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Original article

Trans isomers of long-chain n-3 polyunsaturated fatty acids in tissue lipid classes of rats fed with heated linseed oil

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Abstract – The isomerization of linolenic acid into mono- and di-*trans* isomers takes place during heat treatment. One of these compounds, 18:3Δ9c,12c,15t can be desaturated and elongated to form, in particular, the *trans* isomers of eicosapentaenoic and docosahexaenoic acids. This study was undertaken to observe the incorporation of these *trans* long-chain n-3 polyunsaturated fatty acids into the lipid classes of several tissues. Rats were fed for 8 weeks with heated linseed oil containing high levels of *trans* linolenic acid isomers. The lipid classes of the liver, kidney, heart and adrenals of these rats contained various levels of these compounds. The 20:5Δ5c,8c,11c,14c,17t represented between 80 and 90 % of the total 20:5 in the phospholipids, but 22:5Δ7c,10c,13c,16c, 19t and mainly 22:6Δ4c,7c,10c,13c,16c,19t were only present in small quantities. This may indicate that the *cis* fatty acids are better metabolized in these cases. Among the tissues studied, it was interesting to note a high level of 22:5Δ19t in the adrenals, particularly in cholesterol esters. © Inra/Elsevier, Paris

***trans* polyunsaturated fatty acid / liver / kidney / heart / adrenal / lipid classes**

Résumé – Isomères *trans* d'acides gras polyinsaturés n-3 à longue chaîne dans les classes lipidiques de tissus de rats ayant reçu de l'huile de lin chauffée. Au cours des traitements thermiques, l'acide linolénique est isomérisé et donne naissance à des acides gras comportant des liaisons *trans*. L'un de ces composés néoformés, l'acide 18:3Δ9c,12c,15t peut subir des désaturations et des élongations successives, pour donner en particulier des isomères *trans* des acides eicosapentaenoïque et docosahexaenoïque. Afin de tester l'incorporation de ces acides gras n-3 polyinsaturés à longue chaîne dans les classes lipidiques de plusieurs organes, de l'huile de lin chauffée, contenant des quantités notables d'isomères *trans* de l'acide linolénique, a été administrée à des rats pendant 8 semaines. Les classes lipidiques du foie, des reins, du cœur et des surrénales de ces animaux contenaient à des degrés divers ces acides gras *trans* à longue chaîne. Le 20:5Δ5c,8c,11c,14c,17t représentait entre 80 et 90 % des 20:5 totaux dans les classes de phospholipides, alors que le 22:5Δ7c,10c,13c,16c,19t et surtout le 22:6Δ4c,7c,10c,13c,16c,19t étaient présents à des niveaux bien plus faibles, ce qui laisse penser que dans ce cas, les acides gras *cis* sont utilisés prioritairement. Parmi les organes étudiés, les surrénales se distinguaient par de fortes teneurs en 22:5Δ7c,10c,13c,16c,19t, en particulier dans les esters de cholestérol. © Inra/Elsevier, Paris

acides gras polyinsaturés *trans* / foie / rein / cœur / surrénales / classes lipidiques

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ABBREVIATIONS

20:5Δ17t	20:5Δ5c, 8c, 11c, 14c, 17t
22:5Δ19t	22:5Δ7c, 10c, 13c, 16c, 19t
22:6Δ19t	22:6Δ4c, 7c, 10c, 13c, 16c, 19t
CE	cholesterol ester
DPG	*sn*-1,3-diphosphatidylglycerol (or cardiolipid)
FFA	free fatty acid
HPLC	high performance liquid chromatography
LPC	lysophosphatidylcholine
NPL	non-phosphorus lipid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PI	phosphatidylinositol
PL	phospholipid
PS	phosphatidylserine
PUFA	polyunsaturated fatty acid
SM	sphingomyelin
TG	triglyceride

1. INTRODUCTION

During the heat treatment of vegetable oils, linolenic acid can undergo geometrical isomerization [8]. The main compounds formed are 18:3Δ9c, 12c, 15t, 18:3Δ9t, 12c, 15c and 18:3Δ9t, 12c, 15t [Δ nomenclature (carbon counting beginning by the carboxyl group) was used for *trans* polyunsaturated fatty acids in order to specify the position and the geometry of all the ethylenic bonds]. The relative quantities of these compounds depends on the heating conditions [7, 22]. These *trans* isomers of linolenic acid have been detected in deodorized oils as well as in french fries [20] or in low-calorie spreads [23].

