JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Metabolites of Conjugated Isomers of α-Linolenic Acid (CLnA) in the Rat

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Rumelenic (*cis*-9,*trans*-11,*cis*-15 18:3) acid is a naturally occurring conjugated isomer of α-linolenic acid (CLnA) in milk fat. Metabolism in rats was studied using a synthetic CLnA mixture, composed mainly by equimolar quantities of *cis*-9,*trans*-11,*cis*-15 and *cis*-9,*trans*-13,*cis*-15 CLnA isomers. Their metabolisms were studied by feeding high quantities of CLnA (150 mg/day) for 4 days to rats that had been reared on a fatfree diet for 2 weeks. After this period, animals were sacrificed and liver and epididymal adipose tissue lipids extracted. Six metabolites of the *cis*-9,*trans*-11,*cis*-15 18:3 CLnA isomers were identified as being *cis*-7,*trans*-9,*cis*-13 16:3, *cis*-11,*trans*-13,*cis*-17 20:3, *cis*-8,*cis*-11,*trans*-13,*cis*-17 20:4, *cis*-5,*cis*-8,*cis*-11,*trans*-13,*cis*-17 20:5, *cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:5, and *cis*-4,*cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:6 acids. Two metabolites of *cis*-9,*trans*-13,*cis*-15 18:3 CLnA isomer were also identified by GC-MS as being *cis*-7,*trans*-11,*cis*-13 16:3 and *cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5.

KEYWORDS: Conjugated linolenic acid; fatty acids; lipid metabolism; mass spectrometry

INTRODUCTION

Conjugated fatty acids are present at low levels in a variety of foods and natural products. Because a mixture of naturally occurring conjugated linoleic acids (CLA) has been previously isolated from fried meats, and described as anticarcinogens by Ha et al. (*1*), interest in conjugated fatty acids and in particular in CLA has considerably increased. Biological effects attributed to CLA isomers are many and were recently reviewed by Pariza et al. (*2*). It was recently shown that some conjugated products of α-linolenic acid reduce perirenal and epididymal adipose tissue weight in rats (*3*), have a cytotoxic effect on cultured human tumor cells (*4*), and inhibit the induction and development of mammary tumors in rats (*5*), induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).

Rumelenic (*cis*-9,*trans*-11,*cis*-15 18:3) acid, tentatively identified as an intermediate in the biohydrogenation process of α-linolenic (*cis*-9,*cis*-12,*cis*-15 18:3) acid, catalyzed by Δ^{12}-*cis*,Δ^{11}-*trans*-isomerase, located in the cell envelope of *Butyrivibrio fibrisolvens* (6), was recently tentatively identified in cheese fat (7). This conjugated linolenic acid (CLnA) isomer is converted into *trans*-11,*cis*-15 18:2 by the same enzyme, and both biohydrogenation products were identified in milk fat (Destaillats et al., unpublished results).

It was reported that CLA isomers (*cis*-9,*trans*-11 18:2 and *trans*-10,*cis*-12 18:2) could be metabolized to C16:2 (*trans*-8,*cis*-10 16:2), C18:3 (*cis*-6,*cis*-9,*trans*-11 18:3 and *cis*-6,*trans*-10,*cis*-12 18:3), C20:3 (*cis*-8,*cis*-11,*trans*-13 20:3 and *cis*-8,*trans*-12,*cis*-14 20:3), and C20:4 (*cis*-5,*cis*-8,*cis*-11,*trans*-13 20:4 and *cis*-5,*cis*-8,*trans*-12,*cis*-14 20:4) acids (*8*−*11*), which have been shown to interfere with linoleic acid metabolism (*9*). However, no literature data so far have reported on the presence of metabolites of CLnA, which may interfere with the metabolism of α-linolenic acid. For this reason, we decided to study if rumelenic (*cis*-9,*trans*-11,*cis*-15 18:3) could give rise to metabolites in the rat liver and epididymal adipose tissue. Therefore, we have used a synthetic mixture composed by equimolar quantities of rumelenic acid and *cis*-9,*trans*-13,*cis*-15 CLnA isomer, which has not been reported to occur naturally.

MATERIALS AND METHODS

Animals. Six weanling male Wistar rats were housed in individual stainless steel cages in an animal house maintained at 26 ± 1 °C (light/dark: 12h/12h). They were randomly divided into two experimental groups of three animals, which were fed a fatfree diet for 2 weeks (*8*). At the end of this period, while maintaining for 4 days the animals on the same diet, one group was given intragastrically 150 mg/day of a free fatty acid mixture composed by *cis*-9 18:1 (0.5%), *cis*-9,*cis*-12 (2.4%), *cis*-9,*trans*-11,*cis*-15 18:3 (40.7%), *cis*-9,*trans*-13,*cis*-15 18:3

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10.1021/jf0481958 CCC: $30.25
Published on Web 02/08/2005

