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### ► **To cite this version:**

Lionel Brétilon, Jean-Michel Chardigny, Jean-Louis Sébédio, Jean-Pierre Noël, C.M. Scrimgeour, et al.. Isomerization increases the postprandial oxidation of linoleic acid but not alpha-linolenic acid in men. *Journal of Lipid Research*, 2001, 42 (6), pp.995-997. <hal-02674145>

**HAL Id: hal-02674145**

**<https://hal.inrae.fr/hal-02674145v1>**

Submitted on 31 May 2020

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# Isomerization increases the postprandial oxidation of linoleic acid but not $\alpha$ -linolenic acid in men

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**Abstract** Human lipid intake contains various amounts of *trans* fatty acids. Refined vegetable and frying oils, rich in linoleic acid and/or  $\alpha$ -linolenic acid, are the main dietary sources of *trans*-18:2 and *trans*-18:3 fatty acids. The aim of the present study was to compare the oxidation of linoleic acid,  $\alpha$ -linolenic acid, and their major *trans* isomers in human volunteers. For that purpose, TG, each containing two molecules of [1-<sup>13</sup>C]linoleic acid,  $\alpha$ -[1-<sup>13</sup>C]linolenic acid, [1-<sup>13</sup>C]-9*cis*,12*trans*-18:2, or [1-<sup>13</sup>C]-9*cis*,12*cis*,15*trans*-18:3, were synthesized. Eight healthy young men ingested labeled TG mixed with 30 g of olive oil. Total CO<sub>2</sub> production and <sup>13</sup>CO<sub>2</sub> excretion were determined over 48 h. The pattern of oxidation was similar for the four fatty acids, with a peak at 8 h and a return to baseline at 24 h. Cumulative oxidation over 8 h of linoleic acid, 9*cis*,12*trans*-18:2,  $\alpha$ -linolenic acid, and 9*cis*,12*cis*,15*trans*-18:3 were, respectively, 14.0  $\pm$  4.1%, 24.7  $\pm$  6.7%, 23.6  $\pm$  3.3%, and 23.4  $\pm$  3.7% of the oral load, showing that isomerization increases the postprandial oxidation of linoleic acid but not  $\alpha$ -linolenic acid in men.—Bretillon, L., J. M. Chardigny, J. L. Sébédio, J. P. Noël, C. M. Scrimgeour, C. E. Fernie, O. Loreau, P. Gachon, and B. Beaufrère. Isomerization increases the postprandial oxidation of linoleic acid but not  $\alpha$ -linolenic acid in men. *J. Lipid Res.* 2001. 42: 995–997.

**Supplementary key words** human • oxidation • *trans* fatty acid

Heat treatment of vegetable oils induces the isomerization of essential PUFA, that is, linoleic and  $\alpha$ -linolenic acids (1–3). These geometric isomers of essential fatty acids are accompanied in the diet by *trans* isomers of monounsaturated fatty acids, leading to consumption of *trans* fatty acids from 3.0 to 10.6 g per capita per day in Western countries (4). 9*cis*,12*trans*-18:2 and 9*cis*,12*cis*,15*trans*-18:3 are the major *trans* PUFA found in vegetable and frying oils (1–3). In rats, these compounds are desaturated and elongated into *trans* isomers of arachidonic acid and eicosapentaenoic acid (5–7). From previous studies of rats (8–12), it is concluded that *i)* dietary  $\alpha$ -linolenic acid is oxidized more than

linoleic acid and *ii)* *trans* isomerization increases 18:2 oxidation but does not affect 18:3 oxidation. To test the hypothesis of a similar pattern in humans, we determined postprandial oxidation of <sup>13</sup>C-labeled *cis* or *trans* PUFA given as TG to healthy humans.

