwater value of 17 kJ/g was used for proteins and carbohydrates.

Faecal analysis

The faecal excretion from each animal was mixed with a small portion of water, freeze-dried, ground with a mortar and pestle into an homogenous mixture and stored at −20°C. Lipid was extracted using a modification of the method of Folch *et al.* (1957). A small amount of water (0·25 ml) was added to 0·25 g freeze-dried faeces. Methanol (2·5 ml) and chloroform (5 ml) were added to each tube, which were shaken and heated at 60°C for 1 h (Kamei *et al.* 1995). The samples were centrifuged, reheated and transferred to a second test tube. The extraction was performed twice. KCl in water (8·8 g/l)

was added to each sample prior to centrifugation. The lipid phase was dried under N_2 and put in an exicator to obtain complete dryness (Kaplan & Greenwood, 1998). Faeces from the group fed THFO contained an interphase of non-extracted fat after the first extraction. This interface was saponified by addition of KOH in methanol (2 ml, 150 g/l) and heated at 65°C for 45 min. HCl was added to pH 1–2, and the sample was extracted three times with hexane (3 ml). The fatty acid extract was dried under N_2 and put in an exicator for complete drying. Heating was necessary to complete the extraction in both procedures because of the increased melting point of PHFO and THFO compared with the native oil. The faeces samples from each diet group were further analysed for protein (Kjeldahl analysis) and mineral (ash) content.

Serum analysis

The concentration of triacylglycerol and total cholesterol in serum was measured by enzyme kits obtained from Bio Mérieux (Marcy l'Etoile, France). Total lipids were extracted by the method of Folch *et al.* (1957). Phospholipids and triacylglycerols were separated by TLC (hexane–diethylether–acetic acid (80:20:1, by vol.)).

Fatty acid analysis

The extracted fatty acids from faeces and diets were methylated with benzene and methanolic HCL (3 M) (Supelco Inc., Bellefonte, PA, USA) using the method of Hoshi *et al.* (1973). Tricosanoic acid (23:0) was added as an internal fatty acid standard (corresponding to approximately 10 % fatty acids content). Fatty acids from the serum phospholipid and triacylglycerol fractions were transmethylated (methanolic HCl (3 M), benzene, dimethoxypropane) using the method of Hoshi *et al.* (1973). L-α-Phosphatidylcholine diheptadecanoyl and triheptadacanic acid were used as internal standard for phospholipids and triacylglycerols respectively. The fatty acid composition of diets, faeces, phospholipid and triacylglycerol fractions were analysed by GC (Shimadzu GC-17A; Shimadzu, Kyoti, Japan) with a polar Supelco capillary column (SP-2560, 100 m × 0·25 mm; Supelco Inc.) and flame ionization detection. The carrier gas was He. Retention times were verified with purified standards (Nu Chek Prep Inc., Elysian, MN, USA).

Other assays

Peroxisomes were isolated from liver tissue by subcellular fractionation (Prydz *et al.* 1988) and density-gradient centrifugation (Ruyter *et al.* 1992). The peroxisomal fatty acid oxidation activity was determined by measuring the activity of the rate-limiting enzyme fatty acid oxidase, using a method developed by Small *et al.* (1985) with minor modifications (Spydevold & Bremer, 1989).

Statistical analyses

A one-way ANOVA Bonferroni test for variance was used to compare the group variances, except where data from only two groups were available. In these cases Student's *t* test was used. For all analyses, the acceptable level of significance (type 1 error) was $P \leq 0{\cdot}05$. Statistical

