

Fatty acid composition and conjugated linoleic acid content of different tissues in rats fed individual conjugated linoleic acid isomers given as triacylglycerols☆

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Abstract

Heat treatment of vegetable oils gave rise to four main conjugated linoleic acid (CLA) isomers : the 9c,11t, 9t,11t, 10t,12c and 10t,12t. The diet of male Wistar rats was supplemented with 150 mg/day either 9c,11t-, 9t,11t-, 10t,12c- or 10t,12t CLA isomers for 6 days and their effects on lipid composition were investigated in liver, heart, skeletal muscle *Gastrocnemius*, kidneys, brain and adipose tissue. The incorporation of all isomers was low (< 1.4%) and the level was as follows : adipose tissue > *Gastrocnemius* > liver, kidneys > brain. The main changes in the overall lipid composition were observed in skeletal muscle (*Gastrocnemius*) and in heart and were associated with feeding the 10t,12c and 10t,12t isomers. The diet enriched in 10t,12t CLA decreased the total long chain polyunsaturated fatty acid proportion in *Gastrocnemius* (from 18.4% to 14.4%) and increased that of 20:4 n-6 in heart (from 16.9 to 19.3%). The diet enriched in 10t,12c CLA decreased the monounsaturated fatty acid proportion in *Gastrocnemius* (from 32.0 to 26.1%) and produced an effect similar to the 10t,12t in heart. By contrast, the 9c,11t and 9t,11t isomers did not affect fatty acid composition in all tissues and organs. We concluded that ingestion of 10t,12c and 10t,12t CLA present in oils and in CLA mixtures could change muscle lipid composition. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: CLA isomers; Fatty acid composition; Muscle; Rats

1. Introduction

CLA consist of a group of positional and geometrical isomers that are derived from linoleic acid (9cis,12cis-18:2) or from octadecenoic acid by desaturation [1]. The main dietary sources of CLA are animal products from ruminants (3–4 mg/g in beef, 5–7 mg/g in milk and butter) even though CLA is also present in seed oils (0.01–0.12% of total fatty acids) [2,3]. During oil frying, the CLA content increases and can represent up to 0.5% of the total fatty acids depending on the heating temperature [3]. During heat treatment such as deodorization or frying, cis,trans/trans,cis (c,t/t,c) isomers can undergo isomerization and can be converted into trans,trans (t,t) isomers [4]. Although Chin *et al.* [2] reported that the main CLA isomers in commercial oils

were 9c,11t and 10t,12c (75–91% of total CLA), recent results from our laboratory showed that the main CLA isomers in commercial sunflower oil were trans,trans isomers. The 9t,11t and 10t,12t represented respectively 26.3–27.8% and 25.8–27.6% of the total CLA while their cis, trans and trans,cis homologues were present in lower proportion (7.7–12.4% of 9c,11t and 6.8–11.0% of 10t,12c) [3].

CLA exert a variety of effects in experimental animals including anticancer and antiatherogenic actions [5–8]. However, recent data obtained in mice suggested that CLA supplementation reduced adipose tissue by apoptosis and developed lipodystrophy [9]. This result was confirmed by our laboratory [10]. In addition to the diverse positive influence of CLA, many authors found some beneficial effects of dietary CLA on lipid metabolism such as body fat reduction with enhancement of lean body mass [11–13]. Sakono *et al.* [14] found that CLA influenced fatty acid metabolism not only in the liver but also in extra hepatic tissues. The CLA preparations experimented to date consisted mainly of two isomers present in similar amounts : 9c,11t and 10t,12c.

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Park *et al.* [15] showed that the 10t,12c isomer is involved in the mediation of many biochemical effects attributed to CLA (especially body composition). But no study was carried out with the 9t,11t or 10t,12t CLA isomers, which are the major isomers found in oils submitted to heat treatment (deodorization, frying). Hence, the aim of this study was to determine if feeding each trans,trans isomer (9t,11t and 10t,12t) compared to each cis,trans and trans,cis homologue (9c,11t and 10t,12c) to rats had any effect on lipid composition in some tissues and organs: liver, heart, skeletal muscle *Gastrocnemius*, perirenal adipose tissue, kidneys and brain. After feeding rats with 150 mg/day of individual CLA isomer for 6 days, we determined the total lipid content, the lipid composition and the fatty acid composition of total lipids in these tissues.

2. Materials and methods

2.1. Chemical

The solvents were provided by SDS (Peypin, France) and were distilled before use. All other chemicals were purchased by Sigma-Aldrich (Sigma Co., L'Isle d'Abeau-Chêne, France). The 9c,11t and the 10t,12c CLA were provided by Natural (Hovdebygda, Norway).

2.2. Preparation of 9t,11t and 10t,12t CLA isomers

9t,11t CLA isomer synthesis. 15 g of 9c,11t CLA methyl ester and 2.4 g of free p-toluenesulfonic acid were dissolved in 400 ml of dioxane and refluxed for 4 hr according to Snyder and Scholfield [16]. After the addition of 1L of sodium hydroxide in water (2N), the fatty acid methyl esters (FAME) were extracted with hexane. Gas liquid chromatography (GLC) analysis revealed the presence of many CLA isomers : 13.5% 9c,11t, 14.8% 9t,11c, 3.0% 9c,11c, 60.1% 9t,11t and 8.6% others. The 9t,11t CLA isomer was further purified by a set of low-temperature crystallization.

