Desaturation and chain elongation of [1-\textsuperscript{14}C]mono-trans isomers of linoleic and \(\alpha\)-linolenic acids in perfused rat liver

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Abstract Tran\(\textsuperscript{a}\)unsaturated 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-\textsuperscript{14}C]linoleic acid, 9cis,12trans-18:2, 9trans,12cis-18:2, \(\alpha\)-linolenic acid, 9cis, 12cis,15trans-18:3 and 9trans,12cis,15cis-18:3 were studied using a perfused rat liver model. After perfusion with both trans isomers of 18:2n–6, the \textsuperscript{14}C was equally distributed between phospholipids and triacylglycerols, compared to the 70:30 distribution (phospholipid:triacylglycerols) observed after infusing linoleic acid. The corresponding distribution of \textsuperscript{14}C after perfusion with both trans isomers of 18:3n–3 was comparable to what was obtained for \(\alpha\)-linolenic acid. The products of conversion were analyzed by combination of different radio chromatographic methods. 9cis,12trans-18:2 was 16 times more converted into a C18:3n–6 fatty acid than linoleic acid into \(\gamma\)-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), 9cis,12cis,15trans-18:3 and 9trans,12cis,15cis-18:3 were 2- and 10-times less converted to trans-20:5, respectively, than \(\alpha\)-linolenic acid into eicosapentaenoic acid. Moreover, 9cis,12cis,15trans-18:3 and 9trans,12cis,15cis-18:3 were equally and 2.5-times more elongated into “dead-end products”, respectively, than \(\alpha\)-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, 9trans,12cis-18:2 and 9trans,12cis,15trans-18:3 were elongated into trans “dead-end products” rather than being converted to desaturated and chain elongated trans-metabolites. On the other hand 9cis,12cis,15trans-18:3 was more desaturated and chain elongated into 17trans 20:5 rather than elongated into 17trans 20:3. —Bretillon, L., J-M. Chardigny, J-P. Noël, and J-L. Sébédo. Desaturation and chain elongation of [1-\textsuperscript{14}C]mono-trans isomers of linoleic and \(\alpha\)-linolenic acids in perfused rat liver. J. Lipid Res. 1998. 39: 2228–2236.

Heat treatment of oils leads to the formation of trans isomers of both linoleic and \(\alpha\)-linolenic acids. 9cis, 12trans-18:2, 9trans,12cis-18:2, 9cis,12cis,15trans-18:3, and 9trans,12cis,15cis-18:3 are the major isomers produced by isomerization of linoleic and \(\alpha\)-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 \(\alpha\)-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 9cis,12trans-18:2 to 5cis,8cis,11cis,14trans-20:4 is higher than that of 9trans,12cis-18:2 to 5cis,8cis,11trans,14cis-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 9trans,12cis-18:2 (11trans,14cis-20:2), whereas Ratnayake et al. (18) showed that both 9cis,12trans-18:2 and 9trans,12cis-18:2 could be transformed in 11cis,14trans-20:2 and 11trans,14cis-20:2, respectively. No data are available on the conversion of the trans isomers of \(\alpha\)-linolenic acid (partitioning between formation of trans isomers of eicosapentaenoic acid and docosahexaenoic acid and production of dead-end products).

Supplementary key words tran\(\textsuperscript{a}\)unsaturated fatty acids • desaturation • elongation

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.