## MATERIALS AND METHODS

### Materials

[1-<sup>13</sup>C]linoleic acid, [1-<sup>13</sup>C]linolenic acid, [1-<sup>13</sup>C]-9*cis*,12*cis*,15*trans*-18:3, and [1-<sup>13</sup>C]-9*cis*,12*trans*-18:2 were first synthesized as described elsewhere (13) and used for synthesizing TG following the method of Redden et al. (14). The products were >98% TG as estimated by HPLC and had a labeled fatty acid:oleic acid ratio of between 1.83 and 2.00 as estimated by gas chromatography.

### Human subjects

Ten healthy men (mean age of 25  $\pm$  3 years, body mass index 21.3  $\pm$  1.8 kg/m<sup>2</sup>, mean  $\pm$  SD) were recruited. Blood pressure, cholesterolemia, and triacylglycerolemia were within normal ranges. None were taking any medication, their dietary habits were stable, and they were nonsmokers.

### Protocol

The study had been approved by the local ethics committee (Hôpitaux Civils de Clermont-Ferrand, France) according to the French Hurriet law. Informed written consent of the participants had been obtained. The study was designed as four periods of 2 days, separated one from another by at least 1 week. Six subjects performed the whole study and received the four fatty acids. Two additional subjects were involved in the 18:2 part of the study whereas two others performed the 18:3 study. In each study (18:2 and 18:3), the *cis* or *trans* isomers were administered in a randomized, double-blind fashion. The four study periods were strictly identical except for the nature of the ingested labeled TG.

Abbreviations: V'CO<sub>2</sub>, rate of production of carbon dioxide.

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Subjects entered the experimental unit at 7:00 AM after an overnight fast. Two samples of expired breath were collected in Vacutainer® tubes (Becton Dickinson, Grenoble, France) in order to determine the basal  $^{13}\text{C}$  abundance in the expired  $\text{CO}_2$ . One hour after arrival, the subjects ingested the labeled TG (1.40 g for the 18:2, and 0.87 g for the 18:3) mixed with 30 g of olive oil (Lesieur Alimentaire, Neuilly, France). Expired breath collection was performed as described above 1, 2, 3, 5, and 8 h after TG ingestion. Continuous indirect calorimetry (Deltatrac metabolic monitor; Datex, Helsinki, Finland) was performed 2, 5, and 8 h after TG ingestion for periods of 30 min, in order to measure the  $\text{CO}_2$  production. The subjects were fasted during the first 8 h. A meal containing food items poor in  $^{13}\text{C}$  was served afterward. Expired breath samples were again collected at 12, 24, and 48 h after ingestion but no  $\text{CO}_2$  production measurements were performed at these time points.

### Analytical procedures

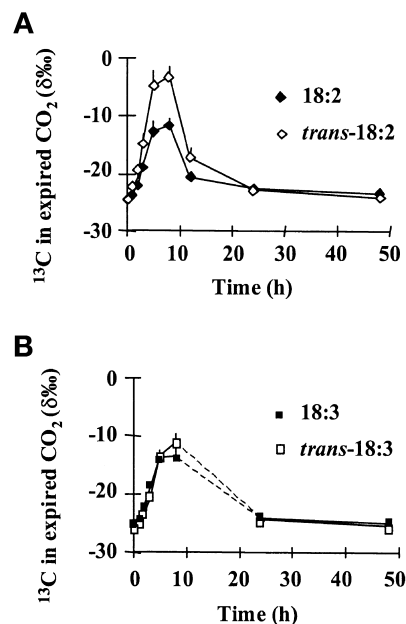
$^{13}\text{C}$ -enrichment analysis of the expired breath was done by gas chromatography-combustion-isotope ratio mass spectrometry. Briefly, 40  $\mu\text{l}$  samples were injected in duplicate into a gas chromatograph (5890; Hewlett-Packard, Palo Alto, CA) equipped with a HAYSEP Q column (Chrompack, Les Ulis, France). The oven temperature was 110°C for 5 min. The  $\text{CO}_2$  peak was then injected on-line into an isotope ratio mass spectrometer ( $\mu\text{G}$ as system; Fisons Instruments, VG Isotech, Middlewich, England). The  $^{13}\text{C}$  enrichment of expired  $\text{CO}_2$  was determined by comparison with a standard of known  $^{13}\text{C}$  abundance (Pee Dee Belemnite, PDB). The rate of  $^{13}\text{CO}_2$  production was obtained by multiplying  $^{13}\text{CO}_2$  enrichment by the  $\text{CO}_2$  production.