Low-temperature crystallization. A flask containing 13 g of the above isomerized FAME mixture dissolved in 130 ml of acetone was immersed in a large acetone bath. The temperature was gradually reduced to -30°C . The resulting crystals were filtered and transferred by washing with solvent at room temperature. The FAME were recovered from the crystals by evaporating the solvent on a rotary film evaporator. From 37 g of FAME mixture, 9.37 g of 94.5% pure 9t,11t CLA methyl esters were recovered. The impurities consisted of 1.3% 9c,12t and 9t,12t linoleic acid, 1.7% 9c,11t CLA, 1.4% 9t,11c CLA and 1.1% 9c,11c CLA.

10t,12t CLA isomer synthesis. For the synthesis of 10t,12t isomer from 10t,12c isomer, the same procedure as that described above for the 9t,11t was used. Before crystallization, the mixture of CLA isomers contained 15.1% 10c,12t, 14.3% 10t,12c, 2.9% 10c,12c, 65.9% 10t,12t and

1.8% others (non-conjugated 18:2 n-6 and c,t isomers). After crystallization, 8.9 g of 10t,12t CLA methyl esters were recovered (91.7% pure). The impurities consisted of 5.9% 10c,12t, 10t,12c, 0.7% 10c,12c and 1.7% of 18:2 n-6 isomers.

Analytical gas liquid chromatography. GLC analyses were performed using a Hewlett Packard HP 5890 Series II (Hewlett Packard Ltd, Wokingham, UK) equipped with a split/splitless injector and a flame ionization detector. The temperature of both the injector and detector was 250°C . Helium was the carrier gas. The FAME were analyzed using a BPX-70 capillary column (50 m x 0.33 mm i.d, 0.25 μm film thickness, SGE Ltd, Melbourne, Australia) under the following temperature program: 60°C (1 min), $20^{\circ}\text{C}/\text{min}$ to 170°C (25 min). Chromatographic data processing was performed with a Borwin workstation (JMBS Developments, Grenoble, France) including an acquisition interface, the software and a computer.

Preparation of triacylglycerols. Triacylglycerols of 9c,11t-, 9t,11t-, 10t,12c- or 10t,12t CLA were obtained by synthesis according to the method of Kodali *et al.* [17] adapted by Martin [18].

Animals and diet. Male Wistar rats (Centre d'Élevage DEPPE, Saint Doulchard, France) weighing 80–90 g were housed in individual stainless steel cages at constant temperature ($22 \pm 1^{\circ}\text{C}$) with a 12-h light-dark cycle (lights on 0h30–12h30 in order rats mainly ate in the afternoon). They were fed *ad libitum* a semi-synthetic diet (control diet) with the following composition : casein 180g/kg; corn starch 460 g/kg; sucrose 230 g/kg; cellulose 20 g/kg; mineral mixture 50 g/kg; vitamin mixture 10 g/kg; lipids 50g/kg as a mixture of sunflower oil, high oleic oil and linseed oil (79:13:8). After a 2-week adaptation period, they were fed daily in two steps. Firstly, at 2:00 p.m., they received 5 g of the experimental diet consisting of the control diet enriched with 150 mg of either the mixture of control oils, 9c,11t isomer (93% pure), 9t,11t isomer (93% pure), 10t,12c isomer (95% pure) or 10t,12t isomer (89% pure) as triacylglycerols for 6 days. Secondly, at 03:30 p.m., after finishing the meal, they were fed *ad libitum* the control diet. As 9t,11t and 10t,12t CLA isomers had to be synthesized, two independent experiments were carried out involving each a control group and two experimental groups (control 1, 9c,11t and 9t,11t; control 2, 10t,12c and 10t,12t).

Lipid analysis. At the end of the experimental period, the diet was withdrawn 12 h before sacrifice. The rats were anesthetized with isoflurane and exsanguinated. Heart, liver, kidneys, brain, *Gastrocnemius* muscle and perirenal adipose tissue were quickly removed and weighed. They were stored in chloroform-methanol (2:1) at -20°C until analysis. Lipids were extracted according to the method of Folch *et al.* [19]. Quantitative lipid class analysis was carried out by the Iatroscan TLC-FID system [20]. FAME were prepared from total lipids using sodium methoxide in methanol (1M) at room temperature followed by boron trifluoride in methanol (14%) according to the method of Glass [21]. The FAME