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The aim of the present study was to establish to what extent the conversion of the [1-\(^{14}\)C]trans isomers of linoleic and \(\omega\)-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]9cis,12cis:18:2, 1.96 GBq \cdot mmol\(^{-1}\)) and \(\omega\)-linolenic acid ([1-\(^{14}\)C]9cis,12cis,15cis:18:3, 1.92 GBq \cdot mmol\(^{-1}\)) were purchased from NEN (Les Ulis, France). Trans fatty acids ([1-\(^{14}\)C]9cis,12trans:18:2, 2.00 GBq \cdot mmol\(^{-1}\), [1-\(^{14}\)C]9trans,12cis:18:2, 1.99 GBq \cdot mmol\(^{-1}\), [1-\(^{14}\)C]9cis,12cis,15trans:18:3, 1.94 GBq \cdot mmol\(^{-1}\), and [1-\(^{14}\)C]9trans,12cis,15cis:18:3, 1.88 GBq \cdot 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 mmol) of the fatty acid was diluted in 5 mL of Krebs-Henseleit 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 (Sigma, L’Isle d’Abeau, France). The vial was then rapidly isolated and perfused with oxygenated recirculating Krebs-Henseleit buffer (pH 7.4) containing 25 mm glucose and 36 mg of bovine serum albumin (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 \(\mu\)mol \cdot min\(^{-1}\). The perfusates 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 \(\cdot\) min\(^{-1}\) were discarded. One milliliter of the perfusate was mixed with 5 mL of scintillation cocktail (Ecocint A, National Diagnostics, Bionis, Clamart, France) and radioactivity was measured using a Tri-carb 2000CA liquid scintillation analyzer (Packard, Groningen, The Netherlands).

**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-Henseleit 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-Henseleit 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 \(\mu\)mol \cdot min\(^{-1}\). The perfusates 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 \(\cdot\) min\(^{-1}\) were discarded. One milliliter of the perfusate was mixed with 5 mL of scintillation cocktail (Ecocint 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, Pepin, 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.

Lipid phospholipids were separated from neutral lipids using Sep-Pak 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/v) (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. Phospholipid classes were separated using the solvent mixture described 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 45:17:30:3 (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\cdot L\(^{-1}\), CaCl\(_2\), 2 mmol\cdot L\(^{-1}\) was added. Digestion was performed at 37°C for 45 min while stirring. The reaction was then stopped by chilling the ice on and adding 2.4 mL water. The products of digestion were extracted twice 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 (\(\gamma\)-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 synthesized fatty acid (28). Similarly, eicosa- pentaeanoic 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 \(\omega\)-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...
TLC on silver nitrate-impregnated silica gel G plates (SDS). The first development was performed using toluene in order to separate saturates, monoenoides, 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 calculating 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 × 0.32 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 14CO2. The radioactivity was determined by counting the 14CO2 after mixing it with argon–methane 9:1. The data were computed using the Laura software (Lablogic).

The metabolites formed from [1,14C]18:2n-6 and [1-14C]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 the radioactive signal and the peak obtained by the FID detector. The data were computed using the Laura software (Lablogic).

**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 after 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 14C 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 14C 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 14C found in triacylglycerols after perfusion with linoleic acid. Results of phospholipase A2 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 14C retention in phosphatidylinostitol was significantly lower after infusion with 9cis,12cis-18:2 than after infusion of linoleic acid or the 9cis,12trans-18:2 isomer (P = 0.006).

The 14C 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 14C retention in phosphatidylcholine was observed...
Fig. 2. $^{14}$C distribution in classes of liver lipids after perfusion with A: [1-14C]linoleic acid, 9cis,12trans-18:2 and 9trans,12cis-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: [1-14C]-α-linolenic acid, 9cis,12cis,15trans-18:3 and 9trans,12cis,15cis-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.

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

Radiolabeled palmitic, γ-linolenic, arachidonic acids and 20:2n–6 were detected in total lipids of livers infused with [1-14C]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 9cis,12trans-18:2 and 9trans,12cis-18:2. Desaturated and chain-elongated products (18:3n–6+20:4n–6+20:2n–6) produced from 18:2n–6, after infusing 9cis,12cis,15trans-18:3 than after infusion of α-linolenic acid and 9trans,12cis,15cis-18:3 (P = 0.018, Fig. 2B). Results of phospholipase A2 digestion of phosphatidylcholine from livers infused with α-linolenic acid, 9cis,12cis,15trans-18:3 and linoleic acid are shown in Fig. 3. The repartition of the radioactivity in the two positions of phosphatidylcholine after infusing 9cis,12cis,15trans-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).

