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Effects of *trans* n-6 fatty acids on the fatty acid profile of tissues and liver microsomal desaturation in the rat

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SUMMARY

Effects of *trans* n-6 fatty acids on the fatty acid profile of tissues and liver microsomal desaturation in the rat

18:2Δ 9c,12t and 18:2 Δ9t,12c are present in our diet, as result of heat treatment of vegetable oils. A nutritional study was carried out in order to obtain more precise information on the conversion of these two isomers into long chain polyunsaturated fatty acids (PUFA) by rat tissues. This *in vivo* study performed using rat fed with small quantities of mono *trans* linoleic acid isomers (0.6% of total energy) showed that 18:2 Δ9c,12t was converted into 20:4 Δ5c,8c,11c,14t while 18:2 Δ9t,12c was only slightly converted into 20:4 Δ5c,8c,11t,14c. Furthermore 18:2 Δ9t,12c was preferentially elongated into 20:2 Δ11t,14c. Each C20 metabolite of these mono *trans* 18:2 isomers was isolated as methyl ester by semi-preparative high-performance liquid chromatography (HPLC) followed by silver nitrate thin layer chromatography ($AgNO_3$-TLC). The structure of the components was identified using partial hydrazine reduction, $AgNO_3$-TLC of the resulting monoenes and gas-liquid chromatography coupled with mass spectrometry (GC-MS) of the 4,4-dimethyloxazoline (DMOX) derivatives. Fourier-transform-infrared spectroscopy (GC-FTIR) confirmed the *trans* geometry.

Gas-liquid chromatography (GC) analyses showed that 18:2 Δ9c,12t and 18:2 Δ9t,12c were present in different tissue lipids (liver, heart, testes, brain and adipose tissue), and without any modification in the amount of 20:4n-6. 20:4 Δ5c,8c,11c,14t was incorporated in different rat tissues except in brain. Furthermore, its incorporation followed that of its structural analogue, 20:3n-9 in liver phospholipid classes (phosphatidylethanolamine, phosphatidylinositol and phosphatidylcholine).

Finally, an *in vitro* study carried out with rat liver microsomes showed that dietary *trans* 18:2 isomers could inhibit the Δ6-desaturation of 18:2n-6 to 18:3n-6 and the Δ5-desaturation of 20:3n-6 to 20:4n-6.

KEY-WORDS: Desaturation – Diet – Linoleic acid – Metabolism – Microsomes – Rat – Trans polyunsaturated fatty acids

Abbreviations: AA, arachidonic acid; ACAT, acyl-CoA: cholesterol acyltransferase; GC, gas-liquid chromatography; GC-FTIR, gas-liquid chromatography coupled with infrared spectroscopy; GC-MS, gas-liquid chromatography coupled with mass spectrometry; CE, cholesteryl esters; DMOX, 4,4-dimethyloxazoline; ECL, equivalent chain length; FAME, fatty acid methyl esters; FFA, free fatty acid; HPLC, high performance liquid chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PUFA, polyunsaturated fatty acid; TG, triglyceride; TLC, thin-layer chromatography.

1. INTRODUCTION

Trans fatty acids are formed as a result of partial hydrogenation or heat treatment of edible oils. Although, margarines may contain high quantities of *trans* monounsaturated fatty acids (Ohlorgge, 1983; Slover et al., 1985; Entressangles, 1986), appreciable quantities of linoleic acid geometrical isomers may be found (Ratnayake *et al.*, 1991). As an example, levels as high as 7.6% of *trans* monoethylenic and 2.8% of *trans* diethylenic isomers were recently reported in some Canadian margarines (Ratnayake *et al.*, 1991). On the contrary, smaller quantities of linolenic geometrical isomers were observed in most of the samples. *Trans* PUFA are more frequently found in deodorized oils (Ackman *et al.*, 1974, Wolff, 1993) and in used frying oils (Sébédio *et al.*, 1987a). While different positional 18:2 isomers are present in margarines (Ratnayake et Pelletier, 1992), only geometrical 18:2 Δ9,12 and 18:3 Δ9,12,15 isomers were identified in deodorized and heated oils (Grandgirard *et al.*, 1984; Sébédio *et al.*, 1988). Some *trans* isomers of the n-3 family such as 18:3 Δ9c,12c,15t and 18:3 Δ9t,12c,15c are known to be metabolized to C20 and C22 PUFA (Grandgirard *et al.*, 1989, Chardigny *et al.*, 1996a) and incorporated into different tissue lipids including those of brain (Grandgirard *et al.*, 1994). Similarly, studies on the *trans* isomers of linoleic acid have shown that at least one *trans* isomer, 18:2 Δ9c,12t could be desaturated and elongated into a *trans* isomer of arachidonic acid, 20:4 Δ5c,8c,11c,14t (Blank and Privett, 1963; Privett *et al.*, 1967; Anderson *et al.*,1975; Ratnayake *et al.*,1994; Beyers and Emken, 1991). However, all

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these studies do not agree as to whether the other mono-*trans* isomer, 18:2 Δ9t,12c, could be converted into a *trans* isomer of arachidonic acid. Moreover, Beyers and Emken (1991) showed that only the 18:2 Δ9t,12c was elongated into the 20:2 Δ11t,14c, while Ratnayake *et al.* (1994) suggested that both mono-*trans* isomers of 18:2 Δ9,12 could be elongated into *trans* isomers of 20:2 Δ11,14. Furthermore the incorporation of the 20:4 isomers into tissue phospholipids was never reported.

Considering these discrepancies and that some of these nutritional studies were performed using high quantities of *trans* 18:2 isomers, we have carried out an *in vivo* study using a much lower dose of the *trans* 18:2 isomers and adequate quantities of linoleic and linolenic acids in the diet in order to determine if both isomers could be desaturated and elongated. Their effects on the desaturation of linoleic acid using liver microsomes of rats fed the different diet also were determined. Two isomers of arachidonic acid (20:4 Δ14t and 20:4 Δ11t) were identified using gas-liquid chromatography coupled with Fourier transform infrared spectroscopy (GC-FTIR) and gas-liquid chromatography coupled with mass spectrometry (GC-MS). However, only one 20:2 isomer the 20:2 Δ11t,14c was detected and identified. Among the different phospholipids classes, 20:4 Δ14t accumulated in liver phosphatidylinositol where higher quantities of its structural analogue, 20:3 n-9 were found.

2. EXPERIMENTAL

Isolation of linoleic acid isomers from heated sunflower oil.

300g of sunflower oil (Lesieur Alimentaire, Neuilly, France), heated at 275°C for 24 hours under nitrogen, was saponified. The unsaponifiable material was removed according to the AOCS procedure (Ca-6a-40). Fatty acids were esterified by refluxing for 5 hours in a solution of 1% H_2SO_4 in methanol. The fatty acid methyl esters (FAME) were then fractionated by urea inclusion. For that purpose, an aliquot (100g) of the resulting methyl esters was dissolved in a hot solution of urea in methanol (300g urea/1.2L methanol). After cooling under nitrogen, the flask was set overnight at 4°C. The urea adduct and non-adduct fractions were then separated by filtration. The crystals were washed with cold hexane. The filtrate was transferred into a separatory funnel, and the methyl esters were extracted with hexane after addition of 2L of water and 30mL of concentrated HCl. The non-adduct fraction was further submitted to a second urea fractionation (urea: fatty acid methyl esters, 1:1, v/v). The non-urea adduct fraction was extracted and methyl esters were further fractionated by column chromatography on silicic acid and preparative high performance liquid chromatography (HPLC) as previously described (Sébédio *et al.*, 1987b) in order to obtain a purified 18:2 fraction. The resulting methyl esters (a mixture of 18:2 Δ9c,12t, 18:2 Δ9t,12c, and 18:2 Δ9t,12t) were saponified with KOH and fatty acids were converted to ethyl esters by refluxing for 5 hours with 4 volumes of 1% H_2SO_4 in ethanol. The esterification was assessed by TLC-FID (Ackman *et al.*, 1981).