Table 3. Apparent fatty acid absorption (mmol) in rats fed on one of four diets for 4 weeks§
(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0·10	0·01	0·23	0·02	16·60*†	0·68	14·90*†‡	0·73
15:0					1·10	0·11	1·73‡	0·10
16:0	7·20	0·56	26·90*	1·14	49·10*†	2·80	50·90*†	3·73
16:1*t*					13·40	0·60	0·62‡	0·03
16:1	0·04	0·01	0·20	0·01	6·60*†	0·26	0·45*†‡	0·03
17:0	0·03	0·01	0·17	0·01	0·83*†	0·07	1·25*†‡	0·13
17:1	0·01	0·00	0·06*	0·01	0·15*†	0·01	0·30*†‡	0·04
18:0	2·00	0·18	7·30*	0·40	11·60*†	1·10	26·90*†‡	3·30
18:1*t*					12·00	1·20		
18:1	14·30	1·10	54·10*	2·50	31·10*†	1·40	21·00*†‡	2·30
19:0					0·50	0·03	0·18‡	0·04
18:2*n*-6	31·90	2·40	120·60*	5·60	42·90*†	1·70	47·90*†	2·96
20:0	0·13	0·02	0·50	0·06	2·90*†	0·30	5·64*†‡	1·13
20:1*t*					7·90	0·90	0·17‡	0·03
20:1+18:3*n*-3	3·60	0·30	13·90*	0·64	11·70*†	0·66	5·60*†‡	0·34
18:4*n*-6	0·01	0·01	0·06*	0·01	0·09*†	0·00		
21:0					0·13	0·04	0·09	0·04
22:0	0·12	0·02	0·49	0·07	2·40*†	0·09	3·62*†‡	1·07
22:1*t*					4·20	0·68	0·10‡	0·09
22:1					3·58	0·43		
Total	59·40	0·42	234·50	0·87	218·80	0·70	181·40	0·95

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil; *t*, *trans*.
Mean values were significantly different from the low-fat group: *$P < 0{\cdot}05$; mean values were significantly different from the SBO group: †$P < 0{\cdot}05$; mean values were significantly different from the PHFO group: ‡$P < 0{\cdot}05$.
§ Ingested fatty acids that are not excreted in the faeces (mmol fatty acid apparently absorbed during the whole feeding period). For details of composition of diets see Tables 1 and 2.

Table 4. Absorption coefficients of fatty acids (%) in rats fed one of four diets for 4 weeks§

(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	84	6	90	4	98*†	1	87‡	4
15:0					88	7	74‡	5
16:0	97	2	98	2	93	4	62*†‡	5
16:1*t*					97	2	74‡	5
16:1	61	14	90*	5	99*	1	88*	5
17:0	56	22	84*	9	88*	6	47†‡	5
17:1	43	28	82*	8	87*	5	62‡	9
18:0	92	5	92	5	84	7	39*†‡	5
18:1*t*					87	7		
18:1	98	1	99	0	96†	3	92*†‡	1
19:0					90	4	21‡	4
18:2*n*-6	99	0	99	0	99*†	1	99*†‡	0
20:0	80	12	85	8	81	7	23*†‡	4
20:1*t*					82	8	53‡	11
20:1+18:3*n*-3	96	3	97	2	84*†	7	85*†	2
18:4*n*-6	48	29	78*	12	99*	1		
21:0					61	16	13‡	5
22:0	79	12	82	8	97*†	1	18*†‡	5
22:1*t*					71	11	21‡	19
22:1					78	8		
Total	98		99		95		61	

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil; *t*, *trans*.

Mean values were significantly different from the low-fat group: $*P < 0{\cdot}05$; mean values were significantly different from the SBO group: $\dagger P < 0{\cdot}05$; mean values were significantly different from the PHFO group: $\ddagger P < 0{\cdot}05$.

§ Ingested fatty acids that are not excreted in the faeces (% fatty acids apparently absorbed during the feeding period). For details of the composition of diets see Tables 1 and 2.

analysis was conducted using SPSS 9.0 (SPSS Inc., Chicago, IL, USA).

