Desaturation and chain elongation of (1-14C) mono-trans isomers of linoleic and alpha-linolenic acids in perfused rat liver

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Desaturation and chain elongation of [1-^{14}C]mono-*trans* isomers of linoleic and α-linolenic acids in perfused rat liver

Lionel Bretillon,* Jean-Michel Chardigny,* Jean-Pierre Noël,† and Jean-Louis Sébédio[1,*]

INRA,* Unité de Nutrition Lipidique, 21034 Dijon Cédex, France; and CEA Saclay,† Service des Molécules Cédex Marquées, Gif-sur-Yvette, France

Abstract ***Trans*** **polyunsaturated fatty acids are produced during heat treatment of oils, such as deodorization and frying. The detailed metabolic pathways of these *trans* isomers are not fully understood. In the present work, the desaturation and chain elongation of [1-^{14}C]linoleic acid, 9*cis*,12*trans*-18:2, 9*trans*,12*cis*-18:2, α-linolenic acid, 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3 were studied using a perfused rat liver model. After perfusion with both *trans* isomers of 18:2n–6, the ^{14}C was equally distributed between phospholipids and triacylglycerols, compared to the 70:30 distribution (phospholipids:triacylglycerols) observed after infusing linoleic acid. The corresponding distribution of ^{14}C after perfusion with both *trans* isomers of 18:3n–3 was comparable to what was obtained for α-linolenic acid. The products of conversion were analyzed by combination of different radio chromatographic methods. 9*cis*,12*trans*-18:2 was 16 times more converted into a C18:3n–6 fatty acid than linoleic acid into γ-linolenic acid. *Trans*-18:2 isomers were more elongated into "dead-end products" when compared to the conversion of linoleic acid into 20:2n–6 (from 2- to 5-times more). 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3 were 2- and 10-times less converted to *trans*-20:5, respectively, than α-linolenic acid into eicosapentaenoic acid. Moreover, 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3 were equally and 2.5-times more elongated into "dead-end products", respectively, than α-linolenic acid into 20:3n–3. The partitioning of the conversion between formation of desaturated and chain elongated products on the one hand and production of "dead-end products" on the other was also calculated. Compared to their *cis* analogs, 9*trans*,12*cis*-18:2 and 9*trans*,12*cis*,15*cis*-18:3 were elongated into *trans* "dead-end products" rather than being converted to desaturated and chain elongated *trans*-metabolites. On the other hand 9*cis*,12*cis*,15*trans*-18:3 was more desaturated and chain elongated into 17*trans* 20:5 rather than elongated into 17*trans*-20:3.—**Bretillon, L., J-M. Chardigny, J-P. Noël, and J-L. Sébédio. **Desaturation and chain elongation of [1-^{14}C]mono-*trans* isomers of linoleic and α-linolenic acids in perfused rat liver. *J. Lipid Res.* 1998.** 39: **2228–2236.**

Supplementary key words *trans* polyunsaturated fatty acids • desaturation • elongation

Heat treatment of oils leads to the formation of *trans* isomers of both linoleic and α-linolenic acids. 9*Cis*, 12*trans*-18:2, 9*trans*,12*cis*-18:2, 9*cis*,12*cis*,15*trans*-18:3, and 9*trans*,12*cis*,15*cis*-18:3 are the major isomers produced by isomerization of linoleic and α-linolenic acids, respectively (1–5).

Essential fatty acids are not only desaturated and elongated, leading to the formation of arachidonic acid from 18:2n–6 and eicosapentaenoic and docosahexaenoic acids from 18:3n–3, but also elongated to "dead-end products" (20:2n–6 from linoleic acid and 20:3n–3 from α-linolenic acid) (6–12). *Trans* polyunsaturated fatty acids probably follow the same biochemical pathways as the essential fatty acids, as C20 and C22 fatty acids containing one *trans* ethylenic bond have been found in tissues of rodents fed *trans* C18 fatty acids (11, 13–20). However, only few data on the splitting between desaturation–elongation on the one hand and elongation on the other of the *trans* fatty acids have been published. It is now well established that the rate for conversion of 9*cis*,12*trans*-18:2 to 5*cis*,8*cis*, 11*cis*,14*trans*-20:4 is higher than that of 9*trans*,12*cis*-18:2 to 5*cis*,8*cis*,11*trans*,14*cis*-20:4 (11, 18, 19), while the formation of the dead-end product (20:2) is still controversial. Beyers and Emken (11) and Berdeaux et al. (19) detected a dead-end product only from 9*trans*,12*cis*-18:2 (11*trans*, 14*cis*-20:2), whereas Ratnayake et al. (18) showed that both 9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2 could be transformed in 11*cis*,14*trans*-20:2 and 11*trans*,14*cis*-20:2, respectively. No data are available on the conversion of the *trans* isomers of α-linolenic acid (partitioning between formation of *trans* isomers of eicosapentaenoic acid and docosahexaenoic acid and production of dead-end products).

Abbreviations: LDH, lactate dehydrogenase; FAME, fatty acid methyl ester; HPLC, high performance liquid chromatography; FID, flame ionization detector; GC-RAM, gas chromatography coupled with a radioactive detection; TLC, thin-layer chromatography.

[1]To whom correspondence should be addressed.

The aim of the present study was to establish to what extent the conversion of the [1-^{14}C]*trans* isomers of linoleic and α-linolenic acids is split between the formation of long chain polyunsaturated fatty acids formed by desaturation and elongation and the production of dead-end products, by comparison with the essential C18 fatty acids. For that purpose, the perfused rat liver model was used. Such an ex vivo experiment was preferred to an in vivo study as the dilution of the radiolabelled precursor in all organs does not occur and metabolic oxidation of the tracer can be minimized.