Animals and diets

Wistar weanling male rats from IFFA CREDO (L'Arbresle, FRANCE) weighing 64±4g were randomly assigned into 2 groups. They were housed in individual cages. The room temperature averaged 22±1°C, with 12 /12h L:D cycles. Rats were fed a semi-liquid diet containing (in g/kg) casein 185, starch 380, sucrose 295, agar 20, cellulose 20, minerals mixture 50, vitamin mixture (Grandgirard *et al.*, 1994) and different oil mixtures. The first mixture was obtained by mixing high oleic sunflower and linseed oils (92.5/7.5, w/w). The second one was a mixture of high oleic sunflower, linseed and sunflower oils (84.5/7.0/8.5, w/w/w). Animals from the experimental group (E) were fed a semi-liquid synthetic diet containing 4.5% by weight of the first oil mixture and 0.5% by weight of linoleic geometrical isomers as ethyl esters. Control group (C) was fed a semi-liquid synthetic diet containing 4.5% by weight of the second oil mixture and 0.5% by weight of ethyl esters of the same oil mixture. The fatty acid composition of dietary oils are reported in Table I. After 4 weeks on the diets, rats were killed by decapitation and their liver, heart, testes, brain and adipose tissue were removed rapidly and placed in a vessel containing a mixture of chloroform/methanol (2:1, v/v). Total lipids were extracted using the method of Folch *et al.* (1957).

Table I
Fatty acid composition of the dietary oils (in wt %)

Fatty acids	Experimental (E)	Control (C)
16:0	4.0	4.7
18:0	4.0	4.1
18:1 n-9	59.5	63.1
18:2 cc	20.0	22.4
18:2 ct	2.2	-
18:2 tc	2.7	-
18:2 tt	0.2	-
18:3 n-3	3.9	3.9
Others	3.5	1.8

Lipid analyses

Phospholipids were separated from neutral lipids using silica cartridges, according to the method of Juanéda and Rocquelin (1985).

The neutral lipid fraction was separated by thin layer chromatography (TLC). Silica gel (0.25 mm thickness, 20 cm x 20 cm) plates (Merck, Darmstadt, Germany) were used. The developing solvent was a mixture of hexane/diisopropyl ether/acetic acid (60:40:4, v/v/v). After 30 min of migration, the plates were dried, sprayed with a solution of 2',7'dichlorofluorescein (0.2% in ethanol) and visualized under ultraviolet light. The different bands containing triacylglycerol (Rf = 0.63) and cholesterol esters (Rf = 0.75) were carefully scraped off the plate and then extracted with a mixture of chloroform/methanol (2:1, v/v).

Liver phospholipid classes were separated by HPLC according to Juanéda *et al.* (1990). The HPLC instrument was a Varian model 9010 (Varian, Les Ulis, France) using a ternary solvent mixture, with a Valco compressed-air injector fitted with a loop (100 µL) and a programmer-integrator. The detector was a Cunow light scattering detector (LSD) Model 10 (Cunow, Cergy, France). The nebulizer air pressure was set at 2.1 bars and the heated pipe temperature was 40°C. The LSD was fitted with a splitter for collecting the phospholipid fractions. A Hibar pre-packed column Lichrosorb Si 60 (5µm, 250mm x 10 mm i.d.) (Merck), was used for both analytical and semi-preparative work. Lipid classes separation was achieved using a ternary gradient as described in Table II. The flow rate was 2.5mL.min^{-1}. It was necessary to re-equilibrate the column for 15 min prior to subsequent injections. A mixture of chloroform and methanol (2:1, v/v) was used as injection solvent. The collected fractions were evaporated to dryness at 40°C using a rotary vacuum apparatus and fatty acids were esterified with boron trifluoride in methanol (BF_3/methanol, 14%, v/v) according to Morrison and Smith (1964), for further GC analysis.

Table II
Ternary gradient of solvents for separation of liver phospholipid classes by HPLC

Time(min)	A	B	C
0	46	50	4
45	36	50	14
90	46	50	4

A:Hexane
B: 2-Propanol/Chloroform (4:1, v/v)
C: 2-Propanol/Water (1:1, v/v)

Gas-liquid chromatography (GC)

The fatty acid composition of different lipid classes and each step of preparation and identification of unknown compounds were monitored by GC of the methyl esters. A Hewlett Packard HP 5890 serie II (Hewlett Packard, Les Ulis France) equipped with a splitless/split injector and a flame ionization detector was used. The temperature of both the injector and detector was 250°C. Helium was the carrier gas. The analyses were performed using two columns of different polarities. The first one was a fused BPX-70 silica capillary column (SGE, Melbourne Australia, 0.25 µm of film thickness, 50 m length, 0.33 mm i.d.). The second one was a fused DB Wax silica capillary column (J and W Scientific, Rancho Cordo, USA, 30 m length, 0.25 mm i.d). The oven temperature was programmed from 60 to 170°C at 20°C/min for the BPX column and from 50 to 190°C at 25°C/min for the DB Wax column. Quantitative data were obtained using an SP 4400 Chromjet (Thermo Separation Products, Les Ulis, France) integrator. FAME were identified by comparison with commercial (Sigma) or synthetised standards (Berdeaux *et al.*, 1995), whenever possible.

Preparation of 4,4-dimethyloxazoline (DMOX) derivatives

FAME were converted to DMOX derivatives by treatment with 2-amino-2-methylpropanol in a sealed ampoule at 170°C for 18 hours (Zhang *et al,.* 1988; Fay and Richli, 1991; Luthria and Sprecher, 1993). The reaction mixture was cooled and dissolved in 3 mL of dichloromethane and further washed twice with 1 mL of water. After drying the organic phase, the solvent was removed under a stream of N_2 and the sample was dissolved in hexane for analysis by GC-MS.

Gas-liquid chromatography - mass spectrometry (GC-MS)

A Hewlett-Packard 5890 gas chromatograph coupled with a 5970 Mass selective detector (Hewlett-Packard) was used for the GC-MS analyses. The GC separation was performed on a BPX-70 capillary column as described above. Helium was used as carrier gas. The oven temperature was programmed from 60 to 190°C at 20°C.min^{-1}. Splitless injection was used with the injection port maintained at 250°C.

Gas-liquid chromatography - Fourier-transform infrared spectroscopy (GC-FTIR)

The gas-phase infrared spectra were obtained with a Bruker IFS 85 Fourier-transform-infrared spectrometer connected to a Carlo Erba (Massy, France) 5160 gas chromatograph equipped with an on-column injector and a flame ionization detector. Both were maintained at 300°C. A fused BPX-70 silica capillary column was used. The interface consisted of a gold-coated light-pipe (20cm x 0.8mm i.d.) maintained at 250°C. Helium was the carrier gas. The oven temperature was programmed from 60 to 250°C at

10°C/min, and then held isothermally for completion of the analyses. The spectra resolution was fixed at $8cm^{-1}$ and 12 interferograms were collected per second.