Results

Apparent lipid absorption

The apparent lipid absorption calculated as the difference between fatty acid content in diet and faeces (Monsma *et al.* 1996; Kaplan & Greenwood, 1998), expressed as mmol as well as percentage absorption (absorption coefficient) are shown in Tables 3 and 4 respectively. For the fatty acids in the THFO diet the absorption coefficient was markedly reduced (61 %) compared with the other groups (low fat 98 %, SBO 99 %, PHFO 95 %; $P < 0{\cdot}05$); however, in terms of amount (mmol), the absorption of LCSFA and VLCSFA (18:0, 20:0 and 22:0) was higher compared with the other groups. The absorption coefficient for the different saturated fatty acids decreased with increasing chain length, a tendency which was most pronounced in the group fed THFO diet. The absorption of VLCSFA was also lower compared with unsaturated fatty acids with the same chain length. In addition to reduced absorption of VLCSFA, there was a reduced absorption of TFA (16:1 *trans*; 18:1 *trans*, 20:1 *trans* and 22:1 *trans*) in the group fed THFO diet compared with the PHFO diet ($P < 0{\cdot}05$). The absorption coefficient of PHFO and THFO may be underestimated due to the supplementation of these diets with SBO to provide a sufficient amount of essential fatty acids.

The difference in absorption coefficients between the four diets results in different energy densities of the diets. The energy available for absorption, assuming that the three high-fat diets were isoenergetic, has been calculated, as well as the energy actually absorbed using the lipid absorption coefficients (Table 5). The calculations of energy actually absorbed were performed taking the differences in protein excretion into consideration (Table 6), but assuming no difference in carbohydrate absorption. The dietary energy intake available for absorption did not differ between the four groups. However, the energy actually absorbed differed among the groups, with the animals fed THFO diet absorbing the lowest amount. The absorbed energy in the THFO group was lower compared with the SBO and PHFO groups ($P < 0{\cdot}05$), but also compared with the low-fat group, although this difference was not significant. The slightly increased food intake did not appear to compensate for the reduced amount of available energy in the diet containing THFO.

Table 5. Experimental variables related to growth and metabolism for rats fed one of the four diets for 4 weeks§

(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Energy available for absorption (kJ/4 weeks)‖	8010	689	8170	499	8288	472	8401	540
Apparent energy absorbed (kJ/4 weeks)¶	7252	642	7520	463	7509	431	6780†‡	441
Body wt gain (g/28 d)	111·4	18·5	121·4	15·2	118·0	9·0	98·0†	15·2
Total food intake (g/28 d)	441·1	38·7	398·4*	24·3	404·1	23·0	409·6	26·3
Relative liver wt (g/kg body weight)	46	46	46	3	48	2	46	2
Total serum cholesterol (mM)	3·9	0·6	4·0	0·3	3·3†	0·3	3·3*†	0·5
Serum triacylglycerol (mM)	2·2‡	0·5	2·5‡	0·7	4·5	1·0	2·0‡	0·3
Fatty acid oxidase**	134·3	44·2	196·7*	72·9	212·2*	79·2	139·6‡	21·2

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Mean values were significantly different from the low-fat group: $*P < 0{\cdot}05$; mean values were significantly different from the SBO group: $\dagger P < 0{\cdot}05$; mean values were significantly different from the PHFO group: $\ddagger P < 0{\cdot}05$.

§ For details of composition of diets see Tables 1 and 2.

‖ Calculation based on the assumption that all high-fat diets provided 20 508 kJ/kg and the low-fat diet 18 066 kJ/kg.

¶ Apparent energy absorbed calculated by using adjusted energy density based on the apparent lipid absorption coefficient and protein absorption measured as protein intake in the diet minus faecal excretion.

** Expressed as nmol dichlorofluorecin oxidized/min per mg protein.

Table 6. Faecal contents of rats fed one of the four diets for 4 weeks§

(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Variable	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Faecal wet wt (g/28 d)	21·9	2·5	19·1	2·3	23·2†	1·6	51·8*†‡	3·9
Water (% wet wt)	23·6	1·7	20·1*	3·0	17·8*	1·7	20·1*‡	2·4
Fat (% dry wt)	5·7	0·8	9·9*	0·4	19·4*†	1·3	47·2*†‡	1·6
Protein (% dry wt)	20·9	4·4	22·0	1·1	19·2*†	2·7	11·7*†‡	3·1
Minerals (% dry wt)	32·4	0·3	29·4	2·0	21·4	0·1	10·9*†‡	0·0

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Mean values were significantly different from the low-fat group: *$P < 0·05$; mean values were significantly different from the SBO group: †$P < 0·05$; mean values were significantly different from the PHFO group: ‡$P < 0·05$.

