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Journal of Nutritional Biochemistry 15 (2004) 741–748
Journal of Nutritional Biochemistry

Isomers of conjugated linoleic acid decrease plasma lipids and stimulate adipose tissue lipogenesis without changing adipose weight in post-prandial adult sedentary or trained Wistar rat

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Received 10 December 2003; received in revised form 9 July 2004; accepted 12 July 2004

Abstract

The respective effects and interactions of supplementation with two conjugated linoleic acid (CLA) isomers and exercise on plasma metabolic profile, activity of lipogenic enzymes and cellularity in two adipose tissue sites, those of the liver and heart, were examined in adult Wistar rats. Rats that were either sedentary or exercise-trained by treadmill running were fed one of four diets: a diet without CLA; a diet with either 1% *cis* 9, *trans* 11 CLA or 1% *trans* 10, *cis* 12 CLA; or a mixture of both isomers (1% of each) for 6 weeks. We observed that the exercise decreased lipogenic enzyme activities in epididymal and perirenal adipose tissue. Plasma cholesterol, insulin, and leptin concentrations were lower in exercise-trained rats than in sedentary rats. The ingestion of either CLA mixture or the *trans* 10, *cis* 12 CLA increased lipogenic enzyme activities in epididymal tissue and more markedly in perirenal adipose tissue, especially in sedentary rats, and without affecting adipose tissue weight or cellularity. A similar effect of *trans* 10, *cis* 12 CLA was observed in regard to malic enzyme activity in the liver. In addition, this isomer decreased plasma lipid and urea concentrations and increased plasma 3-hydroxybutyrate levels. The ingestion of *cis* 9, *trans* 11 CLA increased fatty acid synthase activity in perirenal adipose tissue in sedentary rats and decreased plasma cholesterol and leptin concentrations. These results show that isomers of CLA decrease plasma lipids and stimulate adipose tissue lipogenesis without changing adipose weight in adult sedentary or exercise-trained rat, thus suggesting a stimulation of adipose tissue turnover.
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Keywords: CLA isomers; Exercise; Lipid metabolism; Adipose tissue; Liver; Rat

1. Introduction

Conjugated linoleic acid (CLA) is a mixture of positional and geometric isomers of linoleic acid in which the double bonds are conjugated. Two of these CLA isomers, specifically, *cis* 9, *trans* 11 (*c*9,*t*11 CLA) and *trans* 10, *cis* 12 (*t*10,*c*12 CLA), are known to possess several physiological effects such as enhancing immune response, reducing arteriosclerosis risk, and inhibiting carcinogenesis in animal models [1]. In addition, several in vivo studies on the metabolic effects of CLA in growing animals showed that the ingestion of a mixture of CLA isomers affected body composition (including less body fat and more lean mass) in different species including mice [2–4], Zucker rats [5,6], pigs [7,8], and hamsters [9]. Moreover, in vitro studies on cultured 3T3-L1 adipocytes showed that these effects were specifically due to the *t*10,*c*12 CLA isomer that reduced lipoprotein lipase (LPL) activity, intracellular triacylglycerol, and glycerol and that enhanced glycerol release into medium [2,10]. CLA thus may reduce body fat directly by modifying the activity of key enzymes and processes involved in lipid mobilization and storage both in adipose tissue (AT) and liver [2,11,12]. To our knowledge, there is

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doi:10.1016/j.jnutbio.2004.07.004

no published work on the respective effects in vivo of either *c*9,*t*11 CLA or *t*10,*c*12 CLA isomers on lipid metabolism in adult animals. Endurance training is also known to decrease fat mass and to increase lean body mass; a synergistic action between exercise and CLA consumption on fat and lean body mass could be expected. Thus, the purpose of the present study was to investigate the respective effects and interactions of supplementation with either *c*9,*t*11 CLA and/or *t*10,*c*12 CLA for 6 weeks, and exercise, on lipogenic enzyme activities and cellularity of AT and liver in adult Wistar rats. Changes in blood plasma metabolites and hormones and in heart LPL activity were also measured.

