β-Oxidation of Conjugated Linoleic Acid Isomers and Linoleic Acid in Rats

Jean-Pierre Sergiel[a], Jean-Michel Chardigny[a], Jean-Louis Sébédio[a,*], Olivier Berdeaux[a], Pierre Juaneda[a], Olivier Loreau[b], Bruno Pasquis[a], and Jean-Pierre Noel[b]

[a]INRA, Unité de Nutrition Lipidique, 21065 Dijon Cedex, and [b]CEA-Saclay, Service des Molécules Marquées, 91191 Gif sur Yvette, France

ABSTRACT: To assess the oxidative metabolism of conjugated linoleic acid (CLA) isomers, rats were force-fed 1.5–2.6 MBq of [1-^{14}C]-linoleic acid (9*c*,12*c*-18:2), -rumenic acid (9*c*,11*t*-18:2), or -10*trans*,12*cis*-18:2 (10*t*,12*c*-18:2), and $^{14}CO_2$ production was monitored for 24 h. The animals were then necropsied and the radioactivity determined in different tissues. Both CLA isomers were oxidized significantly more than linoleic acid. Moreover, less radioactivity was recovered in most tissues after CLA intake than after linoleic acid intake. The substantial oxidation of CLA isomers must be considered when assessing the putative health benefits of CLA supplements.

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In animals, conjugated isomers of linoleic acid (CLA) have been reported to have beneficial effects on a range of health parameters, including cancer (1), body composition (2), diabetes (3), immune function (4), and atherogenesis (5,6). However, the last is still controversial as results in mice (7) indicate a proatherogenic effect of CLA, whereas previous data on hamsters and rabbits suggest a preventive effect.

Few studies on the effects of CLA in humans have been published. Vessby and Smedman (8) reported a reduction of fat mass and an increase in urinary excretion of isoprostanes, suggesting enhanced lipid peroxidation (9). On the other hand, Zambell *et al.* (10) reported that CLA had no effect on body composition and energy expenditure in healthy women.

In food, CLA is present as rumenic acid, i.e., 9*cis*,11*trans*-18:2 (9*c*,11*t*-18:2), but synthetic materials are mixtures containing different isomers, 10*trans*,12*cis*-18:2 (10*t*,12*c*-18:2) being one of the major ones. This isomer seems to have specific metabolic effects, mainly on body composition and on desaturase activities (11,12) and related gene expression (13).

However, the effective dosage of CLA is not clearly known. The oxidative metabolism of CLA isomers represents a metabolic pathway that may reduce the bioavailability of CLA for further effects. In the present work, we compared the metabolic oxidation of 9*c*,11*t*- and 10*t*,12*c*-18:2 in rats. The data show that a substantial portion of both CLA isomers is oxidized more than linoleic acid (9*c*,12*c*-18:2).

MATERIAL AND METHODS

Male rats (Janvier, Le Genest Saint Isle, France) weighing 259 ± 6 g (mean ± SEM) were used. The animals were housed under controlled conditions of temperature (22 ± 1°C) and relative humidity (55–60%). A 12-h light–dark cycle (lights on 7:00 A.M.–7 :00 P.M.) was maintained. The animals were fed *ad libitum* with commercial pellets (Extralabo, Provins, France) and had free access to tap water. The day before the experiment at 5:00 P.M., they had access to only 10 g of commercial pellets, for the researchers to get animals at the same fasting status. All the experiments started at the same time (9:00 A.M.). Animal maintenance and handling were performed according to the French guidelines for animal studies (Authorizations A21200 and 3273).

[1-^{14}C]Linoleic acid (2.20 GBq · mmol^{-1}) was purchased from NEN (Le Blanc Mesnil, France). The detailed synthesis of [1-^{14}C]-9*c*,11*t*- (1.97 GBq · mmol^{-1}) and [1-^{14}C]-10*t*,12*c*-CLA (2.00 GBq · mmol^{-1}) isomers is described elsewhere (14). Each fatty acid was dissolved in triolein (Sigma Chemicals, L'Isle d'Abeau, France) and then administered by gastric tubing.

Immediately after intubation, the rats were placed in an airtight Plexiglas metabolic chamber, as described previously (15). Briefly, the $^{14}CO_2$ expired was trapped in a bottle containing Carbosorb (Packard, Groningen, the Netherlands). Air flow (950 mL · mmol^{-1}) was provided by a peristaltic pump.

Without interrupting the bubbling of the expired air through the trapping agent, 1 mL was removed every 30 min during the first 6 h of the experiment, hourly during the next 10 h, and hourly again from 18 to 24 h. As the density of the trapping agent increased during the experiment, the weight of each sample and of the bottle at each sampling time was measured to determine the exact radioactivity expired.