Hydrazine reduction

PUFA were submitted to hydrazine reduction according to Ratnayake (1981). Briefly, 30 mL of 96% ethanol and 1mL of hydrazine (Pierce, Rockford, IL, USA) were added to 3.0-3.5 mg of free fatty acid in a round-bottomed flask (125 mL). The reaction was performed at 40±1°C under a light stream of oxygen with gentle magnetic stirring. At the end of the reaction, 120 mL of distilled water were added and the fatty acids were extracted three times with 40 mL of hexane. The hexane phase was washed with distilled water, dried over anhydrous sodium sulphate and evaporated to dryness.

Isolation of *trans* isomers of arachidonic acid and of 11, 14-eicosadienoic acid

The liver FAME were fractionated by semi-preparative HPLC (25 mg per injection), using a Spectra-Physics (Les Ulis, France) SP 8810 pump, a C18 Nucleosil column (25 cm x 5 mm i.d.) (Shandon, Eragny, France), a differential refractometer R 401 (Waters, Milford, MA, USA). The mobile phase was a mixture of acetonitrile and acetone (90:10, v/v) at a flow rate of 4 $mL.min^{-1}$. Different fractions were collected and the solvent evaporated. The composition of the fractions was obtained by GC. The HPLC fraction containing the mixture of methyl arachidonate geometrical isomers was submitted to silver-nitrate thin layer chromatography ($AgNO_3$-TLC). Silica gel plates (Merck, 0.25 mm thickness, 20 cm x 20 cm) were impregnated by dipping them for 30 minutes in a 10% solution of silver nitrate in acetonitrile. The developing solvent was a mixture of diethyl ether/methanol (95:5, v/v) as described by Morris (1966). Similarly the HPLC fraction containing methyl eicosadienoate was submitted to $AgNO_3$-TLC and the developing solvent was a mixture of toluene/diethyl ether (95:5, v/v). After spraying with a 0.2% solution of 2',7'-dichlorofluorescein in ethanol, bands were detected under ultraviolet light at 254 nm, scraped off the plate and then placed into glass centrifuge tubes. A 1% solution of sodium chloride in 90% ethanol was added until the red color of the silver-dichlorofluorescein complex disappeared, as described by Hill *et al.* (1968). The methyl esters were extracted with hexane after addition of water. An aliquot of the fraction containing the *trans* isomers of methyl arachidonate or methyl 11,14-eicosadienoate with the *trans* double bond were converted into DMOX derivatives and analysed by GC-FTIR and by GC-MS .

Structural determination of the *trans* isomers of arachidonic acid

The isolated fraction containing the *trans* isomers of methyl arachidonate was saponified and then submitted to hydrazine reduction as described above. After 8 hours of reaction, water was added and the fatty acids extracted with hexane. Fatty acids were esterified with BF_3/methanol (14%, v/v). Each reduced fraction, containing a mixture of 20:0, monoenes, dienes, trienes and unreacted 20:4 isomers, was fractionated by $AgNO_3$-TLC, as described above, using toluene as mobile phase. Methyl esters were extracted from silica gel and the resulting monoene fractions (Rf = 0.27, 0.38 and 0.51) were converted to DMOX derivatives and then analysed by GC-FTIR and by GC-MS.

Structural determination of the *trans* isomer of 11, 14-eicosadienoic

The isolated fraction containing the *trans* isomer of methyl eicosadienoate was saponified and then submitted to hydrazine reduction. After 1.5 hours of reaction, water was added and the fatty acids were extracted with hexane. The fatty acids were esterified with BF_3/methanol (14%, v/v). Each reduced fraction, which contained a mixture of 20:0, monoenes and unreacted 20:2 isomers, was fractionated by $AgNO_3$-TLC, as described above. Methyl esters were extracted from silica gel and the monoene fraction was converted to DMOX derivatives and analysed by GC-FTIR and by GC-MS.

Desaturation assays

Unlabelled all *cis* 18:2 n-6 and 20:3 n-6 fatty acids were purchased from Sigma Chemicals (l'Isle d'Abeau, France). [1-^{14}C] 18:2 n-6 and 20:3 n-6 were purchased from NEN (Les Ulis, France). Coenzymes and chemicals were supplied by Sigma.

Liver microsomes from rats fed the experimental (E) and the control (C) diets were prepared at 4°C as previously reported (Blond and Bézard, 1991). Briefly, about 3.5 g of each liver was homogenised in 0.25M sucrose and 0.05M phosphate buffer (pH 7.4). After a first centrifugation at 13,000 x g for 20 min, the pellet was discarded and the supernatant was centrifuged at 105,000 x g for 60 min. The second pellet was resuspended in 0.4 mL of supernatant and 0.8 mL of homogenizing solution. Microsomal proteins were quantified according to the method of Layne (1957). 5 mg of microsomal proteins were then incubated in an open flask with [1-^{14}C] 18:2 n-6 (30, 60, 120 nmoles) or [1-^{14}C] 20:3 n-6 (20, 40, 80 nmoles). Incubations were performed at 37°C in a shaking waterbath for 15 min with a total volume of 2.1 mL incubation medium in phosphate buffer (pH 7.4), as already described (Cao *et al.*, 1993). The incubations were stopped by addition of chloroform/methanol (2:1, v/v). After evaporation of the

lower phase to dryness, the fatty acids were esterified using boron trifluoride in methanol (14%, v/v) as before. The conversion of substrates into their Δ6 and Δ5 products (18:3 and 20:4 n-6, respectively) was determined after separation by reversed phase HPLC (Narce et *al.*, 1988). The fractions containing the substrate and metabolites were then counted using a Packard A 300CD (Packard, Les Ulis, France) liquid scintillation counter.

Statistical analysis

Results are expressed as means ± SD. They were analysed using the Statistical Analysis System (SAS Institute, Carry, NC, USA). The PROC ANOVA procedure was used for analysis of variance. P values of less than 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

The rats which received the diet containing the 18:2 geometrical isomers showed in their liver lipids two unknown fatty acids (X and Y) which were not present in the liver lipids of rats fed with the control diet (Figure 1). While component Y was eluted between 20:2n-9 and 20:2n-6 on a BPX column, component X was eluted just ahead of 20:4n-6.

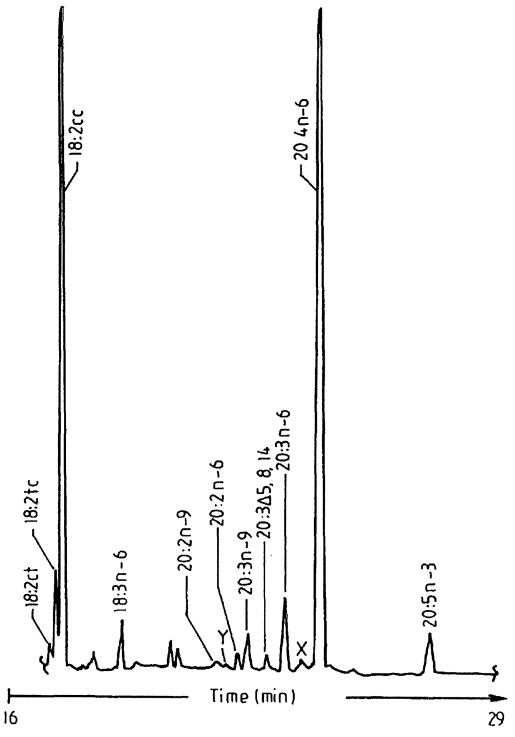

Figure 1
Partial chromatogram of fatty acid methyl esters from liver lipids of rats fed with the experimental diet (BPX-70 column, 170°C)

Identification of C20 metabolites

In order to identify these two unknown components, the total FAME of liver lipids were submitted to an HPLC fractionation on a semi-preparative C18 reversed phase column. Two fractions were collected. The first fraction (F_1) contained component X while the second fraction (F_2) contained component Y (Table III). Fraction F_1 was further submitted to $AgNO_3$-TLC. The band of Rf = 0.85 (40 mg) was scraped off the plate, and methyl esters were extracted as described by Hill *et al.* (1968). GC analysis of this band on a BPX column or on a DB Wax column revealed the presence of two unknown components, X and X' (Figure 2). The latter was not detected in the original sample as it was tailing the large peak of 20:4n-6. The equivalent chain length (ECL) values on BPX 70 column at 180°C of X and X' were 21.13 and 21.33 respectively and on a DB Wax at 190°C, 21.40 and 21.66 respectively.