§ For details of composition of diets see Tables 1 and 2.

Variables related to growth and metabolism

A summary of the body-weight gain, food intake, relative liver weight, total serum cholesterol, serum triacylglycerol and liver fatty acid oxidase activity is shown in Table 5. Animals fed the THFO diet had a lower weight gain compared with the group fed SBO diet ($P < 0·05$). Total food intake during the experimental period was higher for the group fed the low-fat diet compared with the animals fed SBO diet ($P < 0·05$). However, there was no significant difference in food intake between the THFO group and the other groups. The relative liver weights did not differ among the four dietary groups. Animals fed PHFO and THFO diets had lower serum total cholesterol concentration compared with the group fed SBO diet ($P < 0·05$), and concentrations in the THFO group were also lower compared with the low-fat group ($P < 0·05$). The animals fed the PHFO diet had increased serum triacylglycerol concentrations compared with the other groups ($P < 0·05$). Fatty acid oxidase activity was increased in the groups fed SBO and PHFO diets compared with the low-fat group ($P < 0·05$), and was also increased in the PHFO group compared with the THFO group ($P < 0·05$).

Faeces analysis

Faecal excretion (wet weight) as well as percentage of fat, protein and minerals in faeces is shown in Table 6. Faecal excretion (wet weight) was markedly increased for the THFO group compared with the other groups ($P < 0·05$) and also increased in the PHFO group compared with the SBO group ($P < 0·05$). Faeces excreted by rats fed THFO diet contained a higher percentage of fat (47 %) than the faeces excreted by the PHFO fed animals (19 %), which in turn, contained a higher percentage than that from the animals fed SBO (10 %) and low-fat (6 %) diets ($P < 0·05$). The percentage protein was decreased for the PHFO and THFO groups compared with the other groups ($P < 0·05$), in addition to the THFO group having reduced protein content compared with the PHFO group ($P < 0·05$). The percentage faecal mineral content was reduced in the THFO group compared with all the other groups ($P < 0·05$).

Table 7. Fatty acid composition (g/100 g) of the serum triacylglycerol fraction in rats fed one of four diets for 4 weeks§

(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	1·3	0·5	1·1	1·5	5·8*†	0·4	4·2*†	1·6
14:1	0·1	0·0			0·1	0·0	0·1	0·0
15:0	0·3	0·1	0·3	0·3	0·6	0·0	0·7*†	0·3
16:0	32·4	2·9	22·2*	5·0	26·0*	1·5	28·8†	4·1
16:1*t*	0·7	0·1	0·4	0·3	6·4*†	0·4	1·0†‡	0·3
16:1	6·1	1·8	1·1*	0·5	3·1*†	0·4	2·2*	1·6
17:1*t*	0·1	0·0	2·0	5·3	0·2	0·0	0·2	0·1
17:1	0·1	0·1	0·1	0·1	0·1	0·0	0·1	0·1
18:0	2·4	0·3	6·6	8·3	3·4	0·3	8·4	2·7
18:1*t*					6·3	0·3	0·5‡	0·3
18:1	31·0	4·8	24·3	7·1	19·8*	0·9	22·4*	3·3
19:0					0·4	0·2		
18:2*n*-6	20·3	4·0	33·1	13·5	14·8†	1·9	23·9	8·8
20:0	0·1	0·0	0·5	0·8	0·6	0·1	1·5*†‡	0·6
18:3*n*-3	0·1	0·0	0·1	0·1	0·1*†	0·0	0·1‡	0·0
20:1*t*					2·2	0·2		
20:2*n*-6,9*tt*					1·0	0·2		
20:1	1·9	0·7	2·1	0·9	3·5*†	0·4	2·1‡	0·7
20:2*n*-6	0·1	0·1	0·3	0·1	0·1†	0·0	0·1†	0·1
20:4*n*-6	1·5	1·4	4·2	8·2	0·4	0·1	1·0	0·3
21:0	0·1	0·0	0·1	0·1	0·2*†	0·1	0·1‡	0·0
22:0	0·1	0·0	0·4	0·7	0·3	0·1	1·0*†‡	0·4
22:1*t*					1·0	0·2		
22:2*tt*+22:2*tc*					0·2	0·1		
22:1					1·1	0·2		
22:4*n*-6	0·3	0·2	0·3	0·2	0·1*†	0·0	0·2	0·1
22:6*n*-3	0·3	0·2	0·6	1·0	0·1	0·0	0·3	0·1
24:0	0·1	0·0	0·1*‡	0·0	0·1	0·0	0·1†	0·0