Table 1
Lipid composition of experimental diets

	Diet			
	Control	*c*9,*t*11 CLA*	*t*10,*c*12 CLA†	CLA mixture
Ingredient (mg/100 g lipids)				
High oleic sunflower oil	98.4	88.4	88.4	78.4
Linseed oil	1.6	1.6	1.6	1.6
*c*9,*t*11 CLA	0	10	0	10
*t*10,*c*12 CLA	0	0	10	10
Fatty acid‡				
14:0	0.20	0.18	0.18	0.16
16:0	6.02	5.42	5.50	4.90
18:0	4.31	3.88	3.91	3.48
18:1 n-9	68.31	61.70	61.43	54.82
18:2 n-6	20.29	18.25	18.25	16.21
18:3 n-3	0.87	0.87	0.87	0.87
18:2 *c*9,*t*11*		9.12	0.30	9.42
18:2 *c*9,*c*11§		0.06		0.06
18:2 *t*10,c12†		0.35	9.41	9.77
18:2 *t*,*t*¶		0.17	0.11	0.28

* *Cis* 9, *trans* 11 conjugated linoleic acid (CLA).
† *Trans* 10, *cis* 12 CLA.
‡ Expressed as number of carbons : number of double bonds, and given in mg/100 g of fatty acid.
§ *Cis* 9, *cis* 11 CLA.
¶ *Trans*, *trans* CLA.

2. Methods and materials

2.1. Animals, diets, and exercise

A total of 48 male Wistar rats (17 weeks of age, weighing 465 ± 22 g), purchased from IFFA CREDO (L'Arbresle, France) were housed in individual wire-bottomed cages in a temperature-controlled room (21°C) with 12-h light–dark cycle (light on from 20:00 to 08:00). The institution's guide for the care and use of laboratory animals was used. During the first week (adaptation period), rats were fed ad libitum a semi-liquid diet (control diet) containing (g/kg): corn starch 430, sugar 210, chlorhydric casein 180, cellulose 20, fat 100 (high-oleic sunflower oil 98.4 and linseed oil 1.6), mineral mix 50 and vitamin + DL-methionine 10 mix [13]. Water was provided ad libitum. The 48 rats were then divided into two groups. The first group (exercise-trained rats, $n = 24$) was exercised by treadmill running. They were progressively adapted for the first week to run for 1 hour at approximately 22 $m.min^{-1}$ (i.e., no more than 50% VO_2 max). Then they were trained for 6 days per week for 6 weeks during the dark period (at 15:00). The other group (sedentary rats, $n = 24$) was not exercised. In each group, six animals were assigned to one of the four dietary groups: a diet without CLA (control diet), a diet with either 1% *c*9,*t*11 CLA or 1% *t*10,*c*12 CLA, and a diet with 1% *c*9,*t*11 and 1% *t*10,*c*12 CLA (CLA mixture) (Table 1). The diets were fed in a semi-liquid form ad libitum at 8:00 AM for 6 weeks. The CLA isomers were provided by Natural Lipids (Industriveien, 6160 Hovdebygda, Norway) as triacylglycerols (>90% of total lipids) with >90% of fatty acids as either *c*9,*t*11 C18:2 or *t*10,*c*12 C18:2 and were substituted to 10 (for *c*9,*t*11 or *t*10,*c*12 CLA) or 20 (CLA mixture) g/100 g of lipids (Table 1). Fatty acids from oils were esterified using sodium methylate (0.5 N). The resulting fatty acid methyl esters were then analyzed by gas chromatography using an 5890 sere II chromatograph (Hewlett Packard, Palo Alto, CA) fitted with a split-splitless injector (250°C) and an flame ionization detector (FID) (280°C). The column was a CP Sil 88 (100 m × 0.25 mm inner diameter, 0.2 μm film thickness; Varian S.A, Les Ulis, France). After injection in the splitless mode, the oven temperature was programmed from 60°C to 190°C at 20°C/min, and maintaining during 65 minutes. Hydrogen was used as carrier gas (0.7 mL/min at 60°C). Quantitative analyses were performed using a Borwin integrated system (JMBS, Grenoble, France). At the end of the experiment, rats were anesthetised with sodium pentobarbital (40 mg/kg of body weight, i.p.) 5 hours after feeding. Blood was collected by exsanguination into tubes containing EDTA and centrifuged. Plasma was collected and frozen at −20°C until analysis (non-esterified fatty acid (NEFA), triglycerides, total and free cholesterol, glycerol, acetate, 3-hydroxybutyrate, urea, lactate, glucose, insulin, and leptin). Perirenal and epididymal AT, liver, and heart were quickly excised, weighed and immediately placed at 37°C for adipocyte volume determination or frozen in liquid nitrogen and stored at −80°C until analysis of lipogenic enzyme and LPL activities.

Two rats that died for undetermined reasons were withdrawn from the study and not replaced. One was fed the control diet in the exercised group, the other was fed diet *t*10,*c*12 CLA in the sedentary group.