MATERIALS AND METHODS

Fatty acids

Radiolabeled linoleic acid ([1-^{14}C]9*cis*,12*cis*-18:2, 1.96 GBq·mmol^{-1}) and α-linolenic acid ([1-^{14}C]9*cis*,12*cis*,15*cis*-18:3, 1.92 GBq·mmol^{-1}) were purchased from NEN (Les Ulis, France). *Trans* fatty acids ([1-^{14}C]9*cis*,12*trans*-18:2, 2.00 GBq·mmol^{-1}, [1-^{14}C]9*trans*,12*cis*-18:2, 1.99 GBq·mmol^{-1}, [1-^{14}C]9*cis*,12*cis*,15*trans*-18:3, 1.94 GBq·mmol^{-1}, and [1-^{14}C]9*trans*,12*cis*,15*cis*-18:3, 1.88 GBq·mmol^{-1}) were obtained by total synthesis as described by Eynard et al. (21) and Berdeaux et al. (22).

The day prior to the perfusion, 1.85 MBq (100 nmol) of the fatty acid was diluted in 5 mL of Krebs-Henselheit buffer (pH 7.4) containing 25 mm glucose and 36 mg of bovine serum albumin (Sigma, L'Isle d'Abeau, France). The vial was tightly capped under nitrogen and shaken gently at 37°C overnight to allow binding of the fatty acids to albumin.

Animals

Male Wistar rats (Centre d'Elevage DEPRE, Saint Doulchard, France) weighing 300–350 g were housed in controlled conditions of light (lights on 7:00–19:00), temperature (22 ± 1°C) and humidity (55–60%). Animals were fed a commercial pellet diet (Extralabo, Provins, France).

Perfusion

All the experiments were performed at the same time of day in order to avoid diurnal variations (start at 9:30 am). The rats were anesthetized by intraperitoneal injection of pentobarbital (Sanofi, Libourne, France) (6 mg per 100 g of body weight). The liver was perfused in situ with Krebs-Henselheit buffer (pH 7.4) containing 25 mm glucose, oxygenated with a mixture of O_2/CO_2 (95%/5%) and was then rapidly isolated and perfused with oxygenated recirculating Krebs-Henselheit buffer (pH 7.4) maintained at 37°C and containing 25 mm glucose and 1.5% bovine serum albumine (Sigma, L'Isle d'Abeau, France). Ten minutes after starting the recirculation, the viability of the liver was assessed by measuring the lactate dehydrogenase (LDH) activity in the perfusate using a commercial kit (Biomérieux, Marcy l'Etoile, France). The fatty acid was then infused at a rate of 0.1 μmol·min^{-1}. The perfusions were performed for 2 h. The viability of the post-perfused liver was determined by measuring the LDH activity in the perfusate. Livers with LDH activity higher than 400 units·L^{-1}·min^{-1} were discarded. One milliliter of the perfusate was mixed with 5 mL of scintillation cocktail (Ecoscint A, National Diagnostics, Bionis, Clamart, France) and radioactivity was measured using a Tri-carb 2000CA liquid scintillation analyzer (Packard, Groningen, The Netherlands).

Distribution of radioactivity

Radioactivity in lipid classes. Lipids were extracted from the post-perfused liver according to the method of Folch, Lees, and Sloane Stanley (23). Radioactivity was measured on a 1 mL aliquot of the aqueous phase containing the oxidation products by mixing it with 5 mL of Ecoscint A (National Diagnostics).

Lipid classes were fractionated into phospholipids, diacylglycerols, free fatty acids, triacylglycerols, and cholesteryl esters by TLC on silica gel G plates (SDS, Peypin, France) using a mixture of n-hexane–diethyl ether–acetic acid 60:40:4 (v/v/v). The plates were scanned in a Berthold LB 2852 scanner (Elancourt, France) in order to measure the radioactivity in each class of lipids.

Liver phospholipids were separated from neutral lipids using Sep-Pack silica cartridges (Waters, Milford, MA) according to Juanéda and Rocquelin (24). Briefly, 20 mg total lipids was loaded at the top of the cartridge. Neutral lipids were eluted with 30 mL of chloroform and phospholipids were subsequently eluted with 20 mL of methanol. Phospholipid classes were separated by TLC on silica gel G plates (SDS) using chloroform–methanol–petroleum ether–acetic acid–boric acid 40:20:30:10:1.8 (v/v/v/v/w) (25). Each class of phospholipid was visualized with iodine vapor, scraped off, and transferred into a scintillation vial and a scintillation cocktail (Monoflow 4, National Diagnostics) was added. The radioactivity was assessed using a Tri-carb 2000CA liquid scintillation analyzer (Packard, Groningen, The Netherlands).

The level of phosphorus in total phospholipids was determined according to the method of Bartlett (26).

Distribution of the radioactivity in the sn-*1 and* sn-*2 positions of phosphatidylcholine.* Phospholipids classes were separated using the solvent mixture detailed above. Each class of phospholipid was visualized with iodine vapor. Phosphatidylcholine was scraped off and extracted from the silica gel using a mixture of chloroform–methanol–water–acetic acid 150:117:30:3 (v/v/v/v). Solvent was evaporated to dryness and lipids were redissolved in 3 mL of diethyl ether. One unit of phospholipase A_2 (Sigma) dissolved in 0.6 mL of a buffer (Tris 0.5 mol·L^{-1}, $CaCl_2$, 2 mmol·L^{-1}) was added. Digestion was performed at 37°C for 45 min while stirring. The reaction was then stopped by chilling the tube on ice and adding 2.4 mL water. The products of digestion were extracted two times with a mixture of chloroform–methanol 2:1 (v/v), and then separated by TLC on silica gel G plates (SDS) using a mixture of chloroform–methanol–water 65:25:4 (v/v/v). The plates were scanned in a Berthold LB 2852 scanner (Elancourt, France) to determine the distribution of the radioactivity between lysophosphatidylcholine (*sn*-1 position of phosphatidylcholine) and free fatty acid (*sn*-2 position of phosphatidylcholine).