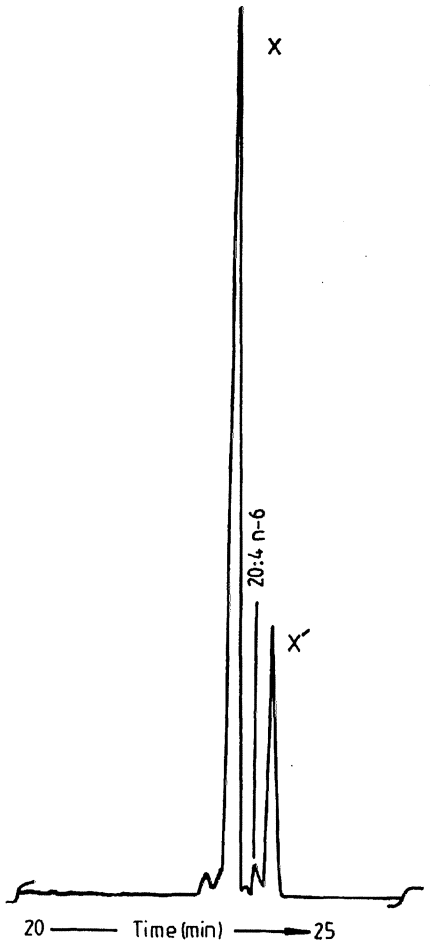

Figure 2
Partial chromatogram of purified mixture of components X and X' (BPX-70 column, 170°C)

Table III
Fatty acid composition of the fraction collected after HPLC fractionation of the liver lipids of rats fed a diet containing 18:2 geometrical isomers (wt %)

Fatty acids	Fraction	
	F1	F2
16:0		3.8
18:1		94.4
20:2n-9		0.05
20:2n-6+Y		1.3
20:4n-6	87.9	
X	3.8	
22:5n-6	1.3	
Others	7.0	0.4

The isolated fraction (F_1) was further submitted to GC-FTIR and an aliquot converted to DMOX derivatives for GC-MS analysis. Both GC-FTIR spectra (Figure 3) revealed the presence of an absorption band characteristic of a *trans* ethylenic bond (966-970 cm^{-1}, δCH) along with the band characteristic of *cis* ethylenic bonds (3022 cm^{-1} and 712 cm^{-1}). GC-MS analysis of DMOX derivatives of 20:4n-6, components X and X' gave the same spectra with an intense molecular ion (*m/z* 357) and the characteristic fragments reported in Figure 4, in agreement with the spectrum of 20:4n-6 reported by Zhang *et al.*, (1988). For example, a mass interval of 12 units instead of 14 units for saturated chain occurred between *m/z* 140 (C4) and 152 (C5), between *m/z* 180 (C7) and 192 (C8), between *m/z* 220 (C10) and 232 (C11), and between *m/z* 260 (C13) and 272 (C14). This indicates the presence of 4 ethylenic bonds in Δ5, Δ8, Δ11, and Δ14 positions. Furthermore, the prominent ion at *m/z* 153 is a diagnostic ion in the spectra of DMOX derivatives with their first double bond at the Δ5 position (Luthria and Sprecher, 1993; Fay and Richli, 1991; Zhang *et al,*. 1988).

In conclusion, GC-MS and GC-FTIR analyses showed that components X and X' are isomers of arachidonic acid, having at least one *trans* ethylenic bond. In order to determine the position of the *trans* ethylenic bond, the band containing both 20:4 isomers (Figure 2) was submitted to hydrazine reduction. With hydrazine, reduction takes place without modification of the geometry or the position of the ethylenic bonds, so that the ethylenic bonds in the monoenes obtained by reduction of the polyenes are representative of the original positions in the parent molecule (Ratnayake, 1981). Hydrazine reduction of components X, X' and 20:4n-6 (Figure 2) gave a complex mixture of 20:0, monoenes, dienes, trienes and some unreacted X, X' and 20:4n-6. This mixture was then fractionated by $AgNO_3$-TLC. Six bands were isolated and further analysed by GC (Figure 5). Band 1 (Rf = 0.62) was composed of 20:0 while bands 5 and 6 were a mixture of dienes, trienes and tetraenes. Bands 2 (Rf = 0.51), 3 (Rf = 0.38) and 4 (Rf = 0.27) were composed of different *cis* and *trans* monoenes. GC-FTIR analyses showed that monoenes C, D, E and F (Figure 5) only contained one *cis* ethylenic bond while monoenes A and B presented an IR band at 968 cm^{-1}, which indicates the presence of a *trans* ethylenic bond. To determine the position of the ethylenic bond in the monoenes, each band (2, 3, and 4) was converted to DMOX derivatives and submitted to GC-MS analysis. The major fragments of monoenes A, B, C, D, E and F