### Calculations and statistical analyses

All results are expressed as mean  $\pm$  standard deviation. The cumulative  $^{13}\text{CO}_2$  production corresponds to the area under the curve and was calculated by the trapezoidal method. Within a pair of studies (18:2 or 18:3), comparisons were made by paired, bilateral *t*-tests. Comparison between the 4 periods was made on the 6 subjects who performed the entire study, by ANOVA followed by a Fisher PLSD test.

## RESULTS

The profile of  $^{13}\text{C}$  enrichment of the expired breath air was the same, regardless of which fatty acid was ingested (Fig. 1A and B). It increased 1 h after TG ingestion, peaked between 5 and 8 h, and decreased thereafter at 12 h to reach the basal  $^{13}\text{C}$  level at 24 and 48 h. Significantly higher  $^{13}\text{C}$  levels were observed after ingestion of *trans*-18:2.  $V'\text{CO}_2$  values were similar between the two 18:2 fatty acids ( $207 \pm 10 \text{ ml}\cdot\text{min}^{-1}$  for linoleic acid and  $202 \pm 10 \text{ ml}\cdot\text{min}^{-1}$  for the *trans*-18:2). Therefore the cumulative amount of tracer excreted into breath was much higher for *trans*-18:2 than for linoleic acid. The fraction of *trans*-18:2 oxidized over 8 h was  $24.7 \pm 6.7\%$  of the oral load, higher than the corresponding fraction of linoleic acid, which was  $14.0 \pm 4.1\%$  ( $P < 0.001$ ; Fig. 2). In contrast to the 18:2 fatty acids, isomerization of 18:3 fatty acid did not affect its oxidation (Fig. 1B), which was similar for both isomers ( $23.6 \pm 3.3$  and  $23.4 \pm 3.7\%$  for  $\alpha$ -linolenic acid and *trans*-18:3, respectively; Fig. 2).  $^{13}\text{CO}_2$  production over the 8 h after ingestion of the  $\alpha$ -linolenic acid ( $23.6 \pm 3.3\%$ ) was almost twice as high as after ingestion of the lin-

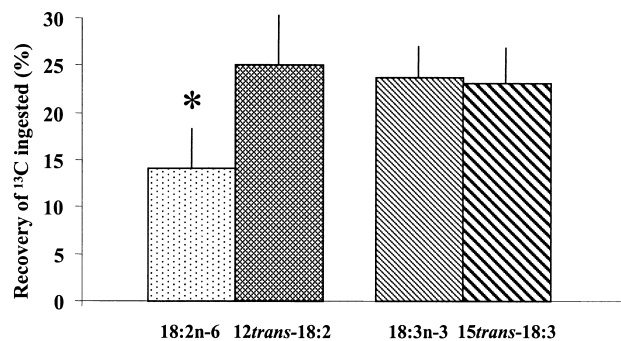


**Fig. 1.**  $^{13}\text{CO}_2$  enrichment ( $\delta \text{‰}$  vs. PDB) after ingestion of linoleic acid (18:2n-6) or 9*cis*,12*trans*-18:2 (12*trans*-18:2) (A), or after ingestion of  $\alpha$ -linolenic acid (18:3n-3) or 9*cis*,12*cis*,15*trans*-18:3 (15*trans*-18:3) (B) by human subjects.

oleic acid ( $14.0 \pm 4.1\%$ ) when expressed as the fraction of tracer recovered ( $P < 0.001$ ; Fig. 2).