2.2. Plasma metabolites and hormones

Metabolites were determined by enzymatic assays with an ELAN auto-analyzer [14]. Urea concentration was analyzed a diagnostic kit from Merck (Chennevières-Lès-Louvres, France). Total and free cholesterol concentrations were analyzed with the Biotrol Diagnostic kit (Chennevières-Lès-Louvres, France). Free glycerol concentration was analyzed with a diagnostic kit from Sigma (St. Louis, MO). Triglycerides (total−free glycerol) were analyzed with the Bio Mérieux kit (Marcy-L'Etoile, France). NEFA concentration was analyzed with the

Wako-N.E. UnipathFA-C kit (Oxoid, Dardilly, France). Acetate concentration was analyzed with the Boehringer Mannheim/R-Biopharm kit (Darmstadt, Germany). Lactate concentration was analyzed with the Bio Mérieux kit (Marcy-L'Etoile, France). Glucose concentration was analyzed with the Merck-Clévenot Kit (Nogent-sur-Marne, France). Concentrations of 3-hydroxybutyrate was analyzed using the 3-hydroxybutyrate dehydrogenase method [15].

Plasma concentration of leptin was assayed using a commercial RIA kit for rats (Linco Research, St. Charles, MO), with a guinea pig anti-rat leptin antibody, rat [^{125} I] leptin, and rat leptin as standard. As recommended by the manufacturer, quantification was carried out in 100 μL of plasma, and all samples were tested in duplicate. The within-assay and between-assays variations were 3.3% and 5.7%, respectively.

Plasma concentration of insulin was assayed using a porcine commercially RIA kit (Cis Bio International, Gif-sur-Yvette, France). This analysis was performed using a guinea pig anti-porcine insulin antibody, porcine [^{125} I] insulin, and porcine insulin as standard. The anti-insulin antiserum showed 90% of cross-reactivity with rat insulin. As recommended by the manufacturer, quantification was carried out in 100 μL of plasma and all samples were tested in duplicate. The within-assay and between-assays variations were 5.4% and 6.4%, respectively.

2.3. Tissue measurements

The LPL activity was measured in epididymal and perirenal AT and in cardiac muscle using an artificial emulsion containing ^{3}H-triolein after a detergent (Deoxycholate-Nonidet P_{40}, Sigma Chemical, Saint-Quentin-Fallavier, France) extraction procedure [16]. The glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), fatty acid synthase (FAS), and glycerol-3-phosphate dehydrogensase (G3PDH) activities were assayed spectrophotometrically in epididymal and perirenal AT and in liver as previously described [17].

Enzyme activities were expressed either as nanomoles of released fatty acids (LPL) or as nanomoles of reduced (G6PDH, ME) or oxidized (FAS, G3PDH) nucleotides per minute and per gram of tissue, per total weight of tissue and on a cellular basis, i.e., per 10^6 adipocytes, for AT.

Adipose tissue cellularity was measured on tissue fixed with osmium tetroxide and digested in urea solution [18].

Table 2
Effects of conjugated linoleic acid on plasma metabolites, insulin and leptin in sedentary and exercise-trained rats*

	Sedentary rats					Exercise-trained rats				
	Control	*c*9,*t*11 CLA†	*t*10,*c*12 CLA‡	CLA mixture	(PSE)§	Control	*c*9,*t*11 CLA	*t*10,*c*12 CLA	CLA mixture	(PSE)¶
Urea (mmol/L)‖	7.61	7.67	7.79	6.98	(0.32)	8.00^{a}	7.56^{ab}	6.86^{b}	7.06^{ab}	(0.32)
Total cholesterol (mmol/L)#	3.36	3.07	2.79	2.83	(0.23)	2.98^{a}	2.77^{ab}	2.94^{a}	2.40^{b}	(0.17)
Free cholesterol (mmol/L)**	1.09	1.06	0.82	0.89	(0.12)	1.02^{ab}	1.08^{a}	0.96^{ab}	0.71^{b}	(0.11)
Triglycerides (mmol/L)††	3.48^{ab}	3.94^{a}	2.58^{ab}	2.40^{bc}	(0.46)	3.41^{ab}	4.50^{b}	2.47^{ac}	2.44^{ad}	(0.56)
Free glycerol (mmol/L)	0.64	0.59	0.63	0.61	(0.05)	0.61	0.63	0.60	0.47	(0.06)
NEFA (mmol/L)	0.18	0.17	0.19	0.15	(0.02)	0.17	0.14	0.18	0.15	(0.02)
3-Hydroxybutyrate (mmol/L)‡‡	0.14^{a}	0.17^{ab}	0.19^{b}	0.17^{ab}	(0.01)	0.14	0.15	0.20	0.17	(0.04)
Acetate (mmol/L)	0.05	0.04	0.10	0.10	(0.03)	0.08	0.06	0.06	0.06	(0.01)
Lactate (mmol/L)§§	6.62	6.85	6.52	5.86	(0.83)	4.84	8.94	7.15	5.96	(1.39)
Glucose (mmol/L)	13.6	13.3	14.0	13.0	(0.40)	13.0	13.4	13.2	13.3	(0.65)
Insulin (pmol/L)¶¶	1183	1000	1625	1222	(258)	986	1122	891	789	(129)
Leptin (nmol/L)‖‖	2.11^{a}	1.81^{bc}	1.88^{ac}	1.70^{c}	(0.09)	1.49	1.61	1.63	1.30	(0.15)