When rats were given a diet containing these *trans* isomers of linolenic acid, some *trans* long-chain n-3 polyunsaturated fatty acids were detected in the liver [10]. An isomer of eicosapentaenoic acid 20:5Δ5c, 8c, 11c, 14c, 17t (abbreviated 20:5Δ17t) and an isomer of docosahexaenoic acid 22:6Δ4c, 7c, 10c, 13c, 16c, 19t (22:6Δ19t) were thus identified. Another compound was also present whose characteristics seemed to indicate that it was the 22:5Δ7c, 10c, 13c, 16c, 19t (22:5Δ19t), a *trans* isomer of 22:5 (n-3). These long-chain *trans* PUFA could be formed in vivo by successive desaturations and elongations from the 18:3Δ9c, 12c, 15t present in the diet.

The purpose of the present study was first, to determine which lipid classes contained these *trans* polyunsaturated fatty acids, and second if other organs, such as the heart, kidneys or adrenals could also incorporate these compounds.

2. MATERIALS AND METHODS

2.1. Animals and diet

In this experiment, 24 specific pathogen free (SPF) Wistar rats from our animal breeding were used after they were weaned. They were all fed during a 6-day pre-experimental period with the control diet, in order to be accustomed to the purified diet. They were then separated into two groups of 12 animals and received the experimental diets for 8 weeks. The composition of this diet was already described [6]. As the preparation of large quantities of purified isomers of linolenic acid is difficult and expensive [9], these compounds were introduced in the diet as heated linseed oil. However, another experiment was undertaken in order to check that the other compounds present in the heated linseed oil did not interfere with the metabolism of *trans* polyunsaturated fatty acids. This work will be published later.

The experimental group (H) received in its diet linseed oil heated under nitrogen at 275 °C for 12 h, as lipids (*table I*). The control group

Table I. Fatty acid composition of linseed oils.

Fatty Acid	Fresh linseed oil F	Linseed oil heated at 275 °C, 12 h, under N_2 H
16:0	5.1	5.5
18:0	4.1	4.7
18:1Δ9t	—	0.4
18:1Δ9c	19.8	16.8
18:2Δ9c,12t	—	3.2
18:2Δ9t,12c	—	3.1
18:2Δ9c,12c	17.2	7.2
18:3Δ9t,12c,15t	—	8.0
18:3Δ9c,12c,15t	—	4.1
18:3Δ9t,12c,15c[a]	—	4.6
18:3Δ9c,12c,15c	51.4	1.1
Other fatty acids	2.3	16.2
Polymerized fatty acids	—	25.1

[a] This peak also contains some 18:3Δ9c,12t,15c.

(F) was fed with fresh linseed oil (Robbe, Compiègne, France). Because of the possible reduction in food intake by the animals receiving this heated oil, the consumption of the control group was restricted by pair feeding. All the animals were fed each day and their food intake was recorded.

After 8 weeks, the animals were killed and the liver, heart, kidneys and adrenals were dissected out, weighed and placed immediately in a mixture of chloroform/methanol 2/1 (V/V) for lipid extraction.

2.2. Lipid extraction and class separations

The organ lipids were extracted according to Folch et al. [4]. All the livers were extracted individually and their lipids were then gathered in three pools. The kidneys, heart and adrenals were pooled before extraction (three pools of four rats).

The separation of phospholipids (PL) and non-phosphorus lipids (NPL) was effected using silica cartridges [13]. The cholesterol esters (CE), triglycerides (TG) and free fatty acids (FFA) were obtained from NPL using the HPLC method of Hamilton and Comai [12]: a pump (Waters F 6000 A), a silica 5-µm column (0.39 cm i.d. × 12.5 cm Waters), and a differential refractometer R401 Waters were used. The mobile phase was a mixture of hexane/2-propanol/acetic acid 100/0.4/0.01 (V/V/V) at 1 mL·min^{-1}.

The PL were fractionated in classes with the HPLC method of Juaneda and Rocquelin [14], using a Varian 5000 HPLC system and a Pye Unicam PU 4020 UV detector. A first column, 25 cm long, internal diameter 0.75 cm, containing a 5-µm phase (Lichrosorb Li 60, Merck) was used, with a solvent gradient of 2-propanol/hexane/water varying from 54/41/5 (V/V/V) to 52/39/9 (V/V/V) in 10 min, at 2 mL·min^{-1}. This allowed the separation of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS) and lysophosphatidylcholine (LPC). The phosphatidylcholine (PC) and sphingomyelin (SM) were collected together and separated on a second column, 25 cm long, internal diameter 0.48 cm, containing a 10-µm phase (Lichrosorb Li 60, Merck), with acetonitrile/methanol/water 71/21/8 (V/V/V) running isocratically. For fatty acid identification, all the classes were collected using a fraction collector Gilson 201.