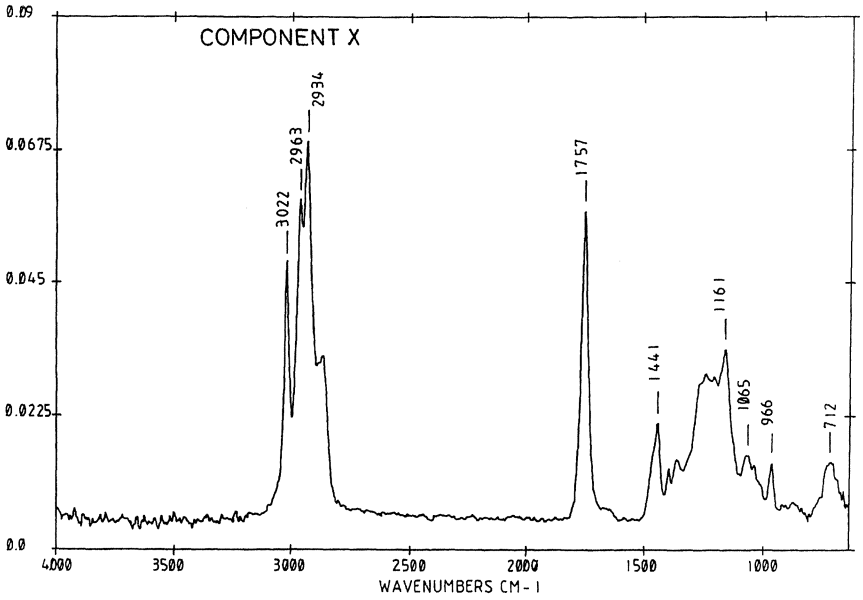

Figure 3
Infrared spectrum of methyl ester of component X

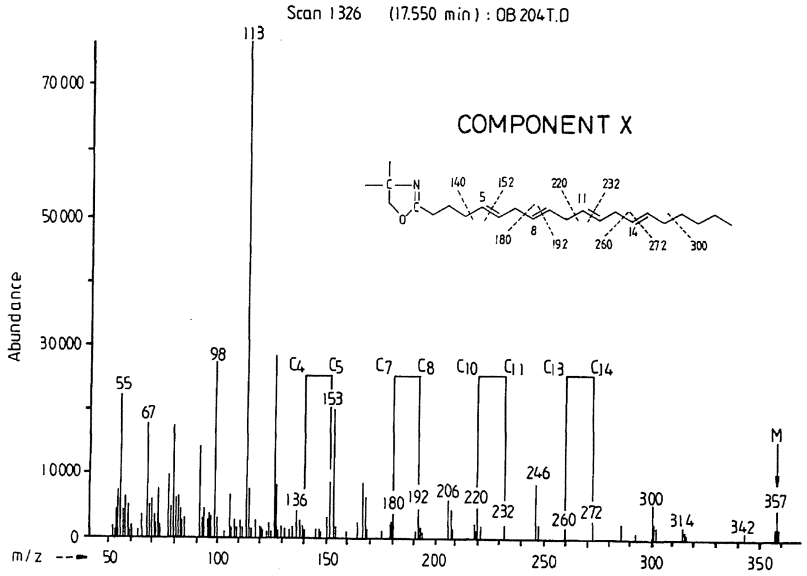

Figure 4
Mass spectrum of 4,4-dimethyloxazoline (DMOX) derivative of the isolated component X

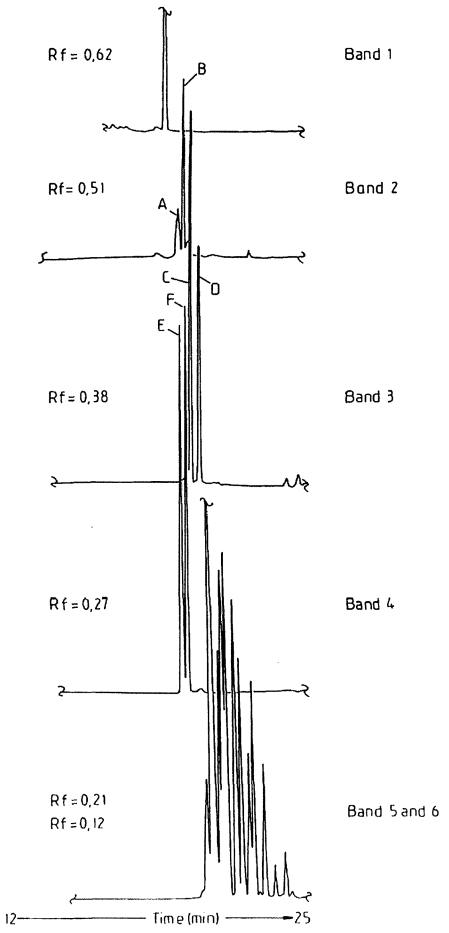

Figure 5
Partial chromatogram of $AgNO_3$-TLC bands resulting from the hydrazine reduction of the mixture of components X and X'

are reported in Table IV. As an example, for compound A, a mass interval of 12 units instead the usual 14 units for saturated chain occurred between *m/z* 224 (C10) and 236 (C11), indicating a double bond in the Δ11 position in the parent molecule. Similarly, components B and D are the 20:1 Δ14 isomers, component C 20:1Δ 11, component E the 20:1 Δ5, and component F the 20:1 Δ8.

All the results, as well as the ratio of monoene A / monoene B (Figure 5) indicate that compounds X and X' are respectively 20:4 Δ5c,8c,11c,14t and 20:4 Δ5c,8c,11t,14c. These two components are the metabolites of the 18:2 Δ9c,12t and 18:2 Δ9t,12c. These data agree with those of Beyers and Emken (1991). However, it is important to note that in previous studies (Privett *et al.*, 1967; Ratnayake *et al.*,1994) the structure of the 20:4 Δ14 *trans* isomer was fully determined, which was not the case for the 20:4 Δ11 *trans* isomer.

For the identification of component Y, the isolated HPLC fraction 2 (Table III) which contained among others, 20:2n-9, 20:2n-6 and Y was further submitted to a $AgNO_3$-TLC. Five bands of Rf 0.12, 0.25, 0.35, 0.59 and 0.76 respectively were obtained. The band of Rf = 0.59 was randomly divided into three parts (D_1, D_2, D_3, Table V) in order to concentrate component Y (Table V) in one fraction. GC-FTIR spectra of the 3 parts (D_1, D_2, D_3) of band D revealed that component Y contained a *trans* ethylenic bond while 20:2n-6 and 20:2n-9 contained only *cis* ethylenic bonds. GC-MS analyses of DMOX derivatives (Table VI) confirmed the structure of 20:2 usual fatty acid isomers present in band D, as 20:2n-6 and 20:2n-9. However, component Y showed the same fragmentation pattern as that of 20:2n-6 which indicates two ethylenic bonds in Δ11 and Δ14 positions. To establish which double

Table IV
Characteristic ions in mass spectra of 4,4-dimethyloxazoline (DMOX) derivatives of principal fragments of the monoenes obtained by hydrazine reduction of the mixture of components X and X'

Bands	Components	M^+ *m/z* (Intensity %)	Fragments *m/z* (intensity %)
2	A	363 (9.9)	113 (100); 126 (84.3); 210 (11.4); 224 (4.1); 236 (3.6); 264 (21.3)
	B	363 (12.7)	113 (100); 126 (85.5): 252 (9.9); 266 (2.0); 278 (2.4); 306 (21.1)
3	C	363 (7.3)	113 (100); 126 (77.8); 168 (9.2); 224 (3.1); 236 (2.9); 264 (16.0)
	D	363 (9.3)	113 (100); 126 (72.8); 252 (8.8); 266 (0.9); 278 (1.7); 306 (2.3)
4	E	363 (1.4)	113 (100); 126 (8.9); 140 (0.3); 152 (1.7); 153 (9.2); 166 (3.8); 180 (9.4)
	F	363 (6.8)	113 (66.2); 126 (100); 168 (25.8); 182 (6.9); 194 (4.0); 222 (24.1)

bond was in the *trans* configuration, band D_1 was submitted to hydrazine reduction and the resulting monoenes were then isolated by $AgNO_3$-TLC. Hydrazine reduction resulted in the formation of three major monoenes G, H and Z. GC-MS studies of DMOX derivatives (Table VII) showed that H and Z were 20:1 Δ11 isomer while the monoene G was the 20:1 Δ14 isomer. GC-FTIR studies also revealed that only monoene Z had a *trans* ethylenic bond. Monoenes G and H are formed from 20:2n-6 while monoenes G and Z are formed from component Y. The unknown component was therefore the 20:2 Δ11t,14c. We can anticipate that this 20:2 isomer is formed by chain elongation of 18:2 Δ9t,12c. This product also has never been fully identified.

These results which are in agreement with those reported by Beyers and Emken (1991), do not support those of Ratnayake *et al.* (1994). *In vivo*, 18:2 Δ9c,12t can be desaturated and elongated into an isomer of arachidonic acid (20:4 Δ14t) while 18:2 Δ9t,12c is rather elongated to 20:2 Δ11t,14c (one fifth as much desaturation product is obtained from 18:2 Δ9t,12c (20:4 Δ11t isomer) compared to that formed from 18:2 Δ9c,12t).

Distribution of *trans* PUFA in tissue lipids.