a, b, c, d Mean values within a row and within sedentary or exercise-trained rats with unlike superscipts letters are significantly different ($P<0.05$).
* Values represent mean for $n=5$ or 6 per diet group.
† *Cis* 9, *trans* 11 conjugated linoleic acid (CLA).
‡ *Trans* 10, *cis* 12 CLA.
§ PSE=pooled standard error for the sedentary rats.
¶ PSE=pooled standard error for the exercise-trained rats.
‖ Effects of *t*10,*c*12 ($P<0.02$) and exercise (E) × *c*9,*t*11 × *t*10,*c*12 interaction ($P<0.10$).
\# Effect of E ($P<0.10$), *c*9,*t*11 ($P<0.10$), and *t*10,*c*12 ($P<0.04$).
** Effect of *t*10,*c*12 ($P<0.01$).
†† Effect of *t*10,*c*12 ($P<0.01$).
‡‡ Effect of *t*10,*c*12 ($P<0.07$).
§§ Effect of *c*9,*t*11 × *t*10,*c*12 interaction ($P<0.07$).
¶¶ Effect of E ($P<0.05$) and E × *t*10,*c*12 interaction ($P<0.08$).
‖‖ Effect of E ($P<0.01$) and *c*9,*t*11 ($P<0.07$).

Table 3
Effects of conjugated linoleic acid on tissue weight and cellularity, lipogenic enzyme, and lipoprotein lipase activities in perirenal adipose tissue from sedentary and exercise-trained rats*

	Sedentary rats					Exercise-trained rats				
	Control	c9,t11 CLA†	t10,c12 CLA‡	CLA mixture	(PSE)§	Control	c9,t11 CLA	t10,c12 CLA	CLA mixture	(PSE)¶
Weight (g)‖	20.6	22.0	22.2	21.7	(2.22)	16.2	17.1	18.7	15.9	(1.60)
Adipose volume (pl)#	894	937	848	471	(122)	803	827	910	859	(129)
LPL (μmol/min/AT)**	9.14^{a}	9.76^{a}	14.5^{b}	14.9^{b}	(1.58)	6.56	8.97	9.59	10.2	(1.30)
G6PDH (μmol/min/AT)††	10.4^{a}	11.9^{a}	31.4^{b}	32.6^{b}	(4.71)	7.72^{a}	7.92^{a}	14.7^{b}	13.4^{b}	(1.95)
ME (μmol/min/AT)‡‡	9.74^{a}	11.6^{a}	33.0^{b}	28.5^{b}	(4.64)	6.34^{a}	5.63^{a}	14.3^{b}	12.7^{b}	(2.43)
FAS (μmol/min/AT)§§	1.04^{a}	1.57^{b}	0.97^{a}	1.49^{b}	(0.17)	0.75	0.76	0.88	0.81	(0.12)
G3PDH (μmol/min/AT)¶¶	272^{a}	258^{a}	450^{b}	472^{b}	(63.3)	190^{ab}	182^{a}	263^{b}	227^{ab}	(28.4)

a, b Mean values within a row and within sedentary or exercise-trained rats with unlike superscipts letters are significantly different ($P<0.05$).
* Values represent mean for $n=5$ or 6 per diet group.
† *Cis* 9, *trans* 11 conjugated linoleic acid (CLA).
‡ *Trans* 10, *cis* 12 CLA.
§ PSE=pooled standard error for the sedentary rats.
¶ PSE=pooled standard error for the exercise-trained rats.
‖ Effect of exercise (E) ($P<0.01$).
\# Effect of E × t10,c12 interaction ($P<0.08$).
** Effect of E and t10,c12 ($P<0.01$).
†† Effect of E, t10,c12 and E × t10,c12 interaction ($P<0.01$).
‡‡ Effect of E ($P<0.01$), t10,c12 ($P<0.01$) and E × t10,c12 interaction ($P<0.02$).
§§ Effect of E ($P<0.01$), c9,t11 ($P<0.03$) and E × c9,t11 interaction ($P<0.01$).
¶¶ Effect of E ($P<0.01$), t10,c12 ($P<0.01$) and E × t10,c12 interaction ($P<0.05$).