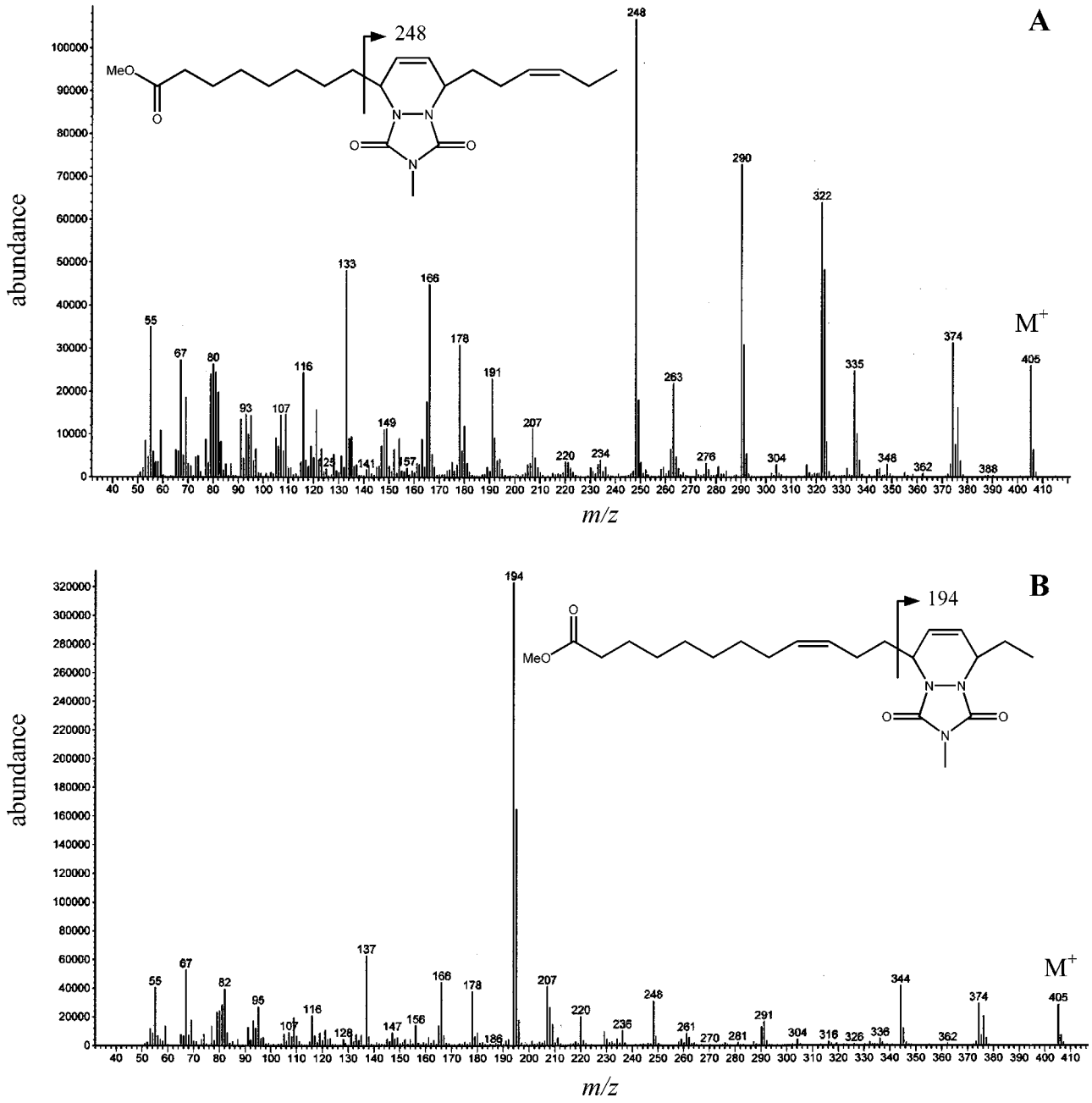

Figure 1. Mass spectra of the MTAD adducts of (**A**) *cis*-9,*trans*-11,*cis*-15 18:3 and (**B**) *cis*-9,*trans*-13,*cis*-15 18:3 acid methyl esters.

(40.7%), *cis*-9,*trans*-11 18:2 (0.7%), and *trans*-10,*cis*-12 18:2 (0.8%) acids prepared by alkaline isomerization and kindly donated by Kemestrie Inc. (Sherbrooke, PQ, Canada).

Lipid Analyses. At the end of the experimental period, the liver and epididymal adipose tissues were quickly excised, blotted on filter paper, and weighed, and lipids were extracted using a mixture of chloroform and methanol (2:1, v/v) (*12*). Quantitative analyses of liver lipid class were carried out by Iatroscan (Iatron, Tokyo, Japan) thin-layer chromatography−flame ionization detection (TLC-FID) system using a mixture of hexane/diethyl ether/acetic acid (97:3:1, v/v/v) as solvent (*13*). Liver lipids were fractionated into phospholipids and neutral lipids using silica cartridges (*14*).