The presence of 18:2 geometrical isomers in the diet of the experimental group (E) as well as the formation of geometrical isomers of arachidonic acid did not induce any modification in the amount of lipids in rat liver, heart, and brain. There was also no change in the non-phosphorus lipids / phospholipids ratio for heart and liver.

The fatty acid compositions of liver, heart, testes, brain and adipose tissue total lipids are listed in Table VIII. For these different tissues, the presence of geometrical isomers of linoleic acid in the experimental group did not induce any modification of the fatty acid composition. The Δ14 *trans* isomer of arachidonic acid was detected in most of the tissues except for brain and adipose tissue. The highest quantity of 20:4 Δ14t was observed in the liver. These results, which are in agreement with those of Privett *et al.* (1967), Beyers and Emken (1991), and Ratnayake *et al.* (1994), who used higher quantities of *trans* 18:2 isomers in their diet, show that even when present in small quantities in the diet (0.6% of the total energy) 18:2 Δ9c,12t can be converted into 20:4 Δ5c,8c,11c,14t. Unfortunately, our analytical conditions did not allow us to quantify in the total lipids the metabolite of the 18:2 Δ9t,12c, the 20:4 Δ11t as this component was tailing the large peak of arachidonic acid as previously described. However, the quantity of 20:4 Δ11t was 4.5 times less than that of 20:4 Δ14t, which is close to what was obtained by Beyers and Emken (1991). We should also mention that the *trans* isomer of 20:2n-6, the 20:2 Δ11t,14c, was only detected in liver and in heart total lipids. However, we have never detected the presence of 20:2 Δ11c,14t, a *trans* fatty acid which could be formed by elongation of 18:2 Δ9c,12t, in any tissue lipids. These results which do not agree with those of Ratnayake et al. (1994), show that only the 18:2 Δ9t,12c can be converted in detectable quantities. Recent *in vitro* studies (Berdeaux, 1996) have shown that 18:2 Δ9t,12c was a better substrate for the elongation process, supporting this *in vivo* experiment. 18:2 Δ9c,12t, and Δ9t,12c were detected in most tissue lipids except for the Δ9c,12t which was only present at a trace level in brain. The largest quantities of 18:2 geometrical isomers were found in the adipose tissue and the lowest value was observed in brain (Table VIII). In adipose tissue (Table IX), the ratio of 18:2 Δ9c,12t/18:2 Δ9t,12c (0.73) was similar to that

Table V
Fatty acid composition (in wt %) of the bands obtained after $AgNO_3$-TLC of fraction 2 isolated by HPLC

Fatty acid	Band A	Band B	Band C	Band D			Band E
				D1	D2	D3	
16:0	-	-	-	-	-	-	98.0
18:1n-9,n-7	-	-	2.8	81.6	96.8	98.7	-
20:2 n-6			69.0	1.2	-	-	-
20:2n-9	-	12.9	2.2	0.3	-	-	-
Y	-	-	-	**8.6**	**2.8**	**1.0**	-
others	-	87.1	0.3	8.3	0.4	0.3	2.0
Rf	0.12	0.25	0.35		0.59		0.76

Table VI
Characteristic ions in mass spectra of 4,4-dimethyloxazoline (DMOX) derivatives of component Y, 20:2n-6 and 20:2n-9

Fatty acids DMOX	M^+ *m/z* (Intensity %)	Fragment *m/z* (Intensity %)
20:2n-6	361 (17.6)	113 (90); 126 (100); 210 (14.4); 224 (5.2); 236 (8.6); 250 (13.6); 264 (16.5); 276 (7.4); 304 (24.4.)
20:2n-9	361 (9.2)	113 (59.7); 126 (100); 168 (20.7); 182 (13.2); 194 (4.3); 208 (35.7) 222 (11.1); 234 (6.5); 262 (11.3)
Y	361 (21.9)	113 (88.3); 126 (100); 210 (13.9); 224 (4.8); 236 (6.9); 250 (14.2); 264 (12.1); 276 (5.9); 304 (25.1)

Table VII
Characteristic ions in mass spectra of 4,4-dimethyloxazoline (DMOX) derivatives of principal fragments of monoenes G, H and Z obtained by hydrazine reduction of the mixture of components Y, 20:2n-6 and 20:2n-9

Fatty acids DMOX	M^+ *m/z* (Intensity %)	Fragment *m/z* (Intensity %)
G	363 (17.6)	113 (100); 126 (83.2); 252 (12.3); 266 (2.9); 278 (3.4); 306 (27.7)
H	363 (15.5)	113 (100); 126 (77); 210 (12.2); 224 (34.7); 236 (4.3); 264 (24.3)
Z	363 (17)	113 (100); 126 (81.2); 210 (9.9); 224 (3.4); 236 (4.5); 264 (24.7)

Table VIII
Fatty acid composition (wt%) of liver, heart, brain, testes and adipose tissue total lipids of rats fed with the control or the experimental diets

	Experimental group					Control group				
	Liver	Heart	Brain	Testes	Adipose tissue	Liver	Heart	Brain	Testes	Adipose tissue
16:0	20.7±0.80	12.5±0.99	19.0±1.71	31.4±0.77	23.6±2.14	21.1±1.93	12.4±0.29	18.1±0.46	30.7±1.08	23.9±2.33
18:0	13.6±0.80	19.2±0.80	19.4±1.74	6.3±0.33	2.5±0.51	14.4±0.72	19.3±0.29	19.0±0.21	6.2±0.06	2.8±0.30
16:1 n-9	0.5±0.10	0.3±0.01	0.2±0.03	0.4±0.14	0.6±0.07	0.5±0.09	0.2±0.03	0.2±0.02	0.4±0.02	0.6±0.05
16:1 n-7	2.8±0.38	1.1±0.22	0.4±0.04	1.5±0.66	7.5±0.68	3.8±0.94	1.0±0.16	0.4±0.02	1.6±0.68	7.5±1.47
18:1 n-9	20.2±0.47	13.4±0.85	18.3±1.83	15.6±1.68	47.6±1.19	21.0±0.58	12.0±1.41	18.9±0.26	16.1±3.38	48.7±2.96
18:1 n-7	4.2±0.44	4.2±0.37	3.3±1.18	2.1±0.5	2.9±0.48	3.3±0.65	4.6±0.70	3.7±0.15	2.3±0.23	2.3±1.39
18:2 n-6	9.1±1.06	18.3±0.73	0.6±0.05	5.0±0.58	9.5±1.6	8.0±0.95	18.8±1.15	0.6±0.05	5.0±0.64	10.0±1.71
18:2 ct	0.2±0.04	0.15±0.02	traces	0.09±0.06	0.82±0.12					
18:2 tc	0.7±0.14	0.66±0.01	0.05±0.01	0.35±0.07	1.13±0.15					
18:3 n-3	0.3±0.11	0.2±0.03	ND[b]	0.2±0.10	1.3±0.18	0.3±0.25	0.2±0.02	ND	0.2±0.13	1.3±0.19
20:2 n-6	0.2±0.05	0.1±0.03	0.2±0.11	0.2±0.04	traces	0.2±0.17	0.1±0.06	0.1±0.04	0.2±0.05	traces
20:2 tc	0.06±0.02	traces[a]	ND[b]	ND	ND					
20:3 n-6	0.9±0.07	0.5±0.04	0.56±0.03	0.9±0.06	traces	1.0±0.12	0.4±0.05	0.4±0.03	0.9±0.22	traces
20:3 n-9	0.5±0.23	0.2±0.06	0.2±0.10	0.2±0.06	ND	0.7±0.13	0.2±0.04	0.2±0.02	0.2±0.08	ND
20:4 n-6	14.5±0.50	16.1±0.71	9.3±0.82	13.7±0.92	traces	14.6±0.87	17.4±1.05	9.0±1.03	13.8±1.57	traces
20:4 ccct	0.15±0.09	0.05±0.01	ND	0.02±0.02	ND					
20:5 n-3	0.1±0.02	0.3±0.04	0.6±0.24	0.1±0.05	traces	0.1±0.02	0.1±0.03	0.5±0.09	0.06±0.01	traces
22:5 n-6	0.5±0.15	0.7±0.11	0.2±0.13	13.3±1.10	traces	0.6±0.08	0.5±0.15	0.3±0.04	13.5±1.35	traces
22:6 n-3	6.3±0.36	5.9±0.36	9.3±5.23	1.7±0.19	traces	7.2±0.46	5.7±0.19	11.0±0.53	1.7±0.18	traces
others	4.5	6.1	18.4	6.94	2.5	3.2	7.1	17.6	7.14	2.9