Analysis of the conversion products

Fatty acid methyl esters (FAME) were prepared from total lipids of the post-perfused livers according to the method of Morrison and Smith (27). FAME were fractionated by reversed-phase HPLC using acetonitrile as a mobile phase. A Nucleosil C18 column (25 cm × 10 mm ID, Shandon HPLC, Cheshire, England) and a Waters R401 refractometer were used. As shown in **Fig. 1**, five fractions, F1 to F5, were collected. Two major desaturated and chain-elongated metabolites of linoleic acid (γ-linolenic acid, 18:3n–6 and arachidonic acid, 20:4n–6) were found in the second collected fraction (F2), while 18:2n–6 was in the third one (F3) and 20:2n–6 (dead-end product produced by chain elongation of the precursor) in F4, as well as palmitic acid which is the main de novo synthetized fatty acid (28). Similarly, eicosapentaenoic acid (20:5n–3) and docosahexaenoic acid (22:6n–3) produced by desaturation and chain elongation of 18:3n–3 were in the first fraction (F1), while α-linolenic acid was in the second one (F2) and 20:3n–3 (dead-end product) in the third one (F3). Each collected fraction was transferred to a scintillation vial and radioactive counting was assessed as described above.

FAME in each HPLC-collected fraction were also separated by

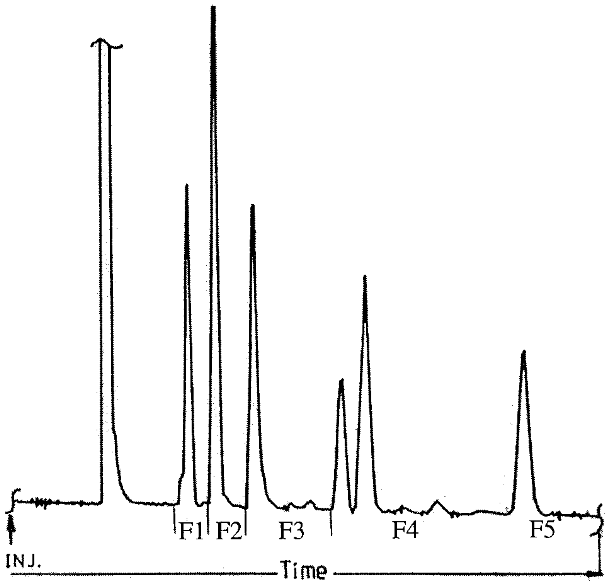

Fig. 1. HPLC separation of FAME of total lipids from rat liver perfused for 2 h with C18:2 or C18:3 isomers (column: Nucleosil C18, 25 cm × 10 mm ID); mobile phase: acetonitrile at 4 ml·min^{-1}). F1, 20:5, 22:6, F2, 18:3, 20:4, F3, 18:2,20:3; F4, 16:0, 18:1, 20:2; F5, 18:0, 20:1.

TLC on silver nitrate-impregnated silica gel G plates (SDS). The first development was performed using toluene in order to separate saturates, monoenes, dienes and trienes from the higher unsaturated fatty acids. A second development was then performed using diethyl ether to separate fatty acids containing four, five and six double bonds. The plates were scanned after each separation in a Berthold LB 2852 scanner. Due to the HPLC separation of the total lipids, a radioactive peak corresponds to the labeling of only one fatty acid, and not to the mixture of two fatty acids with the same unsaturation. Consequently, scanning these plates allowed to quantify a radiolabeled metabolite in the corresponding HPLC fraction. The amount of each labeled metabolite in total lipids was then calculated taking into account the percentage of the five HPLC fractions determined by counting the HPLC fractions.

FAME in each fraction were analyzed on a Hewlett-Packard 5890 series II gas chromatograph (Palo Alto, CA) equipped with a splitless injector and a fused Stabilwax wide bore silica column (60 m × 0.53 mm ID, film thickness: 0.50 μm, Restek, Evry, France). The column was connected to an FID and to a radio-GC detector (GC-RAM, Lablogic, Sheffield, UK). Ten percent of the output flow of the column was split to the FID. Ninety percent of the effluent from the column was oxidized through copper oxide to transform the labeled fatty acids in $^{14}CO_2$. The radioactivity was determined by counting the $^{14}CO_2$ after mixing it with argon–methane 9:1. The data were computed using the Laura software (Lablogic).

The metabolites formed from [1-^{14}C]18:2n–6 and [1-^{14}C] 18:3n–3 were identified by comparison of the retention time of the radioactive signal and the peak obtained by the FID detector. The chain elongation products (20:2 isomers) formed from 9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2 were identified by comparison with authentic standards (29). This was also the case for 5*cis*,8*cis*,11*cis*,14*cis*,17*trans*-20:5 (30) and 5*cis*,8*cis*,11*trans*,14*cis*, 17*cis*-20:5 (31), formed from 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*, 12*cis*,15*cis*-18:3, respectively. All the other fatty acids (20:3 isomers and 6*cis*,9*cis*,12*trans*-18:3) were tentatively identified using the HPLC retention volume, the GC retention time, as well as the R_f values on silver nitrate-impregnated TLC.

The ratios, 18:3n–6+20:4n–6 / 18:3n–6+20:4n–6+20:2n–6 related to C18:2 fatty acids and 20:5n–3 / 20:5n–3+20:3n–3 for the C18:3 fatty acids, were calculated in order to determine to what extent the conversion of the C18 fatty acids was split between the formation of desaturated and elongated products in one hand and the production of dead-end product in the other. Two other ratios (amount of a *trans* metabolite produced from a *trans*-C18 unsaturated fatty acid divided by the amount of the *cis* metabolite formed from linoleic acid or α-linolenic acid) were also calculated in order to compare the conversion of both *trans*-18:2 with linoleic acid and both *trans*-18:3 with α-linolenic acid.