Table 1

Weight, lipid content, lipid and fatty acid composition of liver in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	Liver									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	10.9	11.5	10.9	0.62	0.43	9.1	9.2	9.0	0.90	0.43
Lipids (mg/g)	53.8	53.3	57.6	0.14	1.52	49.1	52.3	48.1	0.22	1.63
% of total lipids										
Phospholipids	69.2	70.7	65.1	0.45	3.02	74.9	68.4	73.6	0.29	2.90
Triacylglycerols	24.8	22.3	27.6	0.42	2.70	19.0	25.7	21.0	0.32	2.99
Diacylglycerols	0.5	0.6	0.8	0.22	0.12	0.6	0.4	0.5	0.14	0.05
Cholesterol	3.8	4.4	3.9	0.11	0.10	3.9	3.4	3.6	0.16	0.19
Cholesterol esters	1.7	2.0	2.6	0.39	0.42	1.6	2.1	1.3	0.07	0.23
% of total methyl esters										
16:0	20.4	19.5	21.7	0.09	0.59	18.7	20.4	19.7	0.31	0.79
18:0	18.3 ab	19.4 a	17.2 b	0.04	0.50	18.2	18.3	18.9	0.72	0.61
Others (1)	1.5 a	1.3 b	1.3 b	0.01	0.04	1.0 a	1.0 a	0.7 b	0.01	0.03
Saturated	40.2	40.2	40.2	0.94	0.29	37.9 b	39.7 a	39.3 a	0.03	0.42
16:1 (n - 9 + n - 7)	2.8	2.4	3.0	0.14	0.20	2.3	2.1	2.1	0.83	0.27
18:1 (n - 9 + n - 7)	20.1	18.0	20.0	0.38	1.09	18.3	19.7	17.8	0.48	1.11
Others (2)	1.0 a	0.9 b	0.8 c	0.0001	0.02	0.5	0.5	0.5	0.23	0.03
Monounsaturated	23.9	21.3	23.8	0.34	1.28	21.1	22.3	20.4	0.59	1.28
18:2 n - 6	8.6	8.3	7.7	0.23	0.37	8.2	7.5	7.5	0.54	0.47
9c, 11t	—	0.4	0.07	—	—	—	—	—	—	—
9t, 11t	—	0.03	0.3	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	0.2	—	—	—
10t, 12t	—	—	—	—	—	—	—	0.1	—	—
20:4 n - 6	18.2	19.5	18.0	0.35	0.70	21.2	18.4	20.6	0.15	0.96
22:4 + 22:5 n - 6	0.4	0.4	0.5	0.40	0.03	0.5	0.6	0.5	0.66	0.06
Others (3)	1.2	1.2	1.2	0.46	0.05	1.4	1.2	1.4	0.40	0.11
n - 6	28.4	29.8	27.8	0.39	0.98	31.3	27.9	30.1	0.18	1.18
18:3 n - 3	0.3 a	0.3 b	0.2 b	0.02	0.02	0.2	0.2	0.2	0.62	0.00
20:5 n - 3	0.6	0.5	0.4	0.88	0.04	0.7 a	0.4 b	0.5 ab	0.03	0.09
22:5 + 22:6 n - 3	5.9	7.2	6.9	0.12	0.41	8.2	9.1	9.0	0.10	0.28
n - 3	6.8	8.0	7.5	0.24	0.44	9.1	9.7	9.7	0.39	0.31
20:3 n - 9	0.5	0.5	0.5	0.99	0.05	0.4	0.3	0.4	0.47	0.07
Polyunsaturated	35.7	38.3	35.8	0.42	1.42	40.8	37.9	40.2	0.37	1.46
DMA	0.2	0.2	0.2	0.93	0.01	0.2 a	0.1 b	0.1 b	0.01	0.00

(1): 14:0 + 15:0 + 17:0 + 20:0 + 22:0 + 24:0; (2): 18:1trans + 20:1 + 22:1; (3): 18:3 + 20:2 + 20:3 + 5, 11, 14 20:3 n - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

were analyzed using a gas chromatograph (HP serie 5890 serie II) equipped with an autosampler and a fused silica capillary column (CPSil 88, 100 m long, 0.25 mm internal diameter, 0.20 μ m film thickness, Chrompack, Middleburg, The Netherlands). The splitless injection mode was used and the injector was heated at 250°C. The oven temperature was held at 60°C for 1min10, increased to 200°C at 20°C/min and then maintained at 200°C until the end of the analysis. The carrier gas was hydrogen. The flame ionization detector temperature was held at 280°C. Data were collected as described above.

Statistical analysis. Data are presented as means of 4 independent determinations. The ANOVA analysis were carried out using the Student-Newmann-Keuls test from SAS software. P values of less than 0.05 were considered significant.

3. Results

3.1. Animals

There was no effect of the CLA isomers on body weight. At the end of the experimental period, the mean weight of the rats was 247 g, 251 g and 243 g in the control 1, 9c,11t and 9t,11t groups, respectively, and 233 g, 222 g and 230 g in the control 2, 10t,12c and 10t,12t groups, respectively. The weights of liver, *Gastrocnemius*, kidneys, brain and adipose tissue were not significantly different (Tables 1, 3, 4, 5, 6), nor was the weight of heart in the 10-,12- groups (Table 2). The weight of heart was significantly lower in the 9t,11t group as compared to the 9c,11t and control 1 groups (0.81 versus 0.88–0.89 g).