Fig. 3. $^{14}$C repartition between sn-1 and sn-2 positions of phosphatidylcholine of livers infused with [1-14C]linoleic acid, α-linolenic acid and 9cis,12cis,15trans-18:3. Data are expressed as means ± SEM (n = 3).
cis,12-trans-18:2 and 9-trans,12cis-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). 6Cis,9cis,12trans-18:3 was detected by GC-RAM in the second reversed-phase HPLC fraction (F2) obtained from total lipids of livers perfused with 9cis,12trans-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 9cis,12trans-18:2 (Fig. 4B) and 9trans,12cis-18:2 (data not shown). The quantity of 11trans,14cis-20:2 formed from 9trans,12cis-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, 11trans,14cis-20:2 from 9trans,12cis-18:2 and 11cis,14trans-20:2 from 9cis,12trans-18:2 accounted for about 85% and 19% of the total of newly synthesized 18:3n–6, respectively (Table 1).

### Conversion of [1-14C]α-linolenic acid, 9cis,12cis,15trans-18:3 and 9trans,12cis,15cis-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 [1-14C]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 9cis,12cis,15trans-18:3 and 9trans,12cis,15cis-18:3 (data not shown). Radiolabeled eicosapentaenoic acid (10.7 nmol), 2 nmol of 5cis,8cis,11cis,14cis,17trans-20:5, and 0.8 nmol of 5cis,8cis,11trans,14cis,17cis-20:5 were produced from 18:3n–3, 9cis,12cis,15trans-18:3, and 9trans,12cis,15cis-18:3, respectively (Table 2).

### Table 1. Radiolabeled fatty acids formed from 100 nmol of [1-14C] linoleic acid or from its trans isomers in total lipids of post-perfused liver

<table>
<thead>
<tr>
<th>Substrate Fatty Acids</th>
<th>9cis,12cis-18:2</th>
<th>9cis,12trans-18:2</th>
<th>9trans,12cis-18:2</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>5.1 ± 0.88</td>
<td>2.9 ± 0.64</td>
<td>2.9 ± 1.05</td>
</tr>
<tr>
<td>18:3n–6 + 20:4n–6</td>
<td>0.4 ± 0.08</td>
<td>6.5 ± 1.27</td>
<td>0.9 ± 0.57</td>
</tr>
<tr>
<td>20:2n–6</td>
<td>0.9 ± 0.10</td>
<td>1.5 ± 0.17</td>
<td>4.5 ± 0.92</td>
</tr>
</tbody>
</table>

Ratios

| Labeled trans-18:3n–6/ labeled 18:3n–6 | 0.32 ± 0.06 | 0.81 ± 0.02 | 0.15 ± 0.05 |
| Labeled trans-20:2n–6/ labeled 20:2n–6 | 16.4 ± 2.16 | 2.4 ± 0.77 | 5.1 ± 0.59 |

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

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

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

Values of labeled γ-linolenic acid and labeled 20:2n–6 were obtained from livers infused with [1-14C] linoleic acid.
Hence, 5cis,8cis,11cis,14cis,17trans-20:5 and 5cis,8cis,11trans,14cis,17cis-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 9trans,12cis,15cis-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 11cis,14cis,17trans-20:3 (2.2 nmol) produced from 9cis,12cis,15trans-18:3 was comparable to the quantity of 20:3n-3 formed from 18:3n-3 (Table 2). 20:5n-3, 5cis,8cis,11cis,14cis,17trans-20:5 and 5cis,8cis,11trans,14cis,17cis-20:5 accounted for 80%, 61%, and 12% of the total 20:5 + 20:3 produced from 18:3n-3, 9cis,12cis,15trans-18:3, and 9trans,12cis,15cis-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 9cis,12trans-18:2 and 9trans,12cis-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 9trans,12cis-18:2 than after perfusions with 18:2n-6 and 9cis,12trans-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]$\Delta^9$-linolenic acid or from its trans isomers in total lipids of post-perfused liver