Statistical analysis

Data were expressed as means ± SEM. Results were analyzed using the Statistical Analysis System (SAS Institute, Cary, NC). The PROC ANOVA procedure was used for the analysis of variance.

RESULTS

Distribution of the radioactivity in lipid and phospholipid classes of the post-perfused livers

About 90% of the radioactivity was recovered in total lipids of the post-perfused livers, whatever the fatty acid infused, indicating a good uptake of the radiolabeled fatty acids. The remainder of the radioactivity was shared between the perfusate (about 9%) and the aqueous phase obtained after the extraction of the total lipids from the livers (about 1%, data not shown). Phosphorus amounts in total phospholipids of all the livers were similar (data not shown).

The ^{14}C distribution in lipid classes 2 h after the infusion of radiolabeled linoleic acid was different from what was observed with its *trans* isomers (**Fig. 2A**). Indeed, about 30% of the radioactivity in the liver was recovered in the triacylglycerols after infusing linoleic acid, whereas this value was higher than 50% after perfusion with its *trans* isomers (P = 0.0033). The remainder of the radioactivity in the post-perfused liver was recovered in phospholipids, mainly in phosphatidylcholine, whatever the fatty acid. Moreover, the ^{14}C retention in phosphatidylcholine after infusing linoleic acid was higher than after perfusion with its *trans* isomers (P = 0.039). This incorporation in phosphatidylcholine balanced the lowest level of ^{14}C found in triacylglycerols after perfusion with linoleic acid. Results of phospholipase A_2 digestion of phosphatidylcholine of livers infused with the C18:2 isomers showed no differences within the distribution of the radioactivity in the two positions of the molecule (data not shown). The ^{14}C retention in phosphatidylinositol was significantly lower after infusion with 9*trans*,12*cis*-18:2 than after infusion of linoleic acid or the 9*cis*,12*trans*-18:2 isomer (P = 0.006).

The ^{14}C repartition was equally split between phospholipids and triacylglycerols after perfusion with the C18:3 fatty acids, whatever the fatty acid studied (Fig. 2B). A higher ^{14}C retention in phosphatidylcholine was observed

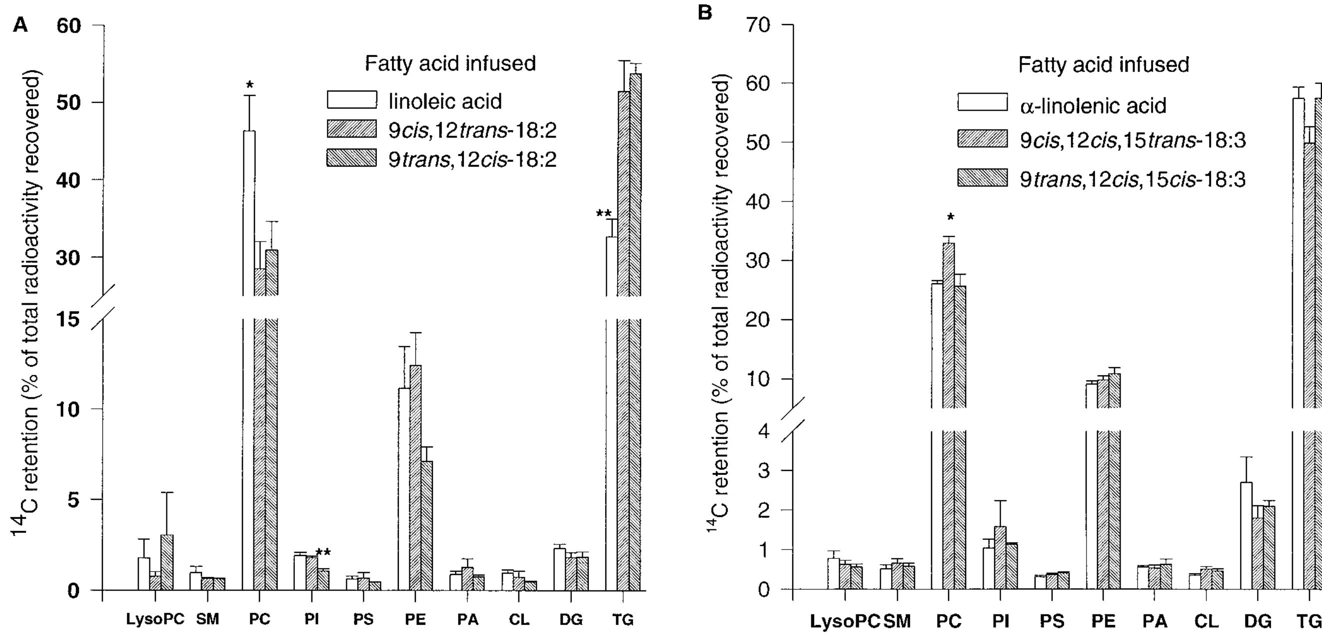

Fig. 2. ^{14}C distribution in classes of liver lipids after perfusion with A: [1-^{14}C]linoleic acid, 9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2. Data are expressed as means of the radioactivity recovered in total lipids ± SEM (n = 3). * and **, significantly different from the corresponding values obtained after perfusion with both other C18:2 isomers at $P < 0.05$ and 0.01, respectively. B: [1-^{14}C]α-linolenic acid, 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3. Data are expressed as means of the radioactivity recovered in total lipids ± SEM (n = 3); *significantly different from the corresponding values obtained after perfusion of both other C18:3 isomers at $P < 0.05$. LysoPC, lysophosphatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PA, phosphatidic acid; CL, cardiolipins; DG, diacylglycerols; TG, triacylglycerols.

after infusing 9*cis*,12*cis*,15*trans*-18:3 than after infusion of α-linolenic acid and 9*trans*,12*cis*,15*cis*-18:3 ($P = 0.018$, Fig. 2B). Results of phospholipase A_2 digestion of phosphatidylcholine from livers infused with α-linolenic acid, 9*cis*,12*cis*,15*trans*-18:3 and linoleic acid are shown in **Fig. 3**. The repartition of the radioactivity in the two positions of phosphatidylcholine after infusing 9*cis*,12*cis*,15*trans*-18:3 was comparable to that of linoleic acid (with a marked selectivity for the *sn*-2 position), while more of α-linolenic acid was in the *sn*-1 position (Fig. 3).