Table 2

Weight, lipid content, lipid and fatty acid composition of heart in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	Heart									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	0.89 a	0.88 a	0.81 b	0.04	0.02	0.83	0.80	0.76	0.33	0.03
Lipids (mg/g)	25.6	26.0	28.0	0.87	1.67	29.9	29.1	30.0	0.87	1.35
% of total lipids										
Phospholipids	75.7	73.0	77.1	0.53	2.48	78.7	84.6	84.3	0.05	1.63
Triacylglycerols	19.5	21.4	17.1	0.51	2.57	16.8	11.0	11.4	0.05	1.59
Diacylglycerols	0.5	0.4	0.3	0.09	0.04	0.4	0.4	0.2	0.42	0.07
Cholesterol	4.3	5.2	5.5	0.04	0.14	4.1	4.0	4.1	0.75	0.18
% of total methyl esters										
16:0	12.4	12.5	11.9	0.41	0.36	12.2	12.4	11.8	0.50	0.38
18:0	18.1	17.9	18.9	0.30	0.44	18.4	19.4	19.7	0.12	0.40
Others (1)	3.2	3.2	3.2	0.78	0.05	3.0 b	3.3 a	3.2 a	0.02	0.05
Saturated	33.7	33.6	34.0	0.52	0.23	33.6	35.1	34.7	0.06	0.40
16:1 (n - 9 + n - 7)	1.7	1.7	1.4	0.38	0.18	1.6	1.1	1.1	0.09	0.17
18:1 (n - 9 + n - 7)	18.4	17.7	15.7	0.24	1.07	17.9 a	14.1 b	14.0 b	0.01	0.81
Others (2)	0.5	0.6	0.5	0.54	0.02	0.6	0.7	0.5	0.19	0.02
Monounsaturated	20.6	20.0	17.6	0.26	1.25	20.1 a	15.9 b	15.6 b	0.01	0.95
18:2 n - 6	16.9	16.7	16.9	0.91	0.48	16.0	14.7	15.5	0.58	0.81
9c, 11t	—	0.3	0.03	—	—	—	—	—	—	—
9t, 11t	—	0.03	0.3	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	0.3	—	—	—
10t, 12t	—	—	—	—	—	—	0.02	0.1	—	—
20:4 n - 6	16.7	17.0	17.9	0.34	0.58	16.9 b	18.7 a	19.3 a	0.04	0.55
22:4 + 22:5 n - 6	1.3	1.1	1.4	0.02	0.05	1.2	1.3	1.4	0.29	0.06
Others (3)	0.9	0.9	0.9	0.22	0.03	0.8	1.1	0.9	0.52	0.05
n - 6	35.8	36.0	37.4	0.34	0.81	34.9	36.1	37.2	0.13	0.71
18:3 n - 3	0.2	0.2	0.2	0.19	0.02	0.2	0.2	0.2	0.10	0.01
20:5 n - 3	0.2	0.2	0.2	0.08	0.01	0.2	0.2	0.2	0.63	0.02
22:5 + 22:6 n - 3	7.9	8.4	9.0	0.29	0.45	9.5	11.0	10.5	0.15	0.55
n - 3	8.3	8.8	9.4	0.32	0.44	9.9	11.4	10.9	0.16	0.54
20:3 n - 9	0.3	0.3	0.3	0.52	0.02	0.3	0.3	0.3	0.88	0.02
Polyunsaturated	44.4	45.1	47.1	0.26	1.11	45.1 b	47.8 a	48.4 a	0.04	0.82
DMA	1.3	1.3	1.3	0.68	0.05	1.2	1.2	1.3	0.58	0.05

(1): 14:0 + 15:0 + 17:0 + 20:0 + 22:0 + 24:0; (2): 18:1trans + 20:1 + 22:1; (3): 18:3 + 20:2 + 20:3 + 5, 11, 14 20:3 n - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

3.2. Lipid composition

The total lipid content in liver, heart, *Gastrocnemius*, kidneys, brain and adipose tissue was not affected by CLA treatments ($P > 0.05$) (Tables 1–6). Average lipid contents ranged from 48.1 to 57.6 mg/g in liver, 25.6 to 30.0 mg/g in heart, 17.3 to 24.6 mg/g in *Gastrocnemius*, 36.3 to 44.1 mg/g in kidneys, 59.0 to 74.7 mg/g in brain and 729.0 to 797.3 mg/g in adipose tissue. The lipid class distribution of liver and heart was not affected by the isomers (Tables 1 and 2). In *Gastrocnemius*, only the 10t,12c and 10t,12t affected the lipid class composition (Table 3). Lipids of the 10t,12c group contained significantly less triacylglycerols and more phospholipids, cholesterol and diacylglycerols than lipids of the 10t,12t group. But the content of lipid classes in the control 2 group was not significantly different from those of the 10t,12c and 10t,12t groups. In kidneys, the influence of CLA isomers was observed for the content of

cholesterol esters in the 10t,12c group which was significantly lower than that in the control 2 group and for the content of cholesterol in the 9c,11t group which was significantly higher than that in the control 1 and 9t,11t groups (Table 4). It was not determined in brain, which contains mainly phospholipids and cholesterol, and in adipose tissue which consists mostly of triacylglycerols.

3.3. CLA incorporation in total lipids

The level of CLA isomers was very low in all organs and tissues. The highest was reported in adipose tissue: 1.4 and 1.2% in the 9c,11t and 9t,11t groups, respectively, and 0.9 and 1.1% in the 10t,12c and 10t,12t groups, respectively, and the lowest in brain (only traces, $< 0.04\%$). The incorporation of 9c,11t isomer was similar in *Gastrocnemius* (0.54%), liver (0.42%) and kidneys (0.42%). It was significantly lower in the heart than in *Gastrocnemius* (0.35