Fatty acids from total liver, phospholipids liver, neutral lipids liver, and adipose tissue were transesterified into fatty acid methyl ester (FAME) derivatives using sodium methylate as a catalyst (*15*). Total liver FAMEs were fractionated by reversed phase high-performance liquid chromatography (HPLC) according to their partition number [PN = (carbon number) − 2*X* (double bond number)] on a 250 mm × 10 mm i.d. Kromasil C18 reversed phase column (Shandon, Eragny, France) using acetonitrile as mobile phase at 4 mL/min (*8*). The first collected fraction was composed of FAMEs of PN = 10 and 12, whereas the second collected fraction contained FAMEs of PN = 14.

Gas−liquid chromatography (GC) analyses of total FAME and FAME fractions were performed on a 5890 series II gas chromatograph (Hewlett-Packard), equipped with a 120 m, 0.25 mm i.d., 0.25 μm film thickness fused-silica BPX-70 (equivalent to 70% cyanopropyl) capillary column (SGE, Melbourne, Australia). Injection (splitless mode) and detection (flame ionization) were performed at 250 °C. Oven temperature programming was 60 °C isothermal for 1 min, increased to 170 °C at 20 °C/min, held isothermal for 60 min, then increased to 210 °C at 5 °C/min, and held isothermal for 15 min. The inlet pressure of the carrier gas (H_2) was 280 kPa at 170 °C.

Identification of Metabolites. Phospholipid liver fractions and total lipids were derivatized into 4,4-dimethyloxazoline (DMOX) derivatives using modified literature procedures (*16*, *17*) by heating intact lipid (10 mg) directly with 2-amino-2-methyl-1-propanol (500 μL) under nitrogen atmosphere at 150 °C overnight.

Table 1. Fatty Acid Compositions (Weight Percent) of Liver Phospholipids (PL), Neutral Lipids (NL), and Total Lipids from Epididymal Adipose Tissue of Rats ($n = 3$) Fed the Control Diet or the Experimental Diet (CLnA)

	liver											
	phospholipids				neutral lipids				adipose tissue			
	control		CLnA		control		CLnA		control		CLnA	
fatty acid	MV[a]	SD[b]	MV	SD	MV	SD	MV	SD	MV	SD	MV	SD
saturated[c]	39.73	0.60	40.57	0.47	39.25	1.07	38.10	1.35	39.61	0.56	38.94	0.51
monunsaturated[d]	18.79	0.31	18.45	1.24	55.97	0.40	56.42	0.92	54.41	1.16	53.49	0.61
diunsaturated[c]	6.42	0.22	6.54	0.65	1.96	0.22	2.03	0.24	3.73	0.53	4.02	0.35
18:3 *n*–7	0.12	0.01	0.15	0.01	0.04	0.01	0.05	0.01	0.01	0.00	0.01	0.00
18:3 *n*–6	0.20	0.04	0.31	0.06	0.09	0.03	0.12	0.03	0.03	0.01	0.03	0.00
18:3 *n*–3	0.04	0.03	0.02	0.02	0.03	0.01	0.04	0.01	0.22	0.06	0.27	0.01
***cis*-9,*trans*-11,*cis*-15 18:3**			**0.08**	**0.01**			**0.12**	**0.01**			**0.36**	0.07
***cis*-9,*trans*-13,*cis*-15 18:3**			**0.11**	**0.01**			**0.64**	**0.06**			**0.62**	0.13
20:3 *n*–9	3.39	0.41	3.50	0.48	0.29	0.08	0.22	0.09	0.06	0.03	0.06	0.01
cis-5,*cis*-11,*cis*-14 20:3	0.49	0.07	0.45	0.03	0.02	0.01	0.02	0.00	0.01	0.00	0.01	0.00
20:3 *n*–6	1.24	0.11	1.53	0.06	0.06	0.02	0.06	0.02	0.02	0.00	0.01	0.00
20:4 *n*–6	19.33	0.55	18.03	0.79	0.81	0.16	0.59	0.09	0.13	0.03	0.10	0.00
20:3 *n*–3	0.02	0.01	0.02	0.00								
20:5 *n*–3	0.38	0.04	0.65	0.07	0.02	0.01	0.03	0.01				
***cis*-11,*trans*-13,*cis*-17 20:3**			**0.07**	**0.01**			**0.03**	**0.01**			**0.03**	0.00
22:3	0.11	0.02	0.11	0.02	0.05	0.02	0.05	0.02	0.01	0.00	0.01	0.00
***cis*-8,*cis*-11,*trans*-13,*cis*-17 20:4**			**0.06**	**0.01**			**0.04**	**0.01**			**0.03**	0.01
***cis*-5,*cis*-8,*cis*-11,*trans*-13,*cis*-17 20:5**			**0.06**	**0.01**			**0.01**	**0.00**				
***cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5**			**0.05**	**0.01**			**0.01**	**0.00**				
22:4 *n*–6	0.23	0.02	0.19	0.00	0.03	0.01	0.02	0.01	0.01	0.00	0.01	0.00
22:5 *n*–6	1.94	0.30	1.58	0.18	0.05	0.01	0.03	0.01				
22:5 *n*–3	0.19	0.02	0.21	0.02	0.01	0.00	0.01	0.00				
22:6 *n*–3	5.59	0.39	5.46	0.27	0.10	0.03	0.08	0.01	0.03	0.01	0.02	0.00
***cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:5**			0.07	0.01								
***cis*-4,*cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:6**			0.07	0.01								
	1.79	0.40	1.68	0.18	1.22	0.11	1.28	0.07	1.72	0.05	1.98	0.78

[a] Mean value. [b] Standard deviation. [c] 14:0 + 16:0 +17:0 +18:0 + 19:0 + 20:0. [b] 16:1 +17:1 + 18:1 + 20:1. [c] 18:2 + 20:2.