Conversion of [1-^{14}C]linoleic acid, 9*cis*,12*trans*-18:2, and 9*trans*,12*cis*-18:2

Radiolabeled palmitic, γ-linolenic, arachidonic acids and 20:2n–6 were detected in total lipids of livers infused with [1-^{14}C]linoleic acid. Similarly, 16:0, as well as 18:3n–6 and 20:2n–6 isomers were detected in total lipids of livers infused with both *trans* 18:2 fatty acids. No 20:4 were detected in livers infused with 9*cis*,12*trans*-18:2 and 9*trans*, 12*cis*-18:2. Desaturated and chain-elongated products (18:3n–6+20:4n–6+20:2n–6) produced from 18:2n–6,

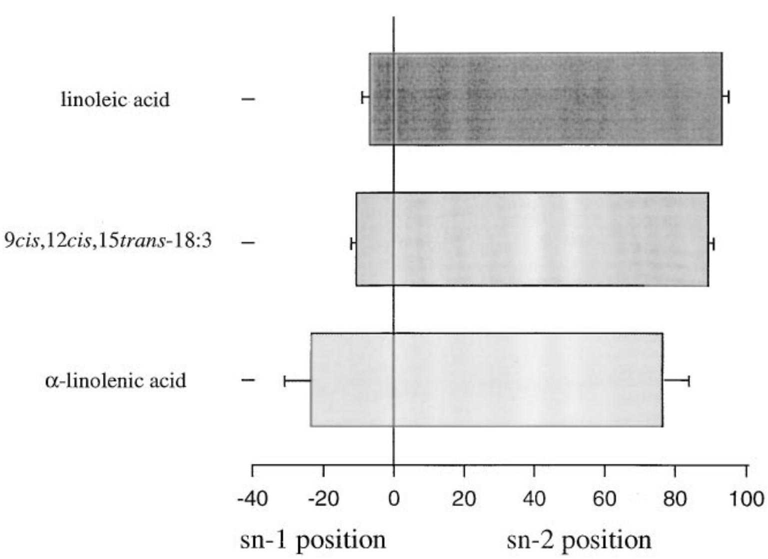

Fig. 3. ^{14}C repartition between *sn*-1 and *sn*-2 positions of phosphatidylcholine of livers infused with [1-^{14}C]linoleic acid, α-linolenic acid and 9*cis*,12*cis*,15*trans*-18:3. Data are expressed as means ± SEM (n = 3).

TABLE 1. Radiolabeled fatty acids formed from 100 nmol of [1-^{14}C] linoleic acid or from its *trans* isomers in total lipids of post-perfused liver

	Substrate Fatty Acids		
Fatty Acids Formed	9*cis*,12*cis*-18:2	9*cis*,12*trans*-18:2	9*trans*,12*cis*-18:2
		nmol ± SEM	
16:0	5.1 ± 0.88	2.9 ± 0.64	2.9 ± 1.05
18:3n–6 + 20:4n–6	0.4 ± 0.08	6.5 ± 1.27^{a}	0.9 ± 0.57
20:2n–6	0.9 ± 0.10	1.5 ± 0.17	4.5 ± 0.92^{c}
Ratios			
18:3n–6+20:4n–6/ 18:3n–6+20:4n–6+20:2n–6	0.32 ± 0.06	0.81 ± 0.02^{b}	0.15 ± 0.05
Labeled *trans*-18:3n–6/ labeled 18:3n–6^{d}		16.4 ± 2.16^{a}	2.4 ± 0.77
Labeled *trans*-20:2n–6/ labeled 20:2n–6^{d}		1.7 ± 0.14	5.1 ± 0.59^{c}

Data are expressed as mean ± SEM (n = 3). Meaning of the ratios is indicated in Materials and Methods.

^{a,b} Significantly different from the corresponding values obtained after infusing linoleic acid and 9*trans*,12*cis*-18:2 at $P < 0.01$ and 0.001, respectively.

^{c} Significantly different from the corresponding values obtained after infusing linoleic acid and 9*cis*,12*trans*-18:2 at $P < 0.01$.

^{d} Values of labeled γ-linolenic acid and labeled 20:2n–6 were obtained from livers infused with [1-^{14}C] linoleic acid.

9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2 accounted for 1.3, 8, and 5.4 nmol, and palmitic acid for 5.1, 2.9, and 2.9 nmol, respectively (**Table 1**). 6*Cis*,9*cis*,12*trans*-18:3 was detected by GC-RAM in the second reversed-phase HPLC fraction (F2) obtained from total lipids of livers perfused with 9*cis*,12*trans*-18:2 (**Fig. 4A**). The amount of this *trans*-18:3n-6 produced (6.5 nmol) was 16 times higher than the amount of γ-linolenic acid formed from linoleic acid (Table 1, $P = 0.003$). Two C20:2 were detected by GC-RAM from total lipids of livers infused with 9*cis*,12*trans*-18:2 (Fig. 4B) and 9*trans*,12*cis*-18:2 (data not shown). The quantity of 11*trans*,14*cis*-20:2 formed from 9*trans*,12*cis*-18:2 (4.5 nmol) was 5 times higher than the quantity of 20:2n–6 (0.9 nmol) produced by elongation of linoleic acid (Table 1). The formation of these dead-end products, 11*trans*,14*cis*-20:2 from 9*trans*,12*cis*-18:2 and 11*cis*,14*trans*-20:2 from 9*cis*,12*trans*-18:2 accounted for about 85% and 19% of the total of newly synthesized 18:3+20:2, respectively (Table 1).