Table 3

Weight, lipid content, lipid and fatty acid composition of *Gastrocnemius* in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	<i>Gastrocnemius</i>									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	1.5	1.6	1.7	0.24	0.05	1.3	1.3	1.4	0.41	0.05
Lipids (mg/g)	20.2	19.3	19.7	0.85	1.14	17.3	18.9	24.6	0.08	1.98
% of total lipids										
Phospholipids	55.5	48.9	51.4	0.18	2.30	56.7 ab	67.9 a	47.5 b	0.04	4.23
Triacylglycerols	40.5	46.4	43.7	0.30	2.52	39.4 ab	27.6 b	49.5 a	0.03	4.34
Diacylglycerols	0.6	0.6	0.6	0.57	0.05	0.5 b	0.6 a	0.4 c	0.001	0.02
Cholesterol	3.4	4.1	4.3	0.13	0.14	3.4 ab	3.9 a	2.6 b	0.02	0.24
% of total methyl esters										
16:0	21.6	21.4	21.5	0.87	0.30	20.6 b	21.5 b	22.9 a	0.01	0.40
18:0	8.8	8.7	9.1	0.63	0.30	9.8 a	10.9 a	8.3 b	0.004	0.36
Others (1)	3.4 b	3.8 a	3.6 ab	0.03	0.08	3.5 a	3.6 a	3.1 b	0.02	0.10
Saturated	33.8	33.9	34.2	0.78	0.44	33.9 b	36.0 a	34.3 b	0.02	0.39
16:1 (n - 9 + n - 7)	4.3	4.2	4.0	0.66	0.22	4.2 a	2.5 b	4.5 a	0.009	0.32
18:1 (n - 9 + n - 7)	29.7	29.1	27.3	0.34	1.15	27.3 b	23.2 c	31.5 a	0.006	1.22
Others (2)	0.5	0.5	0.5	0.82	0.01	0.5	0.4	0.5	0.09	0.02
Monounsaturated	34.5	33.8	31.8	0.35	1.29	32.0 a	26.1 b	36.5 a	0.004	1.40
18:2 n - 6	13.5	12.8	13.7	0.12	0.28	14.1 a	14.6 a	12.8 b	0.03	0.38
9c, 11t	—	0.5	0.04	—	—	—	—	—	—	—
9t, 11t	—	0.02	0.4	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	0.6	—	—	—
10t, 12t	—	—	—	—	—	—	0.03	0.5	—	—
20:4 n - 6	7.3	7.8	7.9	0.52	0.38	7.9 ab	9.1 a	6.3 b	0.01	0.50
22:4 + 22:5 n - 6	0.8	0.8	0.9	0.20	0.05	0.9 a	1.1 a	0.7 b	0.006	0.05
Others (3)	0.8	0.8	0.9	0.11	0.04	0.9	0.9	0.7	0.18	0.07
n - 6	22.4	22.7	23.8	0.26	0.62	23.8 ab	26.3 a	21.0 b	0.01	0.87
18:3 n - 3	0.7	0.6	0.6	0.51	0.04	0.6 a	0.4 b	0.7 a	0.07	0.04
20:5 n - 3	0.3	0.2	0.3	0.51	0.02	0.3	0.3	0.2	0.26	0.04
22:5 + 22:6 n - 3	7.4	7.8	8.3	0.44	0.45	8.4 a	9.7 a	6.5 b	0.002	0.39
n - 3	8.4	8.6	9.2	0.48	0.44	9.3 a	10.4 a	7.4 b	0.002	0.38
20:3 n - 9	0.3	0.3	0.3	0.63	0.02	0.3	0.4	0.3	0.10	0.03
Polyunsaturated	31.1	31.6	33.3	0.28	0.97	33.4 a	37.1 a	28.7 b	0.005	1.20
DMA	0.6	0.7	0.7	0.04	0.02	0.7 a	0.8 a	0.5 b	0.03	0.05

(1): 14:0 + 15:0 + 17:0 + 20:0 + 22:0 + 24:0; (2): 18:1trans + 20:1 + 22:1; (3): 18:3 + 20:2 + 20:3 + 5, 11, 14 20:3 n - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

versus 0.54%). There was no significant difference of incorporation of the 9t,11t isomer between *Gastrocnemius*, liver, kidneys and heart (0.43, 0.28, 0.35, 0.29%, respectively). In the 10t,12c and 10t,12t groups, a similar distribution was observed. The level of incorporation of each isomer was higher in *Gastrocnemius* than in heart, kidneys and liver (0.61% versus 0.34, 0.31 and 0.20%, respectively, in the 10t,12c group and 0.52 versus 0.14, 0.22 and 0.13%, respectively, in the 10t,12t group).

No metabolites of the CLA isomers were detected, probably because of the short-term feeding experiment.

3.4. Effects of CLA on fatty acid composition

The main effects were observed after feeding the 10t,12c or the 10t,12t-CLA-enriched diet in the heart and *Gastrocnemius* (Tables 2 and 3). In *Gastrocnemius*, the 10t,12t

CLA-enriched diet significantly increased the content of C16:0 (from 20.6 to 22.9%) and C18:1 (from 27.3 to 31.5%) and significantly decreased the content of C18:0 (from 9.8 to 8.3%), C18:2 n-6 (from 14.1 to 12.8%), C22:4+C22:5 n-6 (from 0.9 to 0.7%) and C22:5+C22:6 n-3 (from 8.4 to 6.5%). In heart, this diet significantly decreased the content of C18:1 (from 17.9 to 14.0%) and significantly increased that of C20:4 n-6 (from 16.9 to 19.3%). The 10t,12c CLA-enriched diet decreased the content of C18:1 in *Gastrocnemius* (from 27.3 to 23.2%) and in heart (from 17.9 to 14.1%). Moreover, this diet decreased the content of C16:1 in *Gastrocnemius* (from 4.2 to 2.5%) and increased that of C20:4 n-6 in heart (from 16.9 to 18.7%).

Feeding 9c,11t or 9t,11t isomers had no significant effect on fatty acid composition in heart and *Gastrocnemius*. In liver, kidneys, brain and adipose tissue, minor and inconsistent changes were observed (Tables 1, 4–6).