Scintillation cocktail (9 mL; Permafluor E, Packard) was added to each sample, and the radioactivity was determined using a Tri Carb 2000 CA liquid scintillation counter (Packard).

At the end of the 24-h experimental period, the animals were anesthetized. Blood was withdrawn into a heparinized syringe. Tissues (brain, heart, liver, gastrocnemian muscle, lung, kidneys, spleen, adrenals, testes, and epididymal adipose tissue)

*To whom correspondence should be addressed at INRA, Unité de Nutrition Lipidique, 17 rue Sully, BP 86510, 21065 Dijon Cedex, France.

E-mail: sebedio@dijon.inra.fr

Abbreviations: CLA, conjugated linoleic acid; GBq, gigaBecquerel; MBq, megaBecquerel.

were removed, blotted on filter paper, and weighed. The gastrointestinal tract was divided into two parts, as previously described (15). The first part included the stomach and the small intestine. The second part was the large intestine. The carcass of each animal was weighed before homogenization.

Three finely minced portions (30–80 mg) of each tissue finely minced, and five portions of the carcass (50–100 mg) as well as the two parts of the gastrointestinal tract were digested overnight at 50°C using 1 mL of Soluene (Packard). The radioactivity of the samples was then determined by liquid scintillation counting as described previously, after addition of Hionic Fluor (Packard) scintillation cocktail. The radiaoctivity in blood and urine was determined as described (15).

Statistical analysis. Data are presented as means ± SEM of three independent determinations. Analyses of variance were carried out using the SAS software (Cary, NC). *P* values of <0.05 were considered significant.

RESULTS

The weights of rats before administration of the radiolabeled fatty acids were similar. The radioactivity administered to the animals was 2.55 ± 0.04, 1.52 ± 0.04, and 1.57 ± 0.02 GBq for 9*c*,12*c*-, 9*c*,11*t*-, and 10*t*,12*c*-18:2, respectively. At the end of the experiments, 85–95% of the ingested radioactivity was recovered.

$^{14}CO_2$ production was similar for both CLA isomers. At the end of the 24-h experimental period, 71.8 and 70.3% of the dose of radioactivity from 9*c*,11*t*- and 10*t*,12*c*-18:2, respectively, were found in $^{14}CO_2$. These values were significantly higher than that obtained with 9*c*,12*c*-18:2 (60.3%, $P < 0.05$). The cumulative $^{14}CO_2$ production over 24 h is shown in Figure 1. The three curves exhibit a similar pattern with asymptotic profiles. They reached a plateau about 8 h after feeding the labeled fatty acids. The difference between CLA isomers and linoleic acid is borderline significant from 1.5 to 2 h after administration. The *P* values were less than 0.05 from 2.5 h after admistration to the end of the experiment.

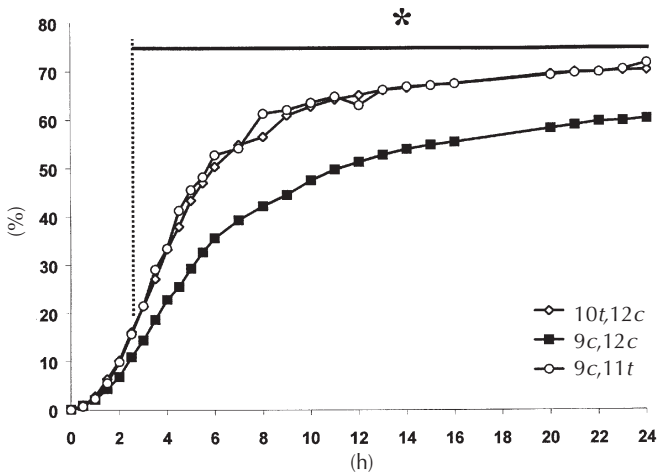

FIG. 1. $^{14}CO_2$ recovery after oral administration to the fasting rats of [1-^{14}C]-linoleic acid (9*c*,12*c*), -rumenic acid (9*c*,11*t*), or -10*trans*,12*cis*-18:2 (10*t*,12*c*). Data were obtained from three male Wistar rats for each fatty acid. Results are expressed as means of the percentage of radioactivity administered, recovered as $^{14}CO_2$, ± SEM. Asterisk (*) indicates period during which the $^{14}CO_2$ production was significantly different between conjugated linoleic acid (CLA) isomers (9*c*,11*t* and 10*t*,12*c*) and the corresponding values for linoleic acid (9*c*,12*c*) ($P < 0.05$).

The incorporated radioactivity per 100 g of tissue and as a fraction of the radioactivity administered at 24 h after the oral administration of the labeled fatty acids is presented in Table 1. In most tissues, the radioactivity recovered was similar whatever the fatty acid administered. However, the incorporation of radioactivity was different between linoleic acid and both the CLA isomers in brain, heart, adrenals, testes, and carcass (Table 1).