2.3. Gas–liquid chromatography of the methyl esters

After the evaporation of the solvents, all classes were methylated using the BF_3/methanol method of Morrison and Smith [15], at 90 °C for 90 min, except for sphingomyelin which was methylated for 12 h. After hexane extraction, the fatty acid methyl esters were analyzed and quantified by gas–liquid chromatography. Three capillary columns were used: a home-made glass Carbowax 20M column (35 m long and 0.35 mm internal diameter), a Silar 10C Alltech glass column (50 m long and 0.25 mm internal diameter) and a fused silica CP Sil 88 Chrompack column (50 m long, 0.32 mm internal diameter and 0.2 µm film thickness). A solid injector [19] was used and helium was the carrier gas. The analyses were performed at 180 °C. The temperature of the injectors and detectors was 240 °C. An Autolab System 4 (Spectraphysics) or a Vista 401 (Varian) integrators was used for the quantitative analyses. The fatty acids were identified by comparison with commercial standards (Nu-Check Prep) or with *trans* long-chain PUFA isolated and previously identified [10].

3. RESULTS

The food intake was not different between the two groups, since the consumption of the control animals was limited by pair feeding. At the end of the experiment, the weight of the animals fed with heated oil was significantly lower (7 %) and their liver and kidneys were heavier (respectively 35 and 24 %) than the control animals, as has already been described in previous studies [3, 17, 18].

No *trans* PUFA were observed in all lipid classes of the four tissues of the animals receiving fresh oil in their diet. Therefore, only the fatty acid composition of the lipid classes of the tissues of experimental rats receiving heated linseed oil in their diet were reported in *tables II, III, IV* and *V*. All the lipid classes of the four tissues contained the *trans* isomers of linolenic acid, except the SM of liver and kidneys and the LPC of adrenals. In these cases, however, linolenic acid was also not detectable. The non-phosphorus lipid classes, in particular the TG, presented the highest levels of *trans* 18:3. Among the phospholipids, the DPG and PC were the main classes containing *trans* 18:3. The PI and PS contained only low quantities of these *trans* compounds, except in the heart.

Trans long-chain n-3 PUFA were also present in tissues, as already observed by Grandgirard et al. [10]. 20:5Δ17t, 22:5Δ19t and 22:6Δ19t were detected in almost all lipid classes. Only the SM of liver, kidneys and heart, as well as the LPC of heart did not contain such isomers.

In order to better evaluate the quantities of *trans* PUFA in lipid classes, their levels were summarized in *figures 1* to *4*. It is first evident that these compounds are not negligible. The CE of adrenals contained about 12 % of them and their average in all classes was 5 %. The 20:5Δ17t was the main long-chain *trans* PUFA. It was present in all classes, except SM and LPC and its level reached 2 % of the total amount of fatty acids in some cases. 22:5Δ19t was never present in large quantities, except in the adrenals. In this organ, it represented the main long-chain *trans* PUFA and its level was very high in CE. When present, the 22:6Δ19t always appeared in small amounts, except in PE and PS.

Among the phospholipids, there were large differences in the incorporation of such compounds. PE and PS were the only classes which contained all the different types of *trans* n-3 isomers. In PC, in most cases, only 20:5Δ17t was found. The cardiolipids (DPG) incorporated very few long-chain *trans* PUFA. The incorporation in PI was better for the long-chain *trans* PUFA than for the *trans* 18:3. There were some unexpected results in LPC. For example, 22:6Δ19t was observed in hepatic LPC and not in hepatic PC. In the

Table II. Polyunsaturated fatty acids in the hepatic lipid classes (% of total fatty acids).