<table>
<thead>
<tr>
<th>Fatty Acid Formed</th>
<th>Substrate Fatty Acid</th>
<th>9cis,12cis,15cis-18:3</th>
<th>9cis,12cis,15trans-18:3</th>
<th>9trans,12cis,15cis-18:3</th>
</tr>
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<tbody>
<tr>
<td>16:0</td>
<td>2.0 ± 0.05</td>
<td>1.1 ± 0.86</td>
<td>3.3 ± 1.73</td>
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<tr>
<td>20:5n-3</td>
<td>10.7 ± 3.76</td>
<td>2.0 ± 0.78</td>
<td>0.8 ± 0.05</td>
<td></td>
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<tr>
<td>20:3n-3</td>
<td>2.5 ± 0.25</td>
<td>2.2 ± 0.51</td>
<td>6.1 ± 1.29</td>
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</table>

**Ratios**

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>20:5/20:5 + 20:3</td>
<td>0.80 ± 0.04</td>
<td>0.61 ± 0.08</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Labeled trans-20:5/ labeled 20:5n-3</td>
<td>0.5 ± 0.17</td>
<td>0.09 ± 0.01</td>
<td>0.5 ± 0.17</td>
</tr>
<tr>
<td>Labeled trans-20:3/ labeled 20:3n-3</td>
<td>0.9 ± 0.12</td>
<td>0.09 ± 0.01</td>
<td>0.5 ± 0.17</td>
</tr>
</tbody>
</table>

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 9trans,12cis,15cis-18:3 at $P < 0.05$.

$^c$ Significantly different from the corresponding values obtained after infusing α-linolenic acid and 9cis,12cis,15trans-18:3 at $P < 0.05$. Values not sharing the same superscript letters ($a$, $b$, and $c$) 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]$\Delta^9$-linolenic acid.
Results of phospholipase A₂ 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 ¹⁴C 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 9cis,12cis,15trans-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 9cis,12cis,15trans-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 9cis,12cis,15trans-18:3 and linoleic acid share common structural features, due to the configuration of the Δ15 double bond in 9cis,12cis,15trans-18:3, these fatty acids would be similarly recognized by the acyl-CoA:phospholipid acyl transferase.

**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 synthesized 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 1-¹⁴C-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 acids 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).

9cis,12trans-18:2 was found to be 16 times more desaturated into 6cis,9cis,12trans-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 desaturase is the most limiting step in the sequence of desaturation and elongation (40). Our data confirmed that the specificity of the Δ6 desaturase towards 9cis,12trans-18:2 may be higher than towards linoleic acid and the 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 9cis,12trans-18:2 to 5cis,8cis,11cis,14trans-20:4 and of linoleic acid to arachidonic acid were identical and both were 5 times higher than conversion of 9trans,12cis-18:2 to 5cis,8cis,11trans,14cis-20:4 (11, 19).

11cis,14trans-20:2 and 11trans,14cis-20:2 were detected in livers infused with 9cis,12trans-18:2 and 9trans,12cis-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 9cis,12trans-18:2, and that the amount of 11trans,14cis-20:2 produced from 9trans,12cis-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 11trans,14cis-20:2 from 9trans,12cis-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 two C20:2 metabolites was performed using a combination of chromatographic separation and mass spectrometry. One could therefore think that minor compounds, such as 11cis,14trans-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 9cis,12cis,15trans-18:3, but not for 9trans,12cis,15cis-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 9cis,12cis,15trans-18:3 and 12% for 9trans,12cis,15cis-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 re-
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, one could conclude 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 polysaturated fatty acids. For example, 5cis,8cis,11cis,14trans stereochemistry exhibits an anti-aggregatory effect on rat platelets when compared to arachidonic acid (42). Similarly, 5cis,8cis,11cis,14trans stereochemistry is retroconverted into linoleic acid, instead of being desaturated into arachidonic acid (6, 7). Moreover, as 20:2n–6 is retroconverted into linoleic acid, instead of being desaturated into arachidonic acid (6, 7).

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REFERENCES


Bretillon et al. Conversion of trans polysaturated fatty acids in perfused rat liver 2235


39. Sheaff Greiner, R. C., Q. Zhang, K. J. Goodman, D. A. Guissani, P. W. Nathanielsz, and J. T. Brenna. 1996. Linoleate, α-linolenate, and docosahexaenoate recycling into saturated and monounsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant rhesus monkeys. J. Lipid Res. 12: 2675–2686.