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil; *t*, *trans*; *c*, *cis*.

Mean values were significantly different from the low-fat group: *$P < 0·05$; mean values were significantly different from the SBO group: †$P < 0·05$; mean values were significantly different from PHFO group: ‡$P < 0·05$.

§ For details of composition of diets see Tables 1 and 2.

Fatty acids in serum triacylglycerol and phospholipid fractions

The percentage arachidic (20:0) and behenic acid (22:0) in the serum triacylglycerol fraction was higher in the group fed THFO diet compared with the other groups ($P < 0·05$) (Table 7). However, in the phospholipid fraction only arachidic acid was increased for the PHFO and THFO groups compared with the low-fat group ($P < 0·05$) (Table 8).

Discussion

This present study shows a low absorption coefficient of fat in the THFO diet compared with the other diets. This observation is in agreement with earlier studies on totally hydrogenated SBO and VLCSFA (Jandacek *et al.* 1993; Kaplan & Greenwood, 1998). The absorption coefficient indicates that nearly 40 % fat in the THFO diet was not absorbed, which is in agreement with the study of Kaplan & Greenwood (1998), who showed a bioavailability coefficient of totally hydrogenated SBO of 41·5 %. In their study, totally hydrogenated SBO contributed 40 % total energy content in the diet, compared with 30 % energy as fat in our study.

Table 8. Fatty acid composition (g/100 g) of the serum phospholipid fraction in rats fed one of four diets for 4 weeks§

(Mean values and standard deviations for eight rats per group)

	Low fat		SBO		PHFO		THFO	
Fatty acid	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0·6	0·2	0·7	0·8	1·1	0·6	0·9	0·6
15:0	0·2	0·0	0·2	0·2	0·3	0·1	0·4*†	0·1
16:0	27·8	3·6	26·5	3·8	22·6*	3·0	25·4	3·8
16:1*t*	0·3	0·1	0·3	0·1	3·1*†	1·3	0·3‡	0·1
16:1	1·1	0·3	0·4*	0·2	0·8	0·5	0·5*	0·4
17:1*t*	0·3	0·1	0·3	0·1	0·3	0·1	0·3	0·0
17:1	0·1	0·0	0·1	0·0	0·1	0·1	0·1	0·0
18:0	21·5	7·8	26·0	3·3	18·5†	2·2	25·8‡	1·4
18:1*t*					6·2	0·9	0·3‡	0·2
18:1	11·4	6·2	8·2	4·2	11·2	2·6	5·9	1·6
19:0					0·3	0·1		
18:2*n*-6	19·7	8·6	20·3	4·1	21·5	4·2	24·2	2·9
20:0	0·1	0·0	0·2	0·2	0·3*	0·1	0·3*	0·1
18:3*n*-3	0·1	0·1	0·1	0·0	0·1	0·1	0·1	0·0
20:1*t*					0·4	0·3		
20:2*n*-6,9*tt*					0·1	0·2		
20:1	0·7	0·6	0·5	0·7	1·2	1·5	0·3	0·4
20:2*n*-6	0·2	0·1	0·4*‡	0·2	0·2	0·1	0·2†	0·1
20:4*n*-6	13·1	6·1	12·6	6·9	8·3	5·3	12·1	3·1
21:0					0·1	0·1		
22:0	0·2	0·1	0·1	0·2	0·1	0·0	0·2	0·1
22:1*t*					0·1	0·1		
22:1					0·2	0·1		
22:4*n*-6	0·2	0·1	0·1	0·1	0·1	0·1	0·1	0·0
22:6*n*-3	1·9	1·0	1·9	1·2	1·8	1·3	1·6	0·6
24:0	0·1	0·0	0·1*†	0·0	0·1	0·0	0·1†	0·0