	TG	CE	FFA	PC	PE	DPG	PI	PS	LPC	SM
18:3Δ9c,12c,15t	0.86 ± 0.04	1.04 ± 0.01	0.83 ± 0.03	0.85 ± 0.05	0.42 ± 0.02	1.48 ± 0.18	0.12 ± 0.02	0.64 ± 0.09	0.57 ± 0.09	nd[b]
18:3Δ9t,12c,15c[a]	1.85 ± 0.08	0.95 ± 0.03	0.99 ± 0.11	0.30 ± 0.07	0.59 ± 0.02	0.64 ± 0.05	0.10 ± 0.01	0.22 ± 0.04	0.61 ± 0.05	nd
18:3Δ9t,12c,15t	2.46 ± 0.05	0.93 ± 0.07	1.55 ± 0.10	1.12 ± 0.15	0.59 ± 0.08	0.64 ± 0.02	0.17 ± 0.01	0.43 ± 0.03	0.73 ± 0.05	nd
Σ trans 18:3	5.17	2.92	3.37	2.27	1.60	2.76	0.39	1.29	1.91	nd
18:3 n-3	0.20 ± 0.02	0.77 ± 0.05	0.30 ± 0.10	0.09 ± 0.01	nd	0.22 ± 0.06	nd	0.08 ± 0.01	nd	nd
20:4 n-6	1.72 ± 0.10	5.83 ± 0.29	5.86 ± 1.95	9.53 ± 0.01	24.51 ± 0.43	4.58 ± 0.25	25.81 ± 0.48	18.44 ± 0.33	14.72 ± 0.12	2.09 ± 0.36
Σ n-6	10.43	16.64	14.59	24.08	30.76	41.38	33.49	23.86	27.39	5.37
20:5Δ17t	0.33 ± 0.02	0.85 ± 0.03	0.58 ± 0.06	1.28 ± 0.06	2.53 ± 0.08	0.41 ± 0.01	0.87 ± 0.04	1.84 ± 0.09	1.99 ± 0.32	nd
20:5 n-3	0.08 ± 0.01	0.48 ± 0.02	0.17 ± 0.04	0.22 ± 0.01	0.52 ± 0.06	0.11 ± 0.03	0.13 ± 0.02	0.51 ± 0.11	0.26 ± 0.05	nd
22:5Δ19t	nd	nd	0.12 ± 0.01	nd	0.31 ± 0.03	nd	0.25 ± 0.04	0.17 ± 0.01	nd	nd
22:5 n-3	nd	nd	0.35 ± 0.06	0.17 ± 0.02	0.87 ± 0.07	0.26 ± 0.01	0.74 ± 0.05	0.67 ± 0.04	0.50 ± 0.19	nd
22:6Δ19t	nd	nd	0.32 ± 0.13	nd	1.09 ± 0.19	0.26 ± 0.05	0.36 ± 0.07	0.95 ± 0.14	0.68 ± 0.12	nd
22:6 n-3	0.36 ± 0.01	0.47 ± 0.02	2.92 ± 0.75	1.60 ± 0.03	9.62 ± 0.56	3.06 ± 0.13	3.25 ± 0.17	9.57 ± 0.34	4.65 ± 0.40	nd

[a] This peak also contains some 18:3Δ9c,12t,15c; [b] nd = not detected (< 0.05).

Table III. Polyunsaturated fatty acids in the renal lipid classes (% of total fatty acids).

	TG	CE	FFA	PC	PE	DPG	PI	PS	LPC	SM
18:3Δ9c,12c,15t	1.08 ± 0.15	1.16 ± 0.16	0.77 ± 0.04	0.59 ± 0.06	0.23 ± 0.01	2.02 ± 0.03	0.09 ± 0.01	0.20 ± 0.04	0.71 ± 0.15	nd[b]
18:3Δ9t,12c,15c[a]	1.54 ± 0.25	1.37 ± 0.14	0.90 ± 0.17	0.81 ± 0.05	0.49 ± 0.02	1.20 ± 0.05	0.22 ± 0.04	0.25 ± 0.01	0.15 ± 0.03	nd
18:3Δ9t,12c,15t	2.89 ± 0.36	1.85 ± 0.28	1.50 ± 0.24	1.59 ± 0.12	0.44 ± 0.01	0.89 ± 0.05	0.25 ± 0.04	0.35 ± 0.03	0.52 ± 0.11	nd
Σ trans 18:3	5.51	4.38	3.17	2.99	1.16	4.11	0.56	0.80	1.38	nd
18:3 n-3	0.34 ± 0.07	0.98 ± 0.11	0.18 ± 0.05	0.07 ± 0.02	nd	0.20 ± 0.05	nd	nd	nd	nd
20:4 n-6	1.43 ± 0.11	8.67 ± 0.26	9.06 ± 0.36	13.89 ± 0.22	35.97 ± 0.33	4.55 ± 0.46	32.62 ± 0.53	30.40 ± 0.21	6.17 ± 0.33	0.31 ± 0.01
Σ n-6	11.17	19.86	18.24	27.85	41.73	59.08	39.15	35.68	13.84	1.23
20:5Δ17t	0.12 ± 0.01	0.82 ± 0.08	0.95 ± 0.05	1.69 ± 0.03	2.75 ± 0.05	0.36 ± 0.09	1.20 ± 0.01	2.65 ± 0.07	0.73 ± 0.07	nd
20:5 n-3	nd	0.75 ± 0.01	0.42 ± 0.04	0.25 ± 0.02	0.54 ± 0.03	0.09 ± 0.01	0.13 ± 0.01	0.54 ± 0.05	0.25 ± 0.05	nd
22:5Δ19t	nd	0.28 ± 0.02	0.15 ± 0.02	nd	0.16 ± 0.01	nd	nd	0.28 ± 0.09	nd	nd
22:5 n-3	nd	0.42 ± 0.14	0.22 ± 0.03	0.21 ± 0.02	0.17 ± 0.02	0.24 ± 0.01	0.22 ± 0.01	0.19 ± 0.05	0.37 ± 0.02	nd
22:6Δ19t	nd	0.17 ± 0.03	0.13 ± 0.03	nd	0.16 ± 0.03	nd	0.09 ± 0.02	0.13 ± 0.01	nd	nd
22:6 n-3	0.06 ± 0.01	1.23 ± 0.17	0.97 ± 0.03	1.21 ± 0.03	1.72 ± 0.05	1.08 ± 0.05	1.06 ± 0.01	0.83 ± 0.09	0.52 ± 0.05	nd