DISCUSSION

In animal models, CLA isomers have been reported to have beneficial effects on some physiological parameters related to health. Their efficacy in humans is still controversial (10). However, the mechanisms by which these fatty acids may act, as well as their metabolic fate, is still unknown. As with linoleic acid, CLA isomers are converted by desaturation and elongation pathways to conjugated 18:3, 20:3, and 20:4 fatty acids (16–18). Besides these conversions to longer and more unsaturated metabolites, the incorporation of CLA in tissues is generally low. Another possible metabolic pathway involves oxidation, either complete or partial. A 16:2 conjugated fatty acid isomer has been detected and identified in rat tissues fed pure CLA isomers (20).

Using radiolabeled CLA, we compared the oxidative metabolism and tissue incorporation of the two major CLA

TABLE 1
Recovery of Radioactivity (% of the administered dosage) per 100 g of Tissue 24 h After Oral Administration of the [1-^{14}C]-Radiolabeled Fatty Acids to Fasting Rats[a]

	9*c*,12*c*-18:2 (linoleic acid)	9*c*,11*t*-18:2 (rumenic (acid)	10*t*,12*c*-18:2	Standard error
Brain	0.33[a]	0.23[b]	0.29[c]	0.009
Carcass	1.19[a]	0.72[b]	0.90[a,b]	0.096
Heart	1.88[a]	0.77[b]	0.94[b]	0.091
Liver	2.27	1.39	1.78	0.252
Gastrocnemius	0.82	0.36	0.41	0.118
Stomach + small intestine	0.21	0.11	0.13	0.065
Large intestine + feces	0.25	0.50	0.22	0.121
Lung	1.19	1.45	1.53	0.228
Kidney	1.55	1.10	1.38	0.111
Spleen	1.66	1.04	1.44	0.192
Blood	0.32	0.25	0.34	0.040
Adrenals	4.72[a]	3.21[b]	2.38[b]	0.289
Testes	0.88[a]	0.24[b]	0.30[b]	0.020
Adipose tissue	1.73	1.91	1.51	0.278
Urine	1.78	1.31	2.00	0.184

[a]Results are expressed as mean ± SEM of three independent determinations. Values having a different roman superscript in rows are statistically significant ($P < 0.05$).

isomers in semifasting rats. Our data showed that CLA isomers produced more $^{14}CO_2$ than linoleic acid. This pattern was close to what we reported for α-linolenic acid, where 70% of the ingested radioactivity was recovered in CO_2 (15). As CLA seemed to be as well absorbed as linoleic acid (19), this difference between linoleic acid and CLA may be due to a higher metabolic utilization by cellular oxidation systems.

In the present study, the radioactivity incorporated in different tissues was similar after intragastric feeding of the three fatty acids. Some differences were observed only in brain, carcass, heart, adrenals, and testes, in which linoleic acid seemed to be better incorporated than CLA isomers. Moreover, our data do not explain why 9*c*,11*t*-18:2 is generally incorporated more than 10*t*,12*c*-18:2 into tissues (20).

When added to the diet, CLA have been reported to induce different physiological effects in several animal models. The mechanisms by which CLA act are not understood. The present data indicate that the catabolism of ingested CLA has to be taken into account with regard to their tissue bioavailability and emphasize that CLA are lipids that may also contribute to energy production.

Further studies have to be carried out to know the CLA metabolic pattern in humans.