Conversion of [1-^{14}C]α-linolenic acid, 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3

Radiolabeled 20:5n–3 and 20:3n–3 were detected by GC-RAM in the first (F1) and third (F3) reversed phase HPLC fraction, respectively, obtained from total lipids of liver infused with [1-^{14}C]α-linolenic acid (**Figs. 5A** and **5B**). Similarly, C20:5 and C20:3 isomers were detected in the first and in the third HPLC fraction, respectively, from total lipids of liver infused with 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3 (data not shown). Radiolabeled eicosapentaenoic acid (10.7 nmol), 2 nmol of 5*cis*,8*cis*,11*cis*,14*cis*,17*trans*-20:5, and 0.8 nmol of 5*cis*,8*cis*,11*trans*,14*cis*,17*cis*-20:5 were produced from 18:3n–3, 9*cis*,12*cis*,15*trans*-18:3, and 9*trans*,12*cis*,15*cis*-18:3, respectively (**Table 2**).

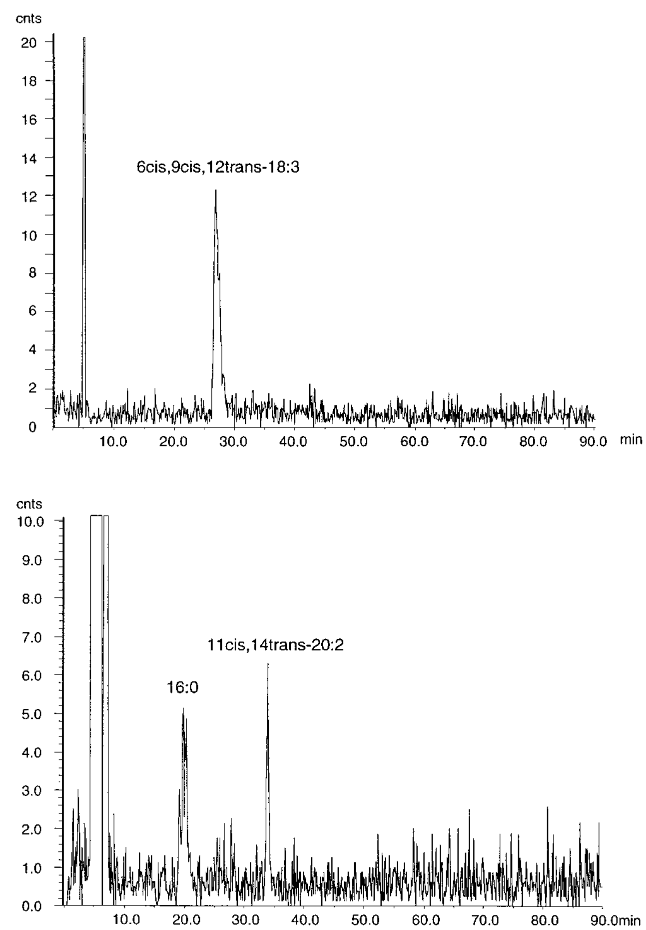

Fig. 4. Radiochromatograms of labeled metabolites produced from [1-^{14}C] 9*cis*,12*trans*-18:2 in 2-h post-perfused rat liver. Top: in the second HPLC fraction (F2). Bottom: in the fourth HPLC fraction (F4).

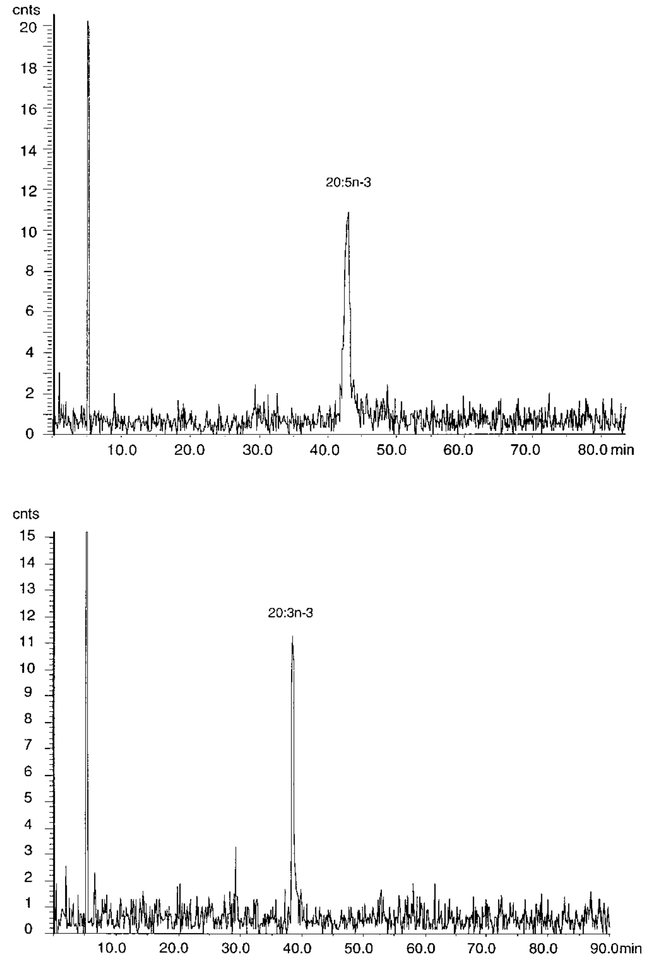

Fig. 5. Radiochromatograms of labeled metabolites produced from [1-^{14}C]α-linolenic acid in 2-h post-perfused rat liver. Top: in the first HPLC fraction (F1). Bottom: in the third HPLC fraction (F3).