Table 4

Weight, lipid content, lipid and fatty acid composition of kidneys in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	Kidneys									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	2.0	2.1	2.0	0.82	0.05	1.8	1.9	1.9	0.92	0.08
Lipids (mg/g)	44.4	39.1	38.1	0.06	1.72	37.3	40.6	36.3	0.19	1.57
% of total lipids										
Phospholipids	65.4	67.1	68.8	0.46	1.83	74.5	70.4	69.9	0.13	1.51
Triacylglycerols	26.5	23.0	22.5	0.31	1.86	17.0	20.8	20.8	0.24	1.68
Diacylglycerols	0.5	0.6	0.4	0.29	0.05	0.3	0.4	0.5	0.13	0.06
Cholesterol	6.9 b	9.0 a	7.6 b	0.01	0.34	7.2	8.1	8.1	0.30	0.39
Cholesterol esters	0.7	0.3	0.7	0.44	0.21	1.0 a	0.3 b	0.7 ab	0.05	0.17
% of total methyl esters										
16:0	22.6	21.7	22.1	0.21	0.35	21.1 b	24.0 a	22.8 ab	0.01	0.53
18:0	12.9	13.6	13.3	0.30	0.31	14.3	13.3	13.8	0.10	0.29
Others (1)	3.5	3.3	3.4	0.32	0.06	2.5	2.7	2.8	0.12	0.09
Saturated	39.0	38.6	38.8	0.58	0.23	37.9 b	40.0 a	39.4 a	0.03	0.44
16:1 (n - 9 + n - 7)	3.7	3.4	3.4	0.57	0.25	2.9	2.7	3.0	0.83	0.29
18:1 (n - 9 + n - 7)	24.7	22.6	23.0	0.22	0.83	22.5	21.7	21.8	0.76	0.71
Others (2)	0.5	0.5	0.5	0.19	0.01	0.5	0.6	0.5	0.34	0.02
Monounsaturated	28.9	26.5	26.9	0.25	1.04	25.9	25.0	25.3	0.78	0.93
18:2 n - 6	7.7	7.4	7.7	0.50	0.17	7.5	7.9	7.3	0.17	0.21
9c, 11t	—	0.4	0.02	—	—	—	—	—	—	—
9t, 11t	—	0.01	0.3	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	0.3	0.02	—	—
10t, 12t	—	—	—	—	—	—	0.02	0.2	—	—
20:4 n - 6	18.8	21.3	20.5	0.12	0.78	22.7	21.2	22.0	0.40	0.78
22:4 + 22:5 n - 6	0.5	0.6	0.6	0.26	0.02	0.7	0.6	0.6	0.56	0.03
Others (3)	1.0	1.0	1.0	0.07	0.03	1.1	0.9	1.1	0.20	0.04
n - 6	28.0	30.7	30.1	0.14	0.89	32.0	30.9	31.2	0.72	0.96
18:3 n - 3	0.3	0.3	0.3	0.14	0.02	0.3	0.3	0.2	0.29	0.01
20:5 n - 3	0.3	0.3	0.3	0.36	0.02	0.4	0.3	0.3	0.65	0.03
22:5 + 22:6 n - 3	2.2	2.3	2.3	0.05	0.06	2.6	2.7	2.7	0.69	0.10
n - 3	2.8	2.9	2.9	0.33	0.06	3.3	3.3	3.2	0.68	0.10
20:3 n - 9	0.2	0.2	0.2	0.56	0.01	0.2	0.2	0.2	0.78	0.02
Polyunsaturated	31.0	33.8	33.2	0.14	0.94	35.5	34.4	34.6	0.16	1.00
DMA	1.1	1.1	1.1	0.79	0.05	0.7	0.6	0.7	0.09	0.03

(1): 14:0 + 15:0 + 17:0 + 20:0 + 22:0 + 24:0; (2): 18:1trans + 20:1 + 22:1; (3): 18:3 + 20:2 + 20:3 + 5, 11, 14 20:3 n - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

4. Discussion

The low content of CLA isomers in all the organs studied here may be due to the low incorporation of these isomers. The greatest incorporation was observed in adipose tissue (0.9–1.4%). This was also noticed by Yamasaki *et al.* [22] who found that the CLA content in white adipose tissue was near 5-fold higher than in liver after feeding 2% CLA in the diet. Tissues with high phospholipid content (liver, heart, skeletal muscle, and kidneys) incorporated low content of CLA. Hence, CLA may be mainly incorporated in triacylglycerols. The total lipids from the skeletal muscle contained significantly higher level of CLA isomers than those of heart, liver and kidneys. Park *et al.* [12] also observed that lipids from skeletal muscles of rats fed either with 9c,11t or 10t,12c CLA isomer for 7 weeks contained more CLA isomer than the lipids of liver.

In our study, the fatty acid composition of *Gastrocnemius* was the most affected by individual CLA isomer feeding, followed by that of the heart and then by those of liver, kidneys, adipose tissue and brain. This is in good agreement with the results of Park *et al.* [11] who found that feeding mice a diet supplemented with CLA enhances fatty acid β -oxidation in skeletal muscles and fat pad but not in liver. However, Sakono *et al.* [14] found an enhanced β -oxidation in the liver of rats fed CLA mixture and Martin *et al.* [23] did not find any effect of the 9c,11t and 10t,12c isomers on lipid metabolizing enzymes in liver, muscle and heart in rat. But these studies were long-term feeding ones (2 and 6 weeks, respectively) compared to our short-term experiment (6 days).