Gas chromatography−mass spectrometry (GC-MS) analysis of DMOX derivatives was carried out by GC-MS on a Hewlett-Packard model 6890 series II gas chromatograph attached to an Agilent model 5973N selective quadrupole mass detector (Palo Alto, CA) under an ionization voltage of 70 eV at 230 °C and connected to a computer with Agilent ChemStation software. The injector (splitless mode) and the interface temperatures were maintained at 250 °C, whereas He was used as carrier gas under constant flow at 2.4 mL/min. GC separation was performed on a 50 m × 0.32 mm i.d., 0.25 μm film thickness BPX-70 capillary column (SGE). Temperature programming consisted of 60 °C isothermal for 1 min, increased to 170 °C at 20 °C/min, and held isothermal for 40 min at 170 °C; then the oven temperature was increased to 210 °C at 5 °C/min and held isothermal for 15 min.

Separation of *cis*-9,*trans*-11,*cis*-15 18:3 and *cis*-9,*trans*-13,*cis*-15 18:3 isomers was achieved as their 4-methyl-1,2,4-triazoline-3,5-dione (MTAD) adduct prepared from the FAME according to a literature procedure (*19*). GC-MS analyses were performed on the previously described apparatus fitted with a 30 m × 0.25 mm i.d., 0.25 μm film thickness DB5-MS capillary column (J&W, Folsom, CA) according to a literature procedure (*8*). Quantifications were achieved by GC analysis of the MTAD adducts on a 5890 gas chromatograph (Hewlett-Packard), equipped with a DB5-MS capillary column. Injection (splitless mode) and detection (flame ionization) were performed at 250 °C, whereas oven temperature programming was 50 °C isothermal for 1 min, increased to 270 °C at 20 °C/min, and held isothermal for 30 min at 270 °C. The flow rate of the carrier gas (H_2) was 35 cm/s at 50 °C.

RESULTS AND DISCUSSION

Animals. The experiment was based on the comparison of the fatty acid composition of liver and adipose tissues of a control group ($n = 3$) fed a fatfree diet and a CLnA group ($n = 3$) fed a fatfree diet plus 150 mg/day of a free fatty acid mixture containing mainly *cis*-9,*trans*-11,*cis*-15 18:3 (40.7%) and *cis*-9,*trans*-13,*cis*-15 18:3 (40.7%) acids. There was no effect of the CLnA isomers on body weight.

At the end of the experimental period, the mean weight of the rats was 170.0 ± 5.7 g in the control group and 171.0 ± 1.2 g for the CLnA group. The weights of livers were not significantly different and were 7.73 ± 0.29 and 7.27 ± 0.45 g, respectively, for the control and CLnA groups. Liver lipid class distributions were also not significantly different and were composed of 61.20 ± 8.13 and 62.50 ± 7.19% of phospholipids and of 35.30 ± 8.49 and 34.30 ± 7.47% of neutral lipid (sum of cholesteryl esters, free fatty acids, triacylglycerols, and diacylglycerols percentages) for the control and CLnA groups, respectively.

CLnA Metabolites. The *cis*-9,*trans*-11,*cis*-15 18:3 and *cis*-9,*trans*-13,*cis*-15 18:3 acids were not resolved as their methyl ester derivatives, and the separation could be achieved, after a second derivatization reaction, as their MTAD adducts. On a nonpolar stationary phase of 5% cyanoalkylpolysiloxane, the *cis*-9,*trans*-11,*cis*-15 18:3 MTAD adduct for which the mass spectrum is given in **Figure 1A** elutes before the MTAD adduct of the *cis*-9,*trans*-13,*cis*-15 18:3 acid methyl ester MTAD, for which the mass spectrum is given in **Figure 1B**. Fatty acid compositions of liver and adipose tissues were achieved by GC on a highly polar 120 m capillary column, and results are given in **Table 1**. CLnA isomers were detected in all lipid samples from the CLnA group; adipose tissue (0.98% of total FA) and neutral lipid (0.76%) contained higher levels of these fatty acids than liver phospholipids (0.19%). Indeed, the *cis*-9,*trans*-13,*cis*-15 18:3 isomer was more accumulated and represented about 84.8 and 63.3% of the total CLnA isomers, in neutral liver lipids and in adipose tissues, respectively (**Table 1**). In the liver phospholipids, the *cis*-9,*trans*-13,*cis*-15 18:3 isomer represented ~57.9% of total CLnA isomers.