SBO, soyabean oil; PHFO, partially hydrogenated fish oil; THFO, totally hydrogenated fish oil; *t*, *trans*; *c*, *cis*.

Mean values were significantly different from the low-fat group: *$P < 0·05$; mean values were significantly different from the SBO group: †$P < 0·05$; mean values were significantly different from the PHFO group: ‡$P < 0·05$.

§ For details of composition of diets see Tables 1 and 2.

The low bioavailability of THFO was mainly due to low absorption of LCSFA and VLCSFA, which decreased with increasing chain length of the fatty acids. In the study by Kaplan & Greenwood (1998), the low absorption of totally hydrogenated SBO was related to the high content of saturated fatty acids, especially stearic acid (18:0) that amounted to 70 %. Furthermore, Apgar *et al.* (1987) showed low absorption of cocoa butter, which was also due to a high content of saturated fatty acids. The content of stearic acid (18:0) in cocoa butter is 300–360 g/kg (Gunstone *et al.* 1986). In our study the THFO diet contained 200 g stearic acid/kg (18:0), which is much higher compared with the other diets (low-fat and SBO 40 g/kg, PHFO 60 g/kg). Consequently, a high content of tristearin in the THFO diet could be the reason for low absorption of stearic acid (18:0) in this group (39 %) compared with the other groups (84–95 %). The mechanism responsible for reduced absorption of stearic acid (18:0) as tristearin could be reduced or slow lipolysis of triacylglycerol containing stearic acid (18:0), as indicated by Bergstedt *et al.* (1990). Increased content of nonadecanoic (19:0) and heneicosanoic acid (21:0), as well as pentadecanoic (15:0) and palmitic acid (16:0) in the diet also resulted in reduced absorption of these fatty acids, as was indicated by Mattson (1959). Jandacek *et al.* (1993) showed lower absorption of triacylglycerol with increasing chain length of the saturated fatty acids. They found behenic acid (22:0) to have absorption of 19–29 %, which is in agreement with our present study of an absorption of 18 % in the group fed THFO. The absorption of arachidic (20:0) and tetracosanoic acid (24:0) was 36–41 % and 2–25 % in Jandacek's study (Jandacek *et al.* 1993), which is higher compared with our study, where the absorption was 23 % for arachidic acid (20:0) and no absorption for tetracosanoic acid (24:0).

Very-long-chain polyunsaturated fatty acids are most often found in position 2 in fish oil triacylglycerol (Sebedio, 1992). Due to hydrogenation, and since pancreatic lipase hydrolyses mainly in *sn*-1 and *sn*-3 positions, this will result in VLCSFA 2-monoacylglycerols. It is to be expected that this will contribute to the reduced absorption of saturated fatty acids in the PHFO and THFO groups.

Peters *et al.* (1997) showed that Olestra, which is a fat substitute, affects the absorption of other compounds, depending on the lipophilic character of the compounds. This could also be a reason for the reduced absorption of saturated fatty acids with shorter chain length in the THFO diet.