## DISCUSSION


In the present work, we have looked at the  $^{13}\text{CO}_2$  production from linoleic and  $\alpha$ -linolenic acids as well as from two of their main *trans* geometric isomers in apparently healthy young men. To our knowledge, this article is the first report of the postprandial oxidation in human volunteers of linoleic and  $\alpha$ -linolenic acids given as TG, which is the dietary form for fatty acid ingestion. As a means of comparison, it would be rather difficult to draw a general



**Fig. 2.** Excretion of  $^{13}\text{CO}_2$  during the 8 h after the ingestion of linoleic acid (18:2n-6), 9*cis*,12*trans*-18:2 (12*trans*-18:2),  $\alpha$ -linolenic acid (18:3n-3), and 9*cis*,12*cis*,15*trans*-18:3 (15*trans*-18:3) by human subjects. Because the amounts of tracer given as 18:2 and 18:3 fatty acids were different,  $^{13}\text{CO}_2$  excretion was normalized for the amount of tracer ingested. \* Significantly different from the value obtained after ingestion of the three other fatty acids given as TG ( $P < 0.05$ ).

conclusion from the previous rare studies of the oxidation of linoleic and  $\alpha$ -linolenic acids in humans (15–18), considering that these were carried out under conditions that were different from each other and from ours. These studies used nondietary forms of the fatty acids (free fatty acids or methyl esters). Hence there may have been low bioavailability, as has already been suggested for the methyl esters (19). In addition, some were performed in men but others were carried out in women or lactating women, using either uniformly labeled or carboxyl-labeled fatty acids. Moreover, to account for incomplete recovery of labeled  $\text{CO}_2$  from the bicarbonate pool, some authors used a correction factor to evaluate the oxidation of labeled tracers (15, 16, 20), whereas others did not (17, 18). The exact value of the correction factor remains controversial, from 0.5 to 0.9 (21). Therefore, our data were not corrected by this factor. Finally, duration of  $\text{CO}_2$  collection and dietary conditions varied from one study to the other.

Nevertheless, despite all the differences, our figures for PUFA oxidation are of the same order of magnitude as most previously published figures. Most importantly, we confirm that  $\alpha$ -linolenic acid is used for energy production in humans to a greater extent than is linoleic acid when the PUFA were given as TG, their naturally occurring dietary form. This difference has already been reported in rats (9, 11, 12) and in humans (17), although with labeled free fatty acids. This suggests that the digestibility of the TG is not involved in the difference observed between oxidation of the two PUFA. Assuming a ratio of linoleic acid to  $\alpha$ -linolenic acid in the diet of between 5 and 10, the greater catabolism of  $\alpha$ -linolenic acid may consistently increase the difference in the availability of these two essential fatty acids for their conversion into long-chain PUFA, which is generally considered to characterize the “essential” nature of linoleic and  $\alpha$ -linolenic acids. The most important result is the greater catabolism of *trans*-18:2 compared with linoleic acid. This is consistent with the results we obtained in rats (12). The difference in oxidation may be due to selective formation of activated substrates (via the acyl-carnitines) for the  $\beta$ -oxidation pathway, or to different affinities for carrier proteins, such as albumin.

In conclusion, the present work demonstrates that the *trans* isomers of essential fatty acids are used for energy production in humans at least as well as their *cis* counterparts. Because these *trans* PUFA are also converted into higher derivatives (2, 5–7, 22), it is conceivable that their metabolic effects might be modulated by their bioavailability for this pathway, which in turn may be dependent of their use for energy production. 

We thank Nurse Liliane Morin (Laboratoire de Nutrition Humaine de Clermont-Ferrand, France) for excellent and skillful technical assistance. This work was supported by a grant from the European Commission (FAIR contract 95-0594, entitled Nutritional and Health Impact of *trans* Polyunsaturated Fatty Acids in European Populations), and by a PhD fellowship from INRA and Lesieur Alimentaire (L.B.).

Manuscript received 22 January 2001 and in revised form 13 February 2001.

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