A representative sample of liver phospholipids from rats of the CLnA group ($n = 3$) was fractionated by reversed

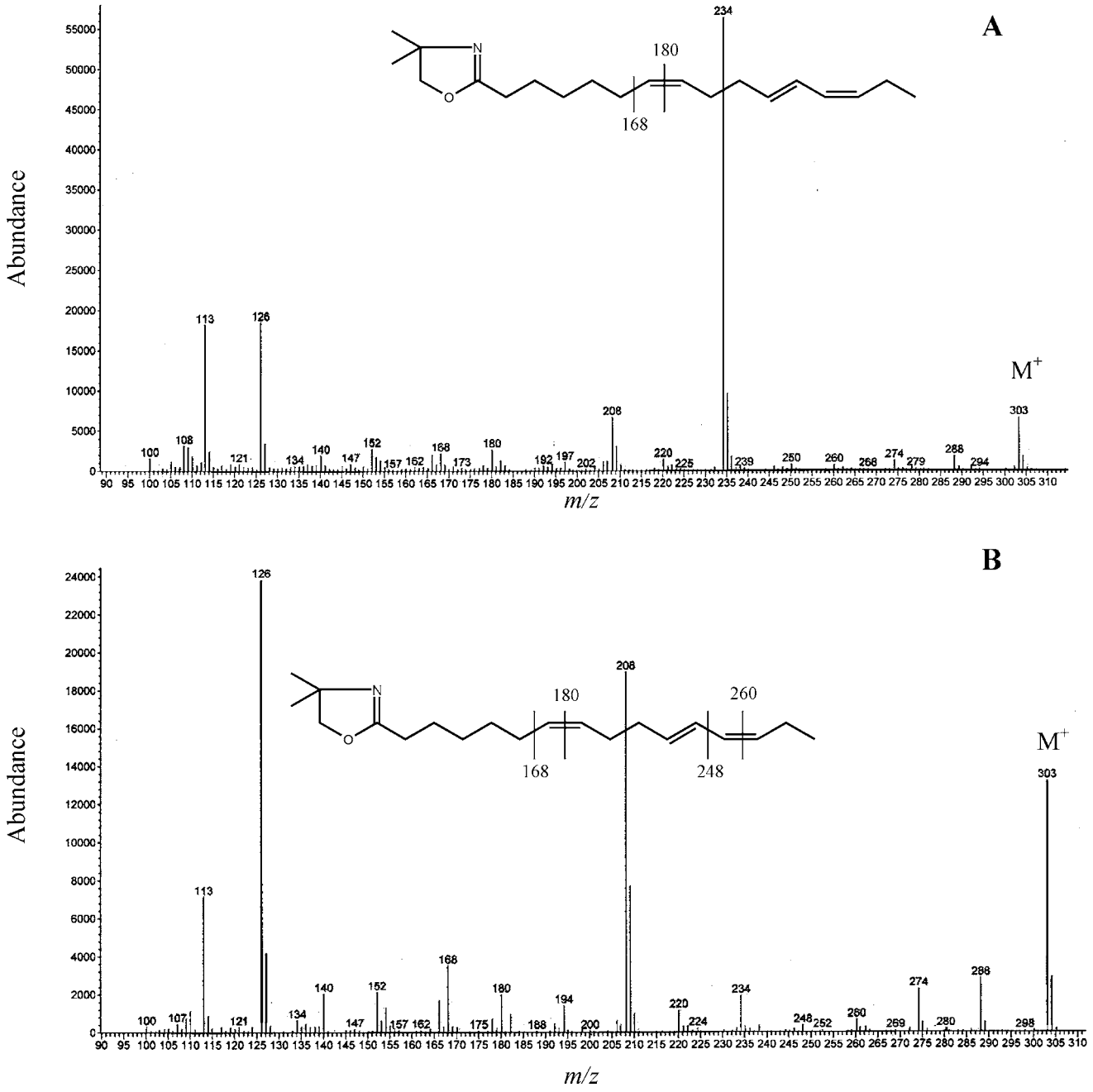

Figure 2. Mass spectra of (**A**) *cis*-7,*trans*-9,*cis*-11 16:3 and (**B**) *cis*-7,*trans*-11,*cis*-13 16:3 acids as their DMOX derivatives.

phase HPLC as their methyl ester derivatives (*8*). Total liver lipids and FAME fractions of PN = 10 + 12 and PN = 14 were analyzed both by GC and by GC-MS and converted into DMOX derivatives for structural identification. Comparative analysis with corresponding materials from rats of the control group revealed the occurrence of new peaks identified by GC-MS to be *cis*-7,*trans*-9,*cis*-13 16:3, *cis*-7,*trans*-11,*cis*-13 16:3, *cis*-11,*trans*-13,*cis*-17 20:3, *cis*-8,*cis*-11,*trans*-13,*cis*-17 20:4, *cis*-5,*cis*-8,*cis*-11,*trans*-13,*cis*-17 20:5, *cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5, *cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:5, and *cis*-4,*cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:6 acids.