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REFERENCES

1. Ip, C., Banni, S., Angioni, E., Carta, G., McGinley, J., Thompson, H.J., Barbano, D., and Bauman, D. (1999) Conjugated Linoleic Acid-Enriched Butter Fat Alters Mammary Gland Morphogenesis and Reduces Cancer Risk in Rats, *J. Nutr. 129*, 2135–2142.
2. Park, Y., Albright, K.J., Liu, W., Storkson, J.M., Cook, M.E., and Pariza, M.W. (1997) Effect of Conjugated Linoleic Acid on Body Composition in Mice, *Lipids 32*, 853–858.
3. Houseknecht, K.L., Vandenheuvel, J.P., Moyacamarena, S.Y., Portocarrero, C.P., Peck, L.W., Nickel, K.P., and Belury, M.A. (1998) Dietary Conjugated Linoleic Acid Normalizes Impaired Glucose Tolerance in the Zucker Diabetic Fatty Fa/fa Rat, *Biochem. Biophys. Res. Commun. 244*, 678–682.
4. Sugano, M., Tsujita, A., Yamasaki, M., Noguchi, M., and Yamada, K. (1998) Conjugated Linoleic Acid Modulates Tissue Levels of Chemical Mediators and Immunoglobulins in Rats, *Lipids 33*, 521–527.
5. Lee, K., Kritchevsky, D., and Pariza, M. (1994) Conjugated Linoleic Acid and Atherosclerosis in Rabbits, *Atherosclerosis 108*, 19–25.
6. Kritchevsky, D., Tepper, S.A., Wright, S., Tso, P., and Czarnecki, S.K. (2000) Influence of Conjugated Linoleic Acid (CLA) on Establishment and Progression of Atherosclerosis in Rabbits, *J. Am. Coll. Nutr. 19*, 472S–477S.
7. Munday, J.S., Thompson, K.G., and James, K.A.C. (1999) Dietary Conjugated Linoleic Acids Promote Fatty Streak Formation in the C57BL/6 Mouse Atherosclerosis Model, *Br. J. Nutr. 81*, 251–255.
8. Vessby, B., and Smedman, A. (1999) Conjugated Linoleic Acid (CLA) Reduces the Body Fat Content in Humans, *Chem. Phys. Lipids 101,* 152.
9. Basu, S., Smedman, A., and Vessby, B. (2000) Conjugated Linoleic Acid Induces Lipid Peroxidation in Humans, *FEBS Lett. 468*, 33–36.
10. Zambell, K.L., Keim, N.L., Van Loan, M.D., Gale, B., Benito, P., Kelley, D.S., and Nelson, G.J. (2000) Conjugated Linoleic Acid Supplementation in Humans: Effects on Body Composition and Energy Expenditure, *Lipids 35*, 777–782.
11. Park, Y., Storkson, J.M., Albright, K.J., Liu, W., and Pariza, M. W. (1999) Evidence That the *trans*-10,*cis*-12 Isomer of Conjugated Linoleic Acid Induces Body Composition Changes in Mice, *Lipids 34*, 235–241.
12. Bretillon, L., Chardigny, J.M., Gregoire, S., Berdeaux, O., and Sébédio, J.L. (1999) Effects of Conjugated Linoleic Acid Isomers on the Hepatic Microsomal Desaturation Activities *in Vitro, Lipids 34*, 965–969.
13. Lee, K.N., Pariza, M.W., and Ntambi, J.M. (1998) Conjugated Linoleic Acid Decreases Hepatic Stearoyl-CoA Desaturase mRNA Expression, *Biochem. Biophys. Res. Commun. 248*, 817–821.
14. Loreau, O., Maret, A., Chardigny, J.M., Sébédio, J.L., and Noel, J.P. (2001) Sequential Substitution of 1,2-Dichloro-ethene: A Convenient Stereoselective Route to (9*Z*,11*E*)-, (10*E*,12*Z*), and (10*Z*,12*Z*)-[1-^{14}C]-Conjugated Linoleic Acid Isomers, *Chem. Phys. Lipids 110,* 57–67.
15. Bretillon, L., Chardigny, J.M., Sébédio, J.L., Poullain, D., Noël, J.P., and Vatèle, J.M. (1998) Oxidative Metabolism of (1-^{14}C) Mono-*trans* Isomers of Linoleic and α-Linolenic Acids in the Rat, *Biochim. Biophys. Acta 1390*, 207–214.
16. Banni, S., Carta, G., Contini, M.S., Angioni, E., Deiana, M., Dessi, M.A., Melis, M.P., and Corongiu, F.P. (1996) Characterization of Conjugated Diene Fatty Acids in Milk, Dairy Products, and Lamb Tissues, *J. Nutr. Biochem. 7*, 150–155.
17. Sébédio, J.L., Juaneda, P., Dobson, G., Ramilison, I., Martin, J.D., Chardigny, J.M., and Christie, W.W. (1997) Metabolites of Conjugated Isomers of Linoleic Acid (CLA) in the Rat, *Biochim. Biophys. Acta 1345*, 5–10.
18. Sébédio, J.L., Juaneda, P., Gregoire, S., Chardigny, J.M., Martin, J.C., and Ginies, C. (1999) Geometry of Conjugated Double Bonds of CLA Isomers in a Commercial Mixture and in Their Hepatic 20:4 Metabolites, *Lipids 34*, 1319–1325.
19. Martin, J.C., Sebedio, J.L., Caselli, C., Pimont, C., Martine, L., and Bernard, A. (2000) Lymphatic Delivery and *in Vitro* Pancreatic Lipase Hydrolysis of Glycerol Esters of Conjugated Linoleic Acids in Rats, *J. Nutr. 130*, 1108–1114.
20 Sébédio, J.L., Angioni, E., Chardigny, J.M., Grégoire, S. Juanéda, P., and Berdeaux, O. (2001) The Effect of Conjugated Linoleic Acid Isomers on Fatty Acid Profiles of Liver and Adipose Tissues and Their Conversion into Isomers of 16:2 and 18:3 Conjugated Fatty Acids in Rats, *Lipids 36*, 575–582.

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