Hence, 5*cis*,8*cis*,11*cis*,14*cis*,17*trans*-20:5 and 5*cis*,8*cis*,11*trans*, 14*cis*,17*cis*-20:5 accounted for only half and 10% of the amount of eicosapentaenoic acid formed from α-linolenic acid, respectively (Table 2). The amount of the dead-end product formed from 9*trans*,12*cis*,15*cis*-18:3 (6.1 nmol) was 2.5 times greater than the amount of 20:3n–3 (2.5 nmol) produced from α-linolenic acid ($P = 0.0241$, Table 2). On the contrary, the amount of 11*cis*,14*cis*,17*trans*-20:3 (2.2 nmol) produced from 9*cis*,12*cis*,15*trans*-18:3 was comparable to the quantity of 20:3n–3 formed from 18:3n–3 (Table 2). 20:5n–3, 5*cis*,8*cis*,11*cis*,14*cis*,17*trans*-20:5 and 5*cis*, 8*cis*,11*trans*,14*cis*,17*cis*-20:5 accounted for 80%, 61%, and 12% of the total 20:5+20:3 produced from 18:3n–3, 9*cis*,12*cis*,15*trans*-18:3, and 9*trans*,12*cis*,15*cis*-18:3, respectively (Table 2).

DISCUSSION

Acylation of the *trans* isomers of linoleic and α-linolenic acids

Some authors showed that linoleate is predominantly acylated in the *sn*-2 position of triacylglycerols, whereas its *trans* isomers are esterified in the *sn*-1 and *sn*-3 positions (32–35). This finding could explain the higher ^{14}C retention in triacylglycerols observed 2 h after infusion with both *trans*-18:2 (Fig. 2A). Indeed, by taking into account that 9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2 can be acylated in two of the three positions of the molecules, compared to only one position for linoleic acid, one would expect the esterification of the *trans* isomers to be higher.

^{14}C retention in phosphatidylinositol was lower after infusing 9*trans*,12*cis*-18:2 than after perfusions with 18:2n–6 and 9*cis*,12*trans*-18:2 (Fig. 2A). As C20 and C22 polyunsaturated fatty acids are found to a great extent in this phospholipid class, a lower rate of conversion of this isomer into higher metabolites can be expected.

TABLE 2. Radiolabeled fatty acids formed from 100 nmol of [1-^{14}C]α-linolenic acid or from its *trans* isomers in total lipids of post-perfused liver

	Substrate Fatty Acid		
Fatty Acid Formed	9*cis*,12*cis*,15*cis*-18:3	9*cis*,12*cis*,15*trans*-18:3	9*trans*,12*cis*,15*cis*-18:3
		nmol ± SEM	
16:0	2.0 ± 0.05	1.1 ± 0.86	3.3 ± 1.73
20:5n–3	10.7 ± 3.76^{a}	2.0 ± 0.78	0.8 ± 0.05
20:3n–3	2.5 ± 0.25	2.2 ± 0.51	6.1 ± 1.29^{c}
Ratios			
20:5/20:5 + 20:3	0.80 ± 0.04^{aa}	0.61 ± 0.08^{bb}	0.12 ± 0.02^{cc}
Labeled *trans*-20:5/labeled 20:5n–3^{d}		0.5 ± 0.17^{b}	0.09 ± 0.01^{c}
Labeled *trans*-20:3/labeled 20:3n–3^{d}		0.9 ± 0.12	2.5 ± 0.29^{c}

Data are expressed as means ± SEM (n = 3). The meaning of the ratios is indicated in Materials and Methods.

[a] Significantly different from the corresponding values obtained after infusing its *trans* isomer at $P < 0.05$.

[b] Significantly different from the corresponding values obtained after infusing α-linolenic acid and 9*trans*,12*cis*,15*cis*-18:3 at $P < 0.05$.

[c] Significantly different from the corresponding values obtained after infusing α-linolenic acid and 9*cis*,12*cis*,15*trans*-18:3 at $P < 0.05$. Values not sharing the same superscript letters (*b*, *c* and *aa*, *bb*, *cc*) were significantly different from each other at $P < 0.001$.

[d] Values of labeled 20:5n–3 and labeled 20:3n–3 were obtained from livers infused with [1-^{14}C]α-linolenic acid.

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Results of phospholipase A_2 digestion of phosphatidylcholine of livers infused with the three C18:2 isomers showed no differences in the distribution between both positions, whatever the geometry of the double bonds (data not shown). Thus, the difference of ^{14}C retention in phosphatidylcholine observed within the C18:2 fatty acids (Fig. 2A) could be explained by a lower acylation of the *trans*-18:2 in both positions of the molecule, in spite of a lower affinity of the acyl-CoA:phospholipid acyl transferase (EC 2.3.1.23) towards the *trans* C18:2 isomers, as this enzyme is involved in the transfer of fatty acyl-CoA derivatives to lysophosphatidylcholine to form phosphatidylcholine and is also responsible for the distribution of the fatty acids in the two positions of phosphatidylcholine.

Linoleic acid and 9*cis*,12*cis*,15*trans*-18:3 showed a marked selectivity for the *sn*-2 position of phosphatidylcholine, while more of α-linolenic acid was in the *sn*-1 position (Fig. 3). A comparable finding has been observed in cardiolipins in which linoleic acid and 9*cis*,12*cis*,15*trans*-18:3 were esterified to both 1(1′′)–/2(2′′)- positions with a marked selectivity for positions 1(1′′). On the other hand, α-linolenic acid was equally esterified in the four positions (36). As 9*cis*,12*cis*,15*trans*-18:3 and linoleic acid share common structural features, due to the configuration of the Δ15 double bond in 9*cis*,12*cis*,15*trans*-18:3, these fatty acids would be similarly recognized by the acyl-CoA:phospholipid acyltransferase.