Six metabolites, having an isolated $n-3$ double bond plus a conjugated double bond system, attributable to the series of the *cis*-9,*trans*-11,*cis*-15 18:3 isomer, were identified, whereas the *cis*-9,*trans*-13,*cis*-15 18:3 was apparently converted into two metabolites (*cis*-7,*trans*-11,*cis*-13 16:3 and *cis*-5,*trans*-8,*cis*-11,*trans*-15,*cis*-17 20:5 acids).

All of the identified metabolites were detected in liver phospholipids, at a level ranging from 0.05 to 0.07% (**Table 1**). However, *cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:5 and *cis*-4,*cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:6 acids were not detected in liver neutral lipid fraction, and only the *cis*-11,*trans*-13,*cis*-17 20:3 and *cis*-8,*cis*-11,*trans*-13,*cis*-17 20:4 fatty acid metabolites were detected in adipose tissue. A putative interrelationship scheme for both series is given in **Figure 3**. The number of detected metabolites and relative distribution of individual CLnA isomers may suggest that the *cis*-9,*trans*-11,*cis*-15 18:3 is better metabolized than the *cis*-9,*trans*-13,*cis*-15 18:3 isomer, which is accumulated (**Table 1**).

Mass fragmentation of these partially conjugated polyunsaturated fatty acids is given in **Table 2**. All of the identified fatty

Table 2. Characteristic Ions in EI Mass Spectra of DMOX Derivatives of Metabolites of *cis*-9,*trans*-11,*cis*-15 18:3 and *cis*-9,*trans*-13,*cis*-15 18:3 Acids

fatty acid DMOX	M^+ *m*/*z* (intensity, %)	diagnostic fragments *m*/*z* (intensity, %)
22:6 (4,7,10,13,15,19)	381 (12)	166 (6), 178 (2), 192 (4), 206 (14), 232 (3), 246 (11), 258 (4), 272 (1), **312**[a] **(48)**, 139 (4)
22:5 (7,10,13,15,19)	383 (34)	168 (5), 180 (9), 208 (10), 220 (6), 248 (37), 260 (7), 274 (3), 300 (6), **314 (65)**, 328 (2), 354 (2)
20:5 (5,8,11,13,17)	355 (24)	180(8), 192 (3), 206 (9), 220 (24), 232 (11), 246 (3), 258 (1), 272 (4), **286 (100)**, 326 (2), 153 (13)
20:5 (5,8,11,15,17)	355 (30)	180 (8), 192 (3), 206 (8), 220 (34), 232 (5), 246 (8), **260 (71)**, 274 (2), 286 (10), 300 (2), 312 (1), 326 (4), 153 (19)
20:4 (8,11,13,17)	357 (40)	182 (4), 208 (1), 222 (12), 234 (5), 248 (4), 274 (4), **288 (100)**, 302 (6), 328 (3)
20:3 (8,11,13)	359 (44)	262 (3), 276 (12), **290 (100)**, 304 (2), 330 (7)
16:3 (7,9,13)	303 (11)	168 (4), 180 (4), 194 (2), 208 (11), 220 (2), **234 (100)**, 260 (1), 274 (2)
16:3 (7,11,13)	303 (56)	168 (15), 180 (9), 194 (6), **208 (78)**, 222 (1), 234 (10), 248 (1), 260 (4), 274 (11), 288 (10)

[a] Diagnostic ion fragment in bold represent cleavage at the center of the bismethylene interrupted double-bond system.

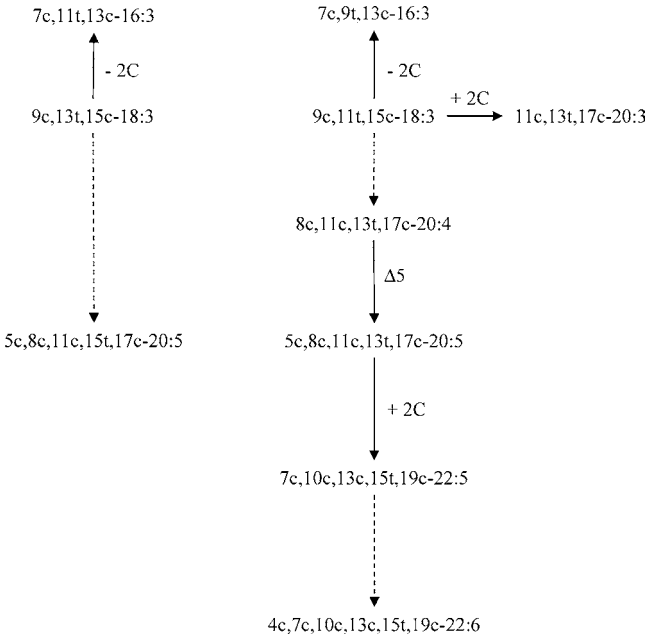

Figure 3. Putative interrelationship pathway between metabolites of conjugated *cis*-9,*trans*-11,*cis*-15 and *cis*-9,*trans*-13,*cis*-15 isomers of α-linolenic acid (CLnA) in the rat.