[a] This peak also contains some 18:3Δ9c,12t,15c; [b] nd = not detected (< 0.05).

Table IV. Polyunsaturated fatty acids in the heart lipid classes (% of total fatty acids).

	TG	CE	FFA	PC	PE	DPG	PI	PS	LPC	SM
18:3Δ9c,12c,15t	1.35 ± 0.06	1.24 ± 0.19	1.17 ± 0.08	0.94 ± 0.06	0.75 ± 0.01	3.35 ± 0.16	0.64 ± 0.03	0.59 ± 0.05	0.28 ± 0.01	0.41 ± 0.07
18:3Δ9t,12c,15c[a]	1.66 ± 0.11	0.27 ± 0.05	0.98 ± 0.13	0.60 ± 0.01	0.83 ± 0.04	0.41 ± 0.04	0.55 ± 0.02	0.61 ± 0.07	0.09 ± 0.01	0.40 ± 0.08
18:3Δ9t,12c,15t	3.48 ± 0.09	0.92 ± 0.18	2.10 ± 0.06	2.43 ± 0.10	2.06 ± 0.05	0.67 ± 0.02	1.36 ± 0.02	1.14 ± 0.06	0.36 ± 0.05	0.35 ± 0.07
Σ trans 18:3	6.49	2.43	4.25	3.97	3.64	4.43	2.55	2.34	0.73	1.36
18:3 n-3	0.49 ± 0.06	0.48 ± 0.07	0.38 ± 0.19	0.11 ± 0.01	0.06 ± 0.01	0.20 ± 0.06	0.06 ± 0.01	0.05 ± 0.01	nd[b]	nd
20:4 n-6	1.16 ± 0.08	10.79 ± 1.63	3.74 ± 1.04	8.47 ± 0.56	21.91 ± 0.55	2.26 ± 0.11	19.06 ± 0.67	12.05 ± 0.52	2.99 ± 0.86	0.76 ± 0.19
Σ n-6	14.14	22.82	13.39	35.42	39.34	78.01	32.89	25.84	10.82	4.44
20:5Δ17t	0.12 ± 0.01	1.00 ± 0.17	0.29 ± 0.04	1.11 ± 0.10	1.64 ± 0.04	0.17 ± 0.01	0.91 ± 0.06	0.93 ± 0.05	nd	nd
20:5 n-3	nd	0.59 ± 0.06	0.05 ± 0.01	0.09 ± 0.02	0.20 ± 0.02	nd	0.08 ± 0.01	0.10 ± 0.03	nd	nd
22:5Δ19t	nd	nd	0.09 ± 0.01	nd	0.32 ± 0.01	nd	nd	0.62 ± 0.03	nd	nd
22:5 n-3	nd	nd	0.22 ± 0.07	nd	0.58 ± 0.02	0.07 ± 0.01	0.24 ± 0.01	0.89 ± 0.04	nd	nd
22:6Δ19t	0.16 ± 0.01	nd	0.20 ± 0.06	nd	0.15 ± 0.01	nd	0.10 ± 0.01	0.39 ± 0.03	nd	nd
22:6 n-3	0.12 ± 0.01	0.38 ± 0.03	0.54 ± 0.05	0.32 ± 0.04	2.87 ± 0.26	0.27 ± 0.04	0.84 ± 0.01	3.48 ± 0.20	0.33 ± 0.09	0.66 ± 0.18

[a] This peak also contains some 18:3Δ9c,12t,15c; [b] nd = not detected (<0.05).