In addition to reduced absorption of saturated fatty acids in the THFO group, the absorption of TFA was reduced compared with fatty acids in *cis* configuration. This difference in absorption of isomeric fatty acids was mainly seen in the group fed THFO, but also in the group fed PHFO. The dietary content of TFA was markedly higher in the PHFO diet compared with the THFO diet, which indicates that the mechanism responsible for the reduced absorption of TFA is different from that of saturated fatty acids. *Cis–trans* configuration affects the polarity of a compound, with *cis* configuration being the more polar. TFA with higher lipophilic character can result in increased aggregation with non-absorptive saturated fatty acids (Peters *et al.* 1997), which can lead, furthermore, to reduced absorption compared with fatty acids in *cis* configuration.

The reduced absorption coefficient of THFO did not result in increased food intake, which is in contrast to the results of Kaplan & Greenwood (1998). A lower content of fat in our study (30 % energy) compared to Kaplan & Greenwood's study (40 % energy), in addition to higher absorption coefficient for THFO (61 %) compared with totally hydrogenated SBO (42 %), could be a reason for this disagreement.

The weight gain can be considered in relation to the available energy content in the diet. The THFO group had lower weight gain compared with the other groups, which may be associated with the reduced content of available energy of this diet.

Our present study showed decreased serum total cholesterol concentration in the PHFO and THFO groups compared with the other groups, which is in agreement with Duthie *et al.* (1988) who showed a lower concentration in rats fed PHFO compared with partially hydrogenated SBO and rapeseed oil. It should be noted that the distribution of HDL- and LDL-cholesterol in rats and man is very different, with rats having the major part of cholesterol present as HDL-cholesterol (Weisgraber *et al.* 1977). In our present study, the reduction in the PHFO

group could be due to the high amount of TFA which reduces HDL-cholesterol (Mensink & Katan, 1989), while in the THFO group it could be due to reduced absorption of cholesterol (Chen *et al.* 1989; Jandacek *et al.* 1993). The PHFO and THFO diets contain higher amounts of stearic (18:0) and behenic acid (22:0), as well as other very-long-chain saturated and monounsaturated fatty acids, which could contribute to the reduced concentration of serum total cholesterol in these groups (Hill *et al.* 1990; Jandacek *et al.* 1993; Kritchevsky, 1994).

The increased serum triacylglycerol concentration after feeding PHFO found in our present study is not in agreement with other studies in rats (Duthie *et al.* 1988) or human subjects (Almendingen *et al.* 1995). The reason for this disagreement is not known.

Incorporation of fatty acids into serum triacylglycerol and phospholipid can be used as a marker for the absorption of fatty acids from the lumen and utilization of the fatty acids in the organism respectively. The difference in incorporation of VLCSFA in serum triacylglycerol (20:0 and 22:0) and phospholipid (20:0) can be due to a selective incorporation of these fatty acids into the phospholipid fraction compared with the triacylglycerol fraction (Larsen *et al.* 1998).

Ishii *et al.* (1980) and Neat *et al.* (1981) showed an induction of the peroxisomal β-oxidation after intake of high-fat diets, which is in agreement with our present study with increased fatty acid oxidase activity in liver peroxisomes of animals fed SBO and PHFO diets compared with the other groups. SBO, PHFO and THFO diets are all high-fat diets, with a fat content of 30 % energy and the increase in peroxisomal activity for the SBO and PHFO groups can be explained by the high-fat content available from these diets. Induction of peroxisomal fatty acid oxidase activity after intake of PHFO has also been shown in other studies (Christiansen *et al.* 1979; Neat *et al.* 1980; Flatmark *et al.* 1988), and has been associated with a high content of TFA (Christiansen *et al.* 1979). The THFO diet cannot be regarded as a high-fat diet due to the low bioavailable fat content.

To summarize, we found reduced absorption of VLCSFA and TFA from the THFO diet compared with unsaturated fatty acids and fatty acids with *cis* configuration. Both serum total cholesterol and triacylglycerol concentrations were reduced for the THFO group compared with the PHFO group, although this was only significant for the triacylglycerol concentration. Except for the high faecal excretion of fat, we did not observe any harmful effects of VLCSFA during the study period. However, before THFO can be used for consumption by man there is a need for more studies on the effects of these fatty acids in human subjects.

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