The contents of 10t,12c and 10t,12t isomers tend to be lower than those of 9c,11t and 9t,11t isomers in the liver, heart and kidneys. We could hypothesize that in these or-

Table 5

Weight, lipid content and fatty acid composition of brain in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	Brain									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	1.87	1.98	1.95	0.05	0.03	1.69	1.77	1.85	0.09	0.04
Lipids (mg/g)	74.7	72.1	70.6	0.76	3.87	59.0	66.4	69.9	0.23	4.20
% of total methyl esters										
15:0	2.7	2.8	2.7	0.08	0.03	2.4	2.5	2.4	0.79	0.06
16:0	17.8 ab	17.6 b	18.0 a	0.04	0.08	18.3	18.3	18.4	0.92	0.20
17:0	5.1	5.1	5.0	0.49	0.04	3.4	3.6	3.8	0.07	0.09
18:0	17.6	17.6	17.7	0.72	0.05	17.7	17.6	17.4	0.22	0.10
Others (1)	1.2	1.3	1.2	0.14	0.03	1.2	1.3	1.2	0.36	0.07
Saturated	44.4	44.4	44.6	0.26	0.06	43.0	43.3	43.2	0.30	0.16
16:1 (n - 9 + n - 7)	0.6	0.6	0.6	0.63	0.01	0.6	0.5	0.6	0.19	0.03
18:1 (n - 9 + n - 7)	21.2	21.2	21.1	0.59	0.05	21.6	21.2	21.6	0.43	0.25
Others (2)	2.8	2.9	2.7	0.11	0.08	2.8	2.9	2.9	0.99	0.08
Monounsaturated	24.6	24.7	24.4	0.13	0.11	25.0	24.6	25.1	0.47	0.30
18:2 n - 6	0.7	0.7	0.6	0.08	0.01	0.7	0.7	0.8	0.53	0.03
9c, 11t	—	0.03	—	—	—	—	—	—	—	—
9t, 11t	—	—	0.01	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	—	—	—	—
10t, 12t	—	—	—	—	—	—	—	—	—	—
20:4 n - 6	9.3	9.3	9.3	0.87	0.05	9.8 a	9.5 b	9.5 b	0.02	0.08
22:4 + 22:5 n - 6	3.3	3.3	3.3	0.93	0.03	3.4	3.4	3.4	0.69	0.04
Others (3)	0.8	0.9	0.9	0.94	0.02	1.0	1.0	1.0	0.57	0.04
n - 6	14.1	14.2	14.1	0.94	0.07	14.9	14.6	14.7	0.11	0.96
18:3 n - 3	0.01	0.02	0.01	0.40	0.01	0.01	0.01	0.01	0.70	0.11
20:5 n - 3	0.02	0.02	0.02	0.40	0.00	0.02	0.01	0.02	0.22	0.00
22:5 + 22:6 n - 3	13.3	13.1	13.4	0.19	0.11	14.2	14.5	13.9	0.23	0.23
n - 3	13.3	13.1	13.4	0.19	0.10	14.2	14.5	13.9	0.24	0.24
20:3 n - 9	0.1	0.1	0.1	0.83	0.005	0.1	0.1	0.1	0.76	0.02
Polyunsaturated	27.5	27.4	27.6	0.37	0.14	29.2	29.2	28.7	0.41	0.28
DMA	3.5	3.5	3.4	0.35	0.05	2.8	2.9	3.0	0.27	0.07

(1): 14:0 + 20:0 + 24:0; (2): 18:1trans + 20:1 + 22:1 + 24:1; (3): 18:3 + 20:2 + 20:3 + 5, 11, 14 20:3 n - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

gans, the 10t,12c and 10t,12t isomers might be more efficiently metabolized by the cells than their 9,11 homologues. Intracellular fatty acids are destined to be esterified into lipids or to undergo β -oxidation. Martin *et al.* [23] found that the 10t,12c isomer was preferentially driven through the β -oxidation pathway compared to the 9c,11t homologue.

The 10t,12c and 10t,12t CLA isomers induced the major changes whereas the 9c,11t and 9t,11t isomers did not influence the fatty acid composition. Park *et al.* [24] explained that the lack of effect was probably due to the position of the double bond. Indeed, a 12-double bond appears to be a key structure for inhibiting stearoyl-CoA desaturase activity, especially when coupled with a 10-double bond, but not with a 9-double bond [24].

In *Gastrocnemius*, the effect of the 10t,12c isomer was slightly different from that of the 10t,12t isomer. Feeding 10t,12t CLA decreased the content of long chain polyunsaturated fatty acids (known to be incorporated mainly in phospholipids) and increased the content of monounsaturated fatty acids (known to be incorporated mainly in tri-

acylglycerols). Two hypothesis could be put forward to explain these changes : (i) these changes in fatty acid profile may just mirror the higher (but non significant) synthesis of triacylglycerols in *Gastrocnemius* of rats fed 10t,12t CLA; (ii) these changes could be attributed to an effect of the 10t,12t isomer on the $\Delta 6$ desaturation. Fatty acid profiles of lipid fractions may provide information whether the changes in fatty acids were merely due to an increase in triacylglycerols in total lipids. Feeding 10t,12c CLA decreased the content of monounsaturated fatty acids. This is consistent with the results obtained *in vitro* by Bretillon *et al.* [25] and those of Park *et al.* in mice [24] showing that the 10t,12c was the isomer responsible for the modification of saturated and monounsaturated levels in tissues from animals fed a CLA mixture by decreasing the $\Delta 9$ desaturation of stearic acid. However, it could also reflect the non-significant decrease of triacylglycerols and the concomitant increase of phospholipids in *Gastrocnemius* of rats fed 10t,12c CLA. Indeed, this effect of 10t,12c was also described in HepG2 cells [26]. This could be related to the