Conversion of the C18:2 and C18:3 isomers

Radiolabeled palmitic acid was produced during the perfusion (Tables 1 and 2). Palmitate is the major fatty acid synthetized de novo and is one of the main components of membrane lipids (28). This labeled fatty acid originated from labeled acetate units, produced during the β-oxidation of the labeled fatty acids, and used for lipogenesis. De novo lipid synthesis from the 1-^{14}C-labeled fatty acids ranged from 1.1 to 5.1% of the total radioactivity (Tables 1 and 2). This finding suggests that formation of saturated fatty acid can account for a substantial conversion of C18:2 and C18:3 isomers, when compared to the formation of desaturated and chain-elongated products, as was previously shown in rats (37) and monkeys (38, 39).

9*cis*,12*trans*-18:2 was found to be 16 times more desaturated into 6*cis*,9*cis*,12*trans*-18:3 than was linoleic acid into γ-linolenic acid. This finding was consistent with previous data obtained in mice fed a diet enriched with a mixture of deuterated *trans*-18:2 for 4 days [11]. Our results gave interesting information on the specificity of the Δ6 desaturase towards the C18:2 fatty acids. Compared to the elongation step, the Δ6 desaturation is the most limiting step in the sequence of desaturation and elongation (40). Our data confirmed that the specificity of the Δ6 desaturase towards 9*cis*,12*trans*-18:2 may be higher than towards linoleic acid and it Δ9*trans* isomer, as observed by Cook and Emken (41). No radiolabeled *trans* isomers of arachidonic acid were detected in lipids of livers infused with both *trans* C18:2 isomers, probably due to the duration of the perfusions (2 h) which was too short. But it was previously shown that rates for conversion of 9*cis*,12*trans*-18:2 to 5*cis*,8*cis*,11*cis*,14*trans*-20:4 and of linoleic acid to arachidonic acid were identical and both were 5 times higher than conversion of 9*trans*,12*cis*-18:2 to 5*cis*,8*cis*,11*trans*, 14*cis*-20:4 (11, 19).

11*cis*,14*trans*-20:2 and 11*trans*,14*cis*-20:2 were detected in livers infused with 9*cis*,12*trans*-18:2 and 9*trans*,12*cis*-18:2, respectively. Our results are in good agreement with those of Ratnayake et al. (18) (in vivo studies) and Berdeaux et al. (29) (in vitro studies) who found that both *trans*-18:2 were elongated into C20:2 fatty acids. In addition, we quantified the formation of these dead-end products and showed that equal quantities of 20:2 were produced from linoleic acid and 9*cis*,12*trans*-18:2, and that the amount of 11*trans*,14*cis*-20:2 produced from 9*trans*, 12*cis*-18:2 was 5.1 times greater than the quantity of 20:2n–6 formed from 18:2n–6 (Table 1). Such data could explain the findings of Beyers and Emken (11) and Berdeaux et al. (19) who only detected 11*trans*,14*cis*-20:2 from 9*trans*,12*cis*-18:2 after feeding *trans* 18:2 isomers. For both of these latter in vivo studies on the conversion of the *trans* C18:2 fatty acids, the identification of the *trans* C20:2 metabolites was performed using a combination of chromatographic separation and mass spectrometry. One could therefore think that minor compounds, such as 11*cis*, 14*trans*-20:2 which can be detected when present as radiolabeled fatty acid, would not always be detectable when given unlabeled.

Desaturation and chain elongation of α-linolenic acid into 20:5 was found to be the preferential pathway compared to elongation into 20:3. This was almost the case for 9*cis*,12*cis*,15*trans*-18:3, but not for 9*trans*,12*cis*,15*cis*-18:3. Indeed, 20:5n–3 accounted for 80% of the total 20:5 + 20:3 produced from 18:3n–3, whereas this value was 61% for 9*cis*,12*cis*,15*trans*-18:3 and 12% for 9*trans*,12*cis*,15*cis*-18:3 (Table 2). The difference between these values was highly significant ($P < 0.001$), suggesting a very different partitioning of these *trans* C18:3n–3 isomers between desaturation into 20:5 and elongation into 20:3.

CONCLUSION

In conclusion, our data detailed the metabolic pathways of *trans* isomers of linoleic and α-linolenic acids in *trans* C20 polyunsaturated fatty acids. We showed that the geometry of the double bonds greatly affected the conversion of the fatty acids, as has already been reported for the 18:2 fatty acids (11, 14, 18, 19). By comparison with linoleic acid, the *trans* geometry in the Δ12 position greatly increased the desaturation of the precursor, while the *trans* geometry in the Δ9 position increased the elongation. Concerning the 18:3n–3 fatty acids, by comparison with α-linolenic acid, the *trans* geometry in the Δ15 position only decreased the desaturation of the fatty acid, while the *trans* geometry in the Δ9 position both decreased the desaturation and increased the elongation of the precursor.

The biological importance of the dead-end products remains obscure. It has been previously shown that 20:2n–6 is retroconverted into linoleic acid, instead of being desaturated into arachidonic acid (6, 7). Moreover, as 20:2n–6 and 20:3n–3 are preferentially acylated into triacylglycerols (10), which is the main class of lipid storage, one could point out that these dead-end products, and also the *trans* dead-end products, might represent a possible source of C18:2n–6 and C18:3n–3 fatty acids. On the other hand, more data are available on the possible effects of the *trans* long chain polyunsaturated fatty acids. For example, 5*cis*,8*cis*,11*cis*,14*trans*-20:4 exhibits an anti-aggregatory effect on rat platetets when compared to arachidonic acid (42). Similarly, 5*cis*,8*cis*,11*cis*,14*cis*,17*trans*-20:5 and 5*cis*,8*cis*,11*trans*,14*cis*,17*cis*-20:5, formed from 9*cis*,12*cis*,15*trans*-18:3 and 9*trans*,12*cis*,15*cis*-18:3 respectively, are equally and more anti-aggregant than is eicosapentaenoic acid (43).

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