Table V. Polyunsaturated fatty acids in the adrenal lipid classes.

	TG	CE	FFA	PC	PE	DPG	PI	PS	LPC	SM
18:3Δ9c,12c,15t	1.32	1.08	0.84	0.19	0.17	0.44	0.07	0.25	nd[b]	0.12
18:3Δ9t,12c,15c[a]	2.17	2.23	0.93	0.16	0.17	0.96	nd	0.22	nd	nd
18:3Δ9t,12c,15t	3.83	3.18	2.06	0.68	0.19	1.63	nd	0.34	nd	0.42
Σ trans 18:3	7.32	6.49	3.83	1.03	0.53	3.03	0.07	0.81	nd	0.54
18:3 n-3	0.50	1.37	0.97	nd	nd	0.06	nd	nd	nd	nd
20:4 n-6	1.87	6.20	7.35	27.48	39.35	13.74	45.14	20.94	9.39	2.75
Σ n-6	13.67	22.77	18.16	37.42	44.72	30.29	46.63	28.64	14.42	4.79
20:5Δ17t	0.20	0.61	0.43	1.61	1.45	0.66	0.42	0.93	0.36	0.13
20:5 n-3	0.07	0.23	0.53	0.17	0.19	0.09	0.14	0.12	0.39	0.12
22:5Δ19t	0.64	3.30	0.79	0.22	0.74	0.57	0.17	1.14	0.24	nd
22:5 n-3	0.24	0.99	0.91	0.13	0.54	0.42	0.09	0.84	0.28	nd
22:6Δ19t	0.09	0.49	0.11	nd	0.12	0.19	nd	0.15	nd	nd
22:6 n-3	0.27	2.14	0.59	nd	0.48	0.83	0.12	1.20	0.30	0.33

[a] This peak also contains some 18:3Δ9c,12t,15c; [b] nd = not detected (< 0.05).

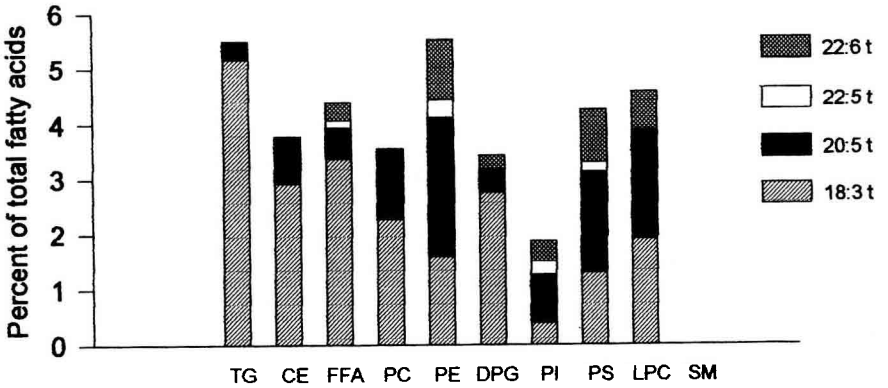

Figure 1. Trans polyunsaturated fatty acids in the liver (TG = triglycerides; CE = cholesterol esters; FFA = free fatty acids; PC = phosphatidylcholine; PE = phosphatidylethanolamine; DPG = diphosphatidylglycerol; PI = phosphatidylinositol; PS = phosphatidylserine; LPC = lysophosphatidylcholine; SM = sphingomyelin).