acids exhibit a bismethylene interrupted double-bond system. In EI GC-MS, this structure gives a highly characteristic intense ion fragment representative of cleavage at the center of the system (*19*, *20*), as shown in the mass spectra of the identified 16:3 metabolites (**Table 2** and **Figure 2**). Diagnostic ion fragments at *m*/*z* 208 and 234 for *cis*-7,*trans*-11,*cis*-13 16:3 (**Figure 2A**) and *cis*-7,*trans*-9,*cis*-13 16:3 (**Figure 2B**) allow unambiguous location of the bismethylene interrupted double-bond system. Indeed, the fragment ions at *m*/*z* $M^+ - 69$ and $M^+ - 95$ are observed for metabolites of the 9,11,15 18:3 and 9,13,15 18:3 acids, respectively.

Both tested CLnA isomers can be converted into 16:3 conjugated fatty acid isomer. This partial degradation has been previously described for linoleic acid, as a peroxisomal β-oxidation process (*21*). As observed for the *cis*-9,*trans*-11 18:2 CLA isomer (*9*−*12*), in the same experimental conditions, the *cis*-9,*trans*-11,*cis*-15 18:3 could be elongated into *cis*-11,*trans*-13,*cis*-17 20:3 acid.

However, the putative elongation product of the *cis*-9,*trans*-13,*cis*-15 18:3 isomer, the *cis*-11,*trans*-15,*cis*-17 20:3 acid, has not been detected. The occurrence of the *cis*-8,*cis*-11,*trans*-13,*cis*-17 20:4 acid suggests that the *cis*-9,*trans*-11,*cis*-15 18:3 could be putatively Δ6-desaturated to give *cis*-6,*cis*-9,*trans*-11,*cis*-15 18:4 acid intermediate, which can lead to the 20:4 metabolite after an elongation step (**Figure 3**). Nevertheless, the 18:4 acid has not been identified from any lipid samples of the experimental CLnA group, as well as the 20:4 acid metabolite from the *cis*-9,*trans*-13,*cis*-15 18:3 isomer. However, two *n*−3 eicosapentaenoic acids, *cis*-5,*cis*-8,*cis*-11,*trans*-13,*cis*-17 20:5 and *cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5 acids, have been identified. The Δ5 double bond was unambiguously located by the occurrence of an odd-numbered ion fragment at *m*/*z* 153 (*15*). These two metabolites should putatively be formed via the Δ5-desaturation of the 20:4 acid precursors (**Figure 3**), similar to the formation of 20:5 *n*−3 from α-linolenic acid (*21*) or for its geometrical isomer (*22*).

The two identified C22 partially conjugated polyunsaturated fatty acids, *cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:5 and *cis*-4,*cis*-7,*cis*-10,*cis*-13,*trans*-15,*cis*-19 22:6 acids, are formed from the dietary *cis*-9,*trans*-11,*cis*-15 18:3 isomer. The Δ4 double bond was unambiguously located by the occurrence of on odd-numbered ion fragment at *m*/*z* 153, formed via a rearrangement of the oxazoline group (*15*). No mass spectrometric data attributable to conjugated C22 metabolites formed from *cis*-9,*trans*-13,*cis*-15 18:3 were recorded. Indeed, we can hypothesize that some constraints prevent their formation from *cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5 or that the formed C22 were additionally converted into superior metabolites or degradated.

We have shown in this study that *cis*-9,*trans*-11,*cis*-15 18:3 acid could be metabolized into a series of conjugated polyunsaturated fatty acids, ranging from the 16:3 to the 22:6 acids, which were all detected in liver phospholipids. Additionally, dietary *cis*-9,*trans*-13,*cis*-15 18:3 acid is converted into *cis*-7,*trans*-9,*cis*-11 16:3 acid and the *cis*-5,*cis*-8,*cis*-11,*trans*-15,*cis*-17 20:5 acid, and this CLnA isomer is more accumulated than rumelenic acid in neutral liver lipids and in epididymal adipose tissue.

ACKNOWLEDGMENT

We thank S. Almanza and J. P. Trottier for technical assistance and Kemestrie Inc. (Sherbrooke, PQ, Canada) for providing the CLnA mixtures.

LITERATURE CITED

(1) Ha, Y. L.; Grimm, N. K.; Pariza, M. W. Inhibition of benz(*a*)-pyrene-induced mouse forestomach neoplasia by conjugated linoleic acid. *Cancer Res.* **1987**, *50*, 1097−1101.

(2) Pariza, M. W.; Park, Y.; Cook, M. E. The biologically active isomers of conjugated linoleic acid. *Prog. Lipid Res.* **2001**, *40*, 283−298.

(3) Koba, K.; Akahoshi, A.; Yamasaki, M.; Tanaka, K.; Yamada, K.; Iwata T.; Kamegai, T. K.; Tsutsumi, Sugano, M. Dietary conjugated linolenic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. *Lipids* **2002**, *37*, 343−350.