[a] Traces <0.02%. Values are means±standard deviation (n=5).
[b] ND: not detected under our analytical conditions.

observed in the experimental diet (0.81). Different ratios ranging from 0.22 to 0.29 were observed for the liver, heart and testes. Adipose tissue lipids usually reflect the lipid composition of the diet, while the ratio in the other tissues could be explained by the preferential transformation of 18:2 Δ9c,12t into the 20:4 Δ5c,8c,11c,14t, or by the fact that such substrate might be oxidized more rapidly than 18:2 Δ9t,12c (Anderson, 1968; Fukuda *et al.*, 1993)

Triglycerides incorporated higher quantities of 18:2 Δ9c,12t and 18:2 Δ9t,12c than the other lipid classes (Table X). Furthermore, the ratio of 18:2 Δ9c,12t/18:2 Δ9t,12c was similar to that observed in the diet (Table I) and in adipose tissue (Table VIII). Both the cholesteryl esters and triglycerides contained only small quantities of 20:4 Δ14t, 0.06 and 0.05% respectively. The low level of 20:4 Δ14t (0.06%) in the cholesteryl esters compared to the amount of 20:4n-6 (8.7%) may be explained by a different affinity of the ACAT (acyl-CoA: cholesterol acyltransferase) for *trans* fatty acids compared to *cis* fatty acids, as shown by Kritchevsky and Baldin (1978) and Sgoutas (1979) for *trans* monoenes. No major modifications of the fatty acid composition of the different phospholipid classes were induced by the presence of the geometrical isomers of linoleic acid in the diet. Higher quantities of 18:2 Δ9c,12t and 18:2 Δ9t,12c were found in the PE and PC, while traces (<0.02%) were detected in the PI. The *trans* 20:4 isomer (20:4 Δ14t) was only detected in three of the phospholipid classes, PE, PI and PC, the highest quantity (0.8%) being found in PI (Table X). It is interesting to note that this phospholipid class also contained the highest quantity of 20:3n-9 (2.8%). The lowest quantity of 20:4 Δ14t (0.2%) was observed in the PE, the phospholipid which contained the lowest quantity of 20:3n-9 (0.4%). The PC showed an intermediate situation. These data seem to indicate that 20:4 Δ14t behaves like its structural analogue, the *trans* Δ14 bond being recognised as a single bond by enzymatic systems. These results agree with those of Wolff *et al.* (1993) who showed that 18:3 Δ9c,12c,15t was esterified in the 1(1") and 2(2")-positions of heart cardiolipins with a marked selectivity for position 1(1") as for its structural analogue, 18:2 Δ9c,12c.

Desaturation of linoleic and dihomo-γlinolenic acids

The fatty acid composition of liver microsomal phospholipids is listed in Table XI. The 18:2 Δ9c,12t and 18:2 Δ9t,12c were incorporated into liver microsomal phospholipids and the ratio of 18:2 Δ9c,12t / 18:2 Δ9t,12c was similar to the ratio observed in liver total lipids (0.38).

Table IX
Some important fatty acids (wt%) in the experimental diet and in the liver, heart, testes, brain and adipose tissue total lipids

	18:2n-6	18:2ct	18:2tc	18:2 ct/tc x 100	20:3n-9	20:4ccct	20:4n-6	20:4 ccct/cccc x100
Liver	9.1	0.2	0.7	0.29	0.9	0.15	14.5	1.03
Heart	18.3	0.15	0.7	0.22	0.2	0.05	16.1	0.31
Testicules	5.0	0.1	0.35	0.28	0.2	0.02	13.7	0.14
Brain	0.6	traces[a]	0.05	NC[c]	0.2	ND[b]	9.3	NC[c]
Adipose Tissue	9.5	0.8	1.1	0.73	traces	ND	traces	NC
Diet E	25.1	2.2	2.7	0.81				

[a] traces (<0.02%)
[b] ND: No detected.
[c] NC: Not calculated: the denominator and/or the numerator are known with insufficient precision (traces).

Table X
Fatty acid composition (wt%) in liver phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), triglycerides (TG) and cholesteryl esters (CE) of rats fed with the control or the experimental diets

	Experimental group					Control group				
	phospholipids			Non phosphorus lipids		Phospholipids			Non phosphorus lipids	
	PE	PI	PC	TG	CE	PE	PI	PC	TG	CE
16:0	12.5±2.68	5.2±0.65	15.1±1.39	25.7±2.22	12.5±2.95	12.5±1.66	7.1±1.05	15.6±1.23	28.6±3.54	12.8±2.28
18:0	22.7±0.96	42.6±2.92	20.6±1.98	2.5±0.29	2.2±0.36	23.5±0.75	43.1±2.51	20.4±0.95	2.9±0.49	2.18±0.32
∑ 16:1	0.4±0.22	0.8±0.11	1.3±0.24	7.5±2.31	9.0±3.2	0.4±0.21	0.8±0.07	1.4±0.33	8.3±2.15	8.5±1.97
∑ 18:1	5.0±0.76	3.5±0.26	9.6±1.35	49.4±1.79	44.7±4.02	5.0±1.32	3.7±0.55	9.0±1.21	45.1±2.4	43.0±5.31
18:2 n-6	3.5±0.74	1.3±0.15	10.8±1.67	7.2±2.69	7.5±2.15	3.1±0.81	1.1±0.12	10.6±1.38	6.4±	7.4±1.06
18:2 ct	0.07±0.02	traces[a]	0.09±0.03	0.37±0.04	0.21±0.07					
18:2 tc	0.35±0.11	traces	0.76±0.11	0.98±0.22	0.39±0.07					
18:3 n-3	ND[b]	ND	0.07±0.02	0.6±0.26	0.6±0.26	ND	ND	0.06±0.02	0.5±0.32	0.6±0.33
20:2 n-6	ND	ND	0.11±0.08	0.05±0.03	traces	ND	ND	0.09+0.01	0.06+0.03	traces
20:2 tc	ND	ND	ND	ND	ND					
20:3 n-6	0.5±0.07	2.2±0.46	1.5±0.09	0.1±0.05	0.3±0.07	0.5±0.11	1.7±0.12	1.6±0.12	0.1±0.03	0.3±0.19
20:3 n-9	0.4±0.12	2.8±0.64	0.7±0.17	0.15±0.02	0.3±0.07	0.3±0.17	2.7±0.22	2.0±0.29	0.15±0.04	0.2±0.06
20:4 n-6	26.3±1.44	33.6±3.41	23.2±1.46	1.2±0.47	8.7±2.32	27.0±1.20	32.9±2.70	23.5±0.95	1.1±0.42	8.6±2.39
20:4 ccct	0.15±0.03	0.83±0.10	0.30±0.07	0.03±0.01	0.06±0.02					
20:5 n-3	0.1±0.02	ND	0.2±0.10	0.2±0.11	0.5±0.13	0.1±0.02	ND	0.2±0.02	0.1±0.03	0.5±0.26
22:5 n-6	0.7±0.40	0.2±0.04	0.4±0.14	traces	traces	0.9±0.41	0.5±0.13	0.4±0.21	traces	traces
22:6 n-3	18.8±1.98	2.4±0.38	7.6±0.95	0.4±0.20	traces	18.4±0.81	2.1±0.61	8.5±0.57	0.4±0.23	traces
others	8.5	4.6	7.2	3.2	13.0	8.3	4.3	6.6	6.3	15.9