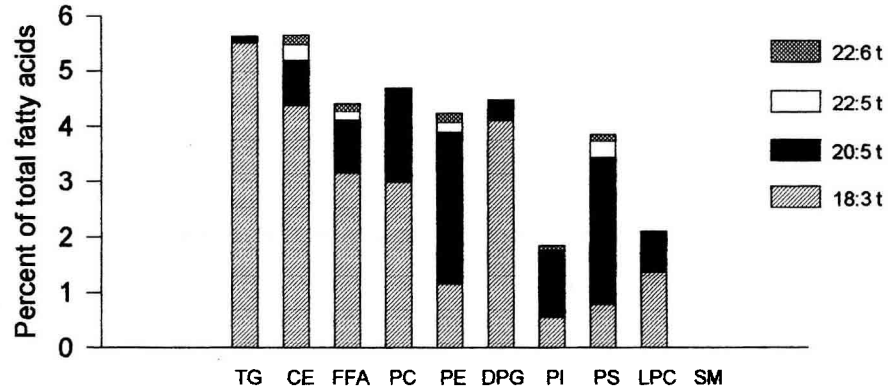

Figure 2. Trans polyunsaturated fatty acids in the kidneys (same legend as in *figure 1*).

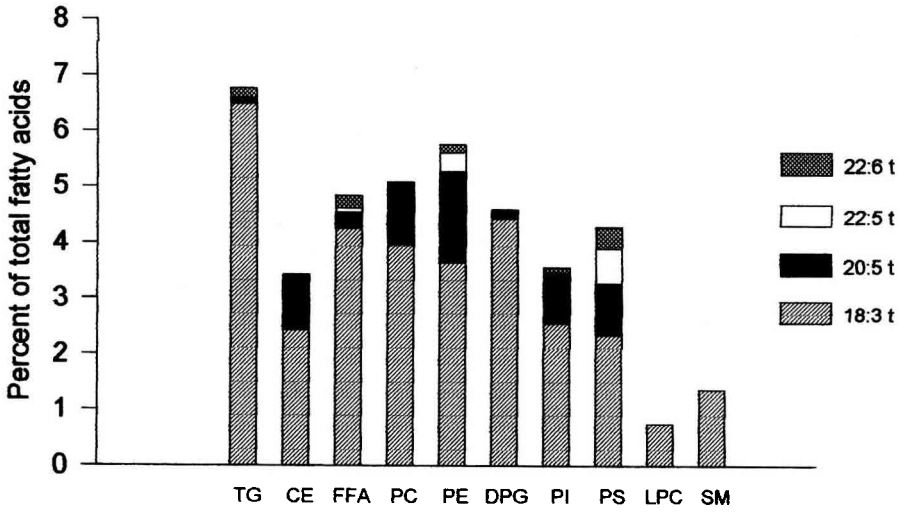

Figure 3. Trans polyunsaturated fatty acids in the heart (same legend as in *figure 1*).

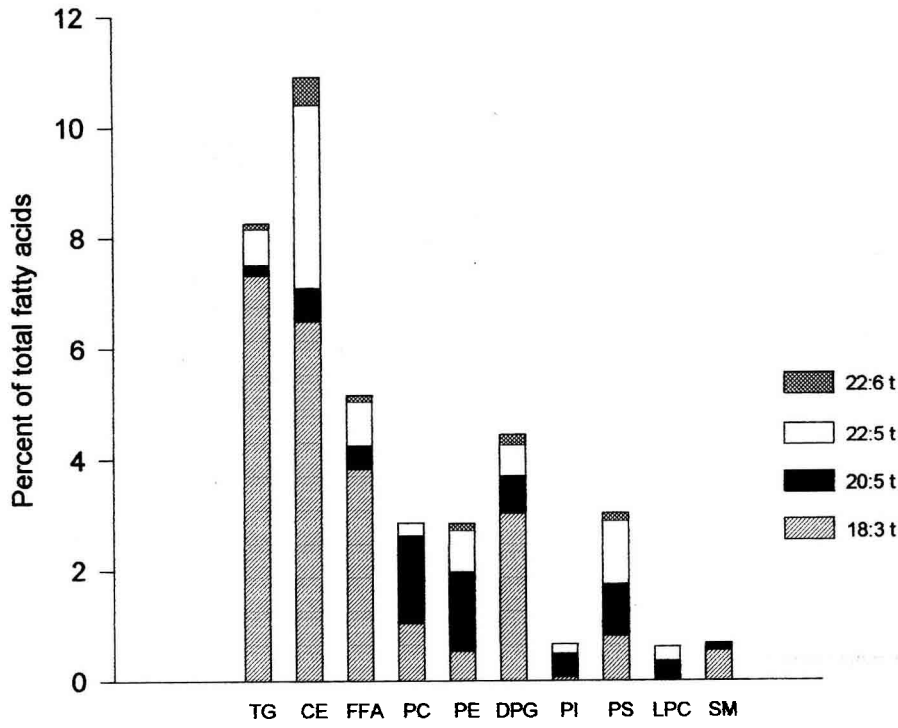

Figure 4. Trans polyunsaturated fatty acids in the adrenals (same legend as in *figure 1*).

liver, the percentage of 22:6 n-3 was three times higher in LPC than in PC. This could result from a little contamination of LPC, due to its very small amount.