(4) Igarashi, M.; Miyazawa, T. Newly recognized cytotoxic effect of conjugated trienoic fatty acids on cultured human tumor cells. *Cancer Lett.* **2000**, *148*, 173−179.
(5) Futakuchi, M.; Cheng, J. L.; Hirose, M.; Kimoto, N.; Cho, Y. M.; Iwata, T.; Kasai, M.; Tokudome, S.; Shirai, T. Inhibition of conjugated fatty acids derived from safflower or perilla oil of induction and development of mammary tumors in rats induced by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). *Cancer Lett.* **2002**, *178*, 131−139.
(6) Kepler, C. R.; Tove, S. B. Biohydrogenation of polyunsaturated fatty acids III: purification and properties of a linoleate 12-*cis*,-11-*trans* isomerase from *Butyrivibrio fibrisolvens*. *J. Biol. Chem.* **1967**, *242*, 5686−5692.
(7) Winkler, K.; Steinhart, H. Identification of conjugated isomers of linolenic acid and arachidonic acid in cheese. *J. Sep. Sci.* **2001**, *24*, 663−668.
(8) Sebedio, J. L.; Juaneda, P.; Dobson, G.; Ramilison, I.; Martin, J. C.; Chardigny, J. M.; Christie, W. W. Metabolites of conjugated isomers of linoleic acid (CLA) in the rat. *Biochim. Biophys. Acta* **1997**, *1345*, 5−10.
(9) Banni, S.; Carta, G.; Angioni, E.; Angioni, E.; Murru, E.; Scanu, P.; Melis, M. P.; Bauman, D. E.; Fischer, S. M.; Ip, C. Distribution of conjugated linoleic acid and metabolites in different lipid fractions in the rat liver. *J. Lipid Res.* **2001**, *42*, 1056−1061.
(10) Banni, S.; Martin, J.-C. Conjugated linoleic acid and metabolites. In *Trans Fatty Acids in Human Nutrition*; Sebedio, J. L., Christie, W. W., Eds.; Oily Press: Dundee, U.K., 1998; pp 261−302.
(11) Berdeaux, O.; Gnadig, S.; Chardigny, J. M.; Loreau, O.; Noel, J. P.; Sebedio, J. L. In vitro desaturation and elongation of rumenic acid by rat liver microsomes. *Lipids* **2002**, *37*, 1039−1045.
(12) Folch, J.; Lees, M.; Stanley, G. H. S. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.
(13) Sebedio, J. L.; Juaneda, P. Quantitative lipid analyses using the new Iatroscan TLC-FID system. *J. Planar. Chromatogr.−Mod. TLC* **1991**, *4*, 35−41.
(14) Juaneda, P.; Rocquelin, G. Rapid and convenient separation of phospholipids and non phosphorus lipids from heart using silica cartridges. *Lipids* **1985**, *20*, 4041.
(15) Shantha, N. C.; Decker, E. A.; Hennig, B. Comparison of methylation methods for the quantitation of conjugated linoleic acid isomers. *J. Assoc. Off. Anal. Chem.* **1993**, *76*, 644−649.
(16) Fay, L.; Richli, U. Location of double bonds in polyunsaturated fatty acids by gas chromatography−mass spectrometry after 4,4-dimethyloxazoline derivatization. *J. Chromatogr.* **1991**, *541*, 89−98.
(17) Garrido, J. L.; Medina, I. One-step conversion of fatty-acids into their 2-alkenyl-4,4-dimethyloxazoline derivatives directly from total lipids. *J. Chromatogr.* **1994**, *673*, 101−105.
(18) Dobson, G. Identification of conjugated fatty acids by gas chromatography mass spectrometry of 4-methyl-1,2,4-triazoline-3,5-dione adducts. *J. Am. Oil Chem. Soc.* **1998**, *75*, 137−142.
(19) Christie, W. W. Gas chromatography mass spectrometry methods for structural analysis of fatty acids. *Lipids* **1998**, *33*, 343−353.
(20) Berdeaux, O.; Wolff, R. L. Gas−liquid chromatography−mass spectrometry of the 4,4-dimethyloxazoline derivatives of delta-5-unsaturated polymethylene-interrupted fatty acids from conifer seed oils. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1323−1326.
(21) Sprecher, H. Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochim. Biophys. Acta* **2000**, *1486*, 219−231.
(22) Chardigny, J.-M.; Sebedio, J. L.; Grandgirard, A.; Martine, L.; Berdeaux, O.; Vatele, J.-M. Identification of novel trans isomers of 20:5n-3 in liver lipids of rats fed a heated oil. *Lipids* **1996**, *31*, 165−168.

Received for review October 29, 2004. Revised manuscript received December 21, 2004. Accepted December 23, 2004. We acknowledge financial support from the Natural Sciences and Engineering Council of Canada and the Fonds FCAR and CORPAQ (Gouvernement du Qubec). We are grateful to the Fondation de l'Université Laval for a Ph.D Scholarship to F.D.

JF0481958