[a] Traces <0.02%. Values are means± standard deviation (n=5).
[b] ND: not detected under our analytical conditions

Table XI
Fatty acid composition (wt%) of microsomal phospholipids of rat fed with the control or experimental diet

	Experimental group (E)	Control group (C)
16:0	18.1±1.79	18.2±1.44
18:0	22.7±0.99	22.2±0.98
16:1 n-9	0.3±0.02	0.3±0.03
16:1n-7	1.2±0.27	1.3±0.37
18:1 n-9	7.5±0.44	7.4±0.07
18:1n-7	4.2±0.38	4.3±0.13
18:2n-6	7.3±0.45	7.5±0.46
18:2Δ9c, 12t	0.20±0.04	–
18:2 Δ9t, 12c	0.52±0.05	–
18:3n-3	traces[a]	traces
20:2n-6	0.3±0.05	0.2±0.02
20:2Δ11t, 12c	ND[b]	–
20:3n-6	1.2±0.09	1.3±0.15
20:3n-9	0.6±0.07	0.6±0.02
20:4n-6	21.0±1.37	21.6±0.49
20:4 Δ5c, 8c, 11c 14t	0.18±0.03	–
20:5n-3	0.7±0.05	0.6±0.05
22:5n-6	0.6±0.17	0.8±0.05
22:6n-3	8.7±0.43	8.9±0.36
Others	4.5	4.7

[a] traces (<0.02%)
[b] ND: No detected

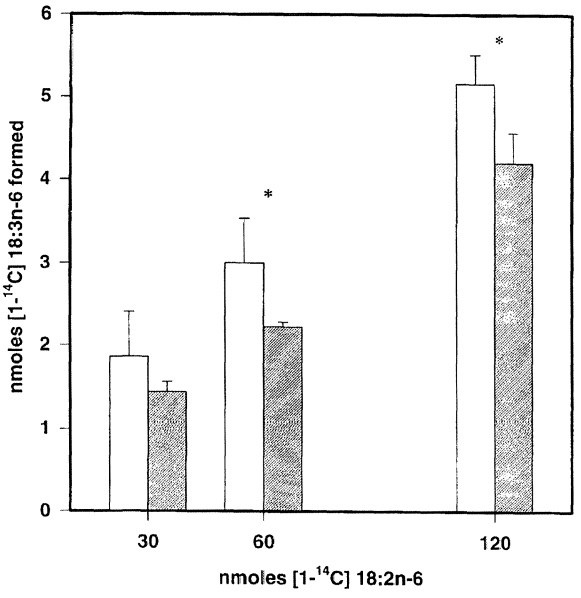

Figure 6
Δ6-desaturation of [1-^{14}C]-linoleic acid to-γlinolenic acid in liver microsomes from rats fed the experimental ■ or the control ❑ diet. Results are expressed as means ±SD (n=4). After analysis of variance, means were compared according to the least difference and classified to decreasing order.
*: P < 0.05 (experimental (E) *versus* control (C)).

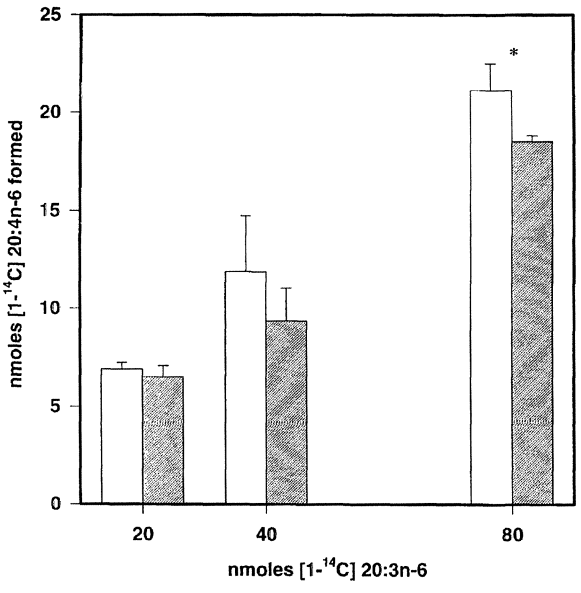

Figure 7
Δ5-desaturation of [1-^{14}C]-dihomo-γlinolenic acid to arachidonic acid in liver microsomes from rats fed the experimental ■ or the control ❑ diet.
Results are expressed as means ±SD (n=4). After analysis of variance, means were compared according to the least difference and classified to decreasing order.
*: P < 0.05 (experimental (E) *versus* control (C)).

Figures 6 and 7 report the *in vitro* determinations of Δ6 and Δ5 desaturation rates of 18:2n-6 and 20:3n-6 respectively. At a lower substrate level, which better reflects the physiological conditions, the rate of Δ6 desaturation, but not of Δ5 desaturation, was decreased in the experimental group, as compared to control. Both desaturation activities were inhibited in group E. Similarly, Cook and Emken (1990) demonstrated in glioma cells that *trans* isomers of 18:2n-6 inhibited the 20:3n-6 synthesis both at desaturation and elongation levels, and the formation of 20:4n-6 after activation of Δ5 desaturase.

However, the presence of 18:2 Δ9c,12t and 18:2 Δ9t,12c in the diet did not affect the all-*cis* n-6 polyunsaturated fatty acid profile of liver (Table IX), suggesting no significant effect *in vivo*.

CONCLUSION

The present data show that, at low concentrations, dietary 18:2 Δ9c,12t is converted into 20:4 Δ14t which was identified. On the other hand, 18:2 Δ9t,12c is converted into another geometrical isomer of arachidonic acid, 20:4 Δ11t, but at a lower rate compared to 18:2 Δ9c,12t. 18:2 Δ9t,12c is preferentially elongated into a 20:2 *trans* isomer which was also totally identified. These results are in agreement with *in vitro* results suggesting that 18:2 Δ9c,12t is Δ6 desaturated at a similar rate as linoleic acid, whereas 18:2 Δ9t,12c is a better substrate for elongation (Berdeaux, 1996). Together, these data confirm that a *cis* double bond at Δ9 position is helpful for Δ6 desaturation of C18 PUFA (Brenner, 1971). However, Δ9 *trans* PUFA are also desaturated, but at a low rate, as already shown with 18:3 Δ9t (Chardigny *et al*, 1996b). The major metabolite from dietary 18:2 isomers has been shown to be 20:4 Δ5c,8c,11c,14t. As we previously observed (Berdeaux *et al*, 1996, in press), 20:4 Δ14t has a significant antiaggregatory effect when compared to arachidonic acid. Therefore, our study indicate that common pathways for usual fatty acids can also operate for *trans* isomers, producing unnatural metabolites with physiological activity of potential interest.

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