Table 6

Weight, lipid content and fatty acid composition of perirenal adipose tissue in rats fed a semi-synthetic diet containing 5% lipids enriched with 150 mg of either TG-control oil (control) or TG-CLA isomers for 6 days

	Adipose tissue									
	Control 1	9c, 11t	9t, 11t	Statistics		Control 2	10t, 12c	10t, 12t	Statistics	
	n = 4	n = 4	n = 4	P value	SE	n = 4	n = 4	n = 4	P value	SE
Weight (g)	0.9	1.2	1.0	0.38	0.14	1.0	1.1	1.0	0.99	0.16
Lipids (mg/g)	729.0	733.1	761.9	0.26	13.91	797.3	750.3	784.5	0.24	18.92
% of total methyl esters										
16:0	24.8	25.0	25.2	0.74	0.38	25.1 b	27.4 ab	28.3 a	0.04	0.79
18:0	3.6 a	3.4 a	3.2 b	0.01	0.07	3.9	3.5	3.8	0.35	0.19
Others (1)	2.1	2.2	2.3	0.21	0.05	2.0 b	2.2 a	2.2 a	0.01	0.04
Saturated	30.5	30.6	30.7	0.94	0.47	31.0	33.1	34.3	0.08	0.92
16:1 (n - 9 + n - 7)	7.5	7.5	7.6	0.98	0.49	7.3	6.5	7.1	0.24	0.32
18:1 (n - 9 + n - 7)	51.3	50.0	50.0	0.30	0.61	51.3	49.7	48.0	0.06	0.87
Others (2)	0.8	0.8	0.7	0.60	0.06	0.7	0.7	0.6	0.28	0.02
Monounsaturated	59.6	58.3	58.3	0.13	0.31	59.3 a	56.9 b	55.7 b	0.008	0.64
18:2 n - 6	8.2	8.0	8.2	0.78	0.18	8.0	7.7	7.4	0.41	0.29
9c, 11t	—	1.45	—	—	—	—	—	—	—	—
9t, 11t	—	0.05	1.2	—	—	—	—	—	—	—
10t, 12c	—	—	—	—	—	—	0.9	0.04	—	—
10t, 12t	—	—	—	—	—	—	0.05	1.1	—	—
20:4 n - 6	0.1	0.1	0.1	0.62	0.02	0.1 a	0.1 b	0.1 b	0.002	0.00
Others (3)	0.2	0.2	0.2	0.05	0.003	0.2	0.1	0.1	0.17	0.01
n - 6	8.5 b	9.8 a	9.7 a	0.002	0.18	8.3	8.8	8.7	0.30	0.29
18:3 n - 3	1.2 a	1.1 b	1.1 b	0.02	0.02	1.2 a	1.0 b	1.1 b	0.01	0.05
20:3 n - 9	0.1	0.1	0.1	—	—	0.1	0.1	0.1	—	—
Polyunsaturated	9.8 b	11.0 a	10.9 a	0.007	0.20	9.6	9.9	9.9	0.64	0.31
DMA	0.1	0.1	0.1	0.11	0.00	0.1	0.1	0.1	0.61	0.00

(1): 14:0 + 15:0 + 17:0 + 20:0 + 22:0 + 24:0; (2): 18:1trans + 20:1 + 22:1; (3): 18:3 + 20:2 + 20:3 - 6.

TG: triacylglycerols; SE: standard error; DMA: dimethylacetals.

Values were compared using the GLM procedure from SAS software. The means with different letters differ significantly ($P < 0.05$).

decrease in $\Delta 9$ desaturation as it was shown that triacylglycerol secretion was highly dependent on the $\Delta 9$ desaturase activity in cultured chicken hepatocytes [27].

The unchanged content in total lipids and fatty acid composition in liver, kidneys and brain of rats fed 10t,12c or 10t,12t could be explained by the very short experimental period during which rats received CLA-isomers (only 6 days). Moreover, it was already noticed that Wistar rats appeared to be poorly responsive to CLA diets towards lipid-metabolizing enzymes [23].

Feeding 9c,11t or 9t,11t had no effect on lipid composition in all organs and tissues in our study. This is in good agreement with results of Park *et al.* [15] who found a minor role for 9c,11t isomer in mediating many of the biochemical effects attributed to CLA.

In conclusion, this study revealed that in rats, during a supplementation of 150 mg/day of 9c,11t or 9t,11t or 10t,12c or 10t,12t isomers for 6 days, the major changes occurred in muscles (*Gastrocnemius* and heart) and changes in lipid composition were associated with the 10t,12c and 10t,12t isomers. We could hypothesize that in CLA mixture, these two CLA isomers may be responsible for lipid composition changes. The geometry of the 12 double bond may be of importance since feeding CLA with a cis12 or a trans12 double bond did not have the same effect in muscle.

Until now, only the 10t,12c was studied. As the 10t,12t was the more prominent in commercial oils, more emphasis should be addressed to this isomer. More research is needed to determine the metabolic pathways by which the isomers affected lipid composition, in particular in muscles and to extend the experimental period to 3 weeks to detect any possible effects in other organs.

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