4. DISCUSSION

The adrenals were different from the other organs. They presented a high level of 22:5Δ19t, in particular in CE. The importance of CE in the adrenals has been known for a long time. Moreover, high levels of 22:5 (n-3) were already detected in several lipid classes [21]. Our results are then not surprising. When the long-chain *trans* PUFA were detected for the first time [5], a compound with the characteristics of 22:5Δ19t was present in large quantity in adrenals.

As a rule, a thorough analysis of our results led us to find that each long-chain *trans* PUFA was important in a lipid class, when the corresponding *cis* compound was present. It is for this reason that we calculated (*table VI*) the proportion of each *trans* n-3 compound in its category (*cis* + *trans*), when possible. It can be seen that each *trans* n-3 isomer represented a constant proportion in all phospholipid classes. For example, the 20:5Δ17t corresponded to 85–90 % of the total 20:5; the 22:5Δ19t reached about 24 % of Σ22:5(n-3) in liver, 39 % in heart, 54 % in kidneys and 60 % in adrenals. The calculation of the proportions of the 22:6 isomers was less valid

Table VI. Proportions of the trans long-chain PUFA in their category.

	PC	PE	DPG	PI	PS
Liver					
20:5t/Σ20:5 (n-3)	85	83	79	87	78
22:5t/Σ22:5 (n-3)	nc	26	nc	25	20
22:6t/Σ22:6 (n-3)	nc	10	8	10	9
Kidneys					
20:5t/Σ20:5 (n-3)	87	84	80	90	83
22:5t/Σ22:5 (n-3)	nc	48	nc	nc	60
22:6t/Σ22:6 (n-3)	nc	9	nc	8	13
Heart					
20:5t/Σ20:5 (n-3)	92	89	nc	92	90
22:5t/Σ22:5 (n-3)	nc	36	nc	nc	41
22:6t/Σ22:6 (n-3)	nc	5	nc	11	10
Adrenals					
20:5t/Σ20:5 (n-3)	90	88	88	75	89
22:5t/Σ22:5 (n-3)	63	58	58	65	58
22:6t/Σ22:6 (n-3)	nc	20	19	nc	11

nc = not calculated (insufficient quantities).

owing to the very low quantities of these compounds. However, it seemed that the 22:6Δ19t represented about 10 % of the total 22:6 in the liver, kidneys and heart, and 17 % in adrenals.

Apparently, the 20:5Δ17t is easily synthesized in vivo from the 18:3Δ9c, 12c, 15t. However, this compound accumulates in all tissues and in the main lipid classes. It is possible that the *cis* compounds are preferentially used to form 22:5 and 22:6 PUFA. This effect has already been observed in brain structures and the retina [11]. All the results obtained suggest that the pathways used to synthesize the *trans* long-chain n-3 PUFA are similar to those of the corresponding *cis* compounds, but that the rate of conversion could be different.

Sterically, the compounds with *trans* double bonds are straightened. So, the *trans* monounsaturated fatty acids look like saturated fatty acids [1]. This point was recently confirmed for PUFA: 18:3Δ9c, 12c, 15t was incorporated in DPG as well as 18:2Δ9c, 12c [24]. Thus, it seems that 18:3Δ9c, 12c, 15t is a good substrate for the enzymic system that ensures the acylation of cardiolipins and that the *trans* ethylenic bond is recognized as a saturated bond. If this was the same for the long-chain n-3 PUFA, for example, the 20:5Δ5c, 8c, 11c, 14c, 17t (20:5Δ17t) would then be recognized as 20:4Δ5c, 8c, 11c, 14c (arachidonic acid). However, our results do not confirm this assumption. These *trans* long-chain n-3 PUFA are found in the classes and the tissues, where their corresponding *cis* isomers are usually found, and they are not similar to n-6 long-chain PUFA. The physiological effects of these compounds are also more comparable to their *cis* n-3 iso-

mers than to those of the n-6 fatty acids. For example, 20:5Δ17t and 22:6Δ19t are less antiaggregant than their corresponding *cis* isomers, but they are not as proaggregant as arachidonic acid [2, 16].

Further studies are needed to determine other possible physiological effects due to the incorporation of these n-3 *trans* long-chain PUFA into most lipid classes of several organs.

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