Cell diameters >12.5 μm were measured with Optimas software (Optimas Corporation, Bothell, WA) and the mean volume was calculated from the individual cell volumes.

2.4. Statistical analysis

Results are expressed as mean ± SD. The data presented in Tables 2–5 underwent analysis of variance using the SAS software program (SAS Institute, Cary, NC) [19]. The statistical model included the effects of exercise (1 df), c9,t11 CLA (1 df), t10,c12 CLA (1 df), and the interactions between c9,t11 CLA and t10,c12 CLA (1 df); c9,t11 CLA and exercise (1 df); t10,c12 CLA and exercise (1 df); and c9,t11 CLA and t10,c12 CLA and exercise (1 df). These effects ($P<0.05$ or $P<0.10$) were tested against the error term (38 df). Data presented in Tables 2–5 for each group of rats (either sedentary or exercise-trained) were further analyzed with the ANOVA procedure of SAS [19] according to a statistical model comparing the four dietary

Table 4
Effects of conjugated linoleic acid on tissue wieght and cellularity and on lipogenic enzyme and lipoprotein lipase activities in epididymal adipose tissue from sedentary and exercise-trained rats*

	Sedentary rats					Exercise-trained rats				
	Control	c9,t11 CLA†	t10,c12 CLA‡	CLA mixture	(PSE)§	Control	c9,t11 CLA	t10,c12 CLA	CLA mixture	(PSE)¶
Weight (g)‖	18.8	15.8	16.3	14.9	(1.17)	12.0	14.1	12.8	10.2	(1.48)
Adipose volume (pl)#	776	832	997	446	(95)	548	653	629	777	(103)
LPL (μmol/min/AT)**	6.67	6.02	8.44	7.30	(0.89)	4.61	5.72	5.18	3.81	(0.72)
G6PDH (μmol/min/AT)††	10.3^{ac}	7.89^{a}	17.6^{b}	15.3^{bc}	(1.91)	5.16^{a}	6.49^{ab}	12.3^{b}	7.50^{ab}	(2.13)
ME (μmol/min/AT)‡‡	5.76^{a}	5.63^{a}	16.0^{b}	12.4^{b}	(2.32)	3.30^{a}	3.12^{a}	7.05^{b}	6.36^{b}	(1.21)
FAS (μmol/min/AT)§§	0.98	0.84	1.03	0.95	(0.15)	0.53	0.64	0.60	0.58	(0.12)
G3PDH (μmol/min/AT)¶¶	243^{ab}	198^{a}	292^{b}	241^{ab}	(27.9)	144	176	158	129	(21.3)

a, b, c Mean values within a row and within sedentary or exercise-trained rats with unlike superscipts letters are significantly different ($P<0.05$).
* Values represent mean for $n=5$ or 6 per diet group.
† *Cis* 9, *trans* 11 conjugated linoleic acid (CLA).
‡ *Trans* 10, *cis* 12 CLA.
§ PSE=pooled standard error for the sedentary rats.
¶ PSE=pooled standard error for the exercise-trained rats.
‖ Effect of exercise (E) ($P<0.01$).
\# Effect of c9,t11 × t10,c12 interaction ($P<0.05$), E × c9,t11 interaction ($P<0.01$), and E × c9,t11 × t10,c12 interaction ($P<0.03$).
** Effect of E ($P<0.01$), t10,c12 ($P<0.08$), and E × t10,c12 ($P<0.06$).
†† Effect of E and t10,c12 ($P<0.01$).
‡‡ Effect of E ($P<0.01$), t10,c12 ($P<0.01$), and E × t10,c12 ($P<0.06$).
§§ Effect of E ($P<0.01$).
¶¶ Effect of E ($P<0.01$), t10,c12 ($P<0.07$), and E × t10,c12 ($P<0.09$).

Table 5
Effects of conjugated linoleic acid on tissue weight and on lipogenic enzyme and lipoprotein lipase activities, respectively, in liver and cardiac muscle from sedentary and exercise-trained rats*

	Sedentary rats					Exercise-trained rats				
	Control	c9,t11 CLA[†]	t10,c12 CLA[‡]	CLA mixture	(PSE)[§]	Control	c9,t11 CLA	t10,c12 CLA	CLA mixture	(PSE)[¶]
Liver										
Weight (g)[‖]	16.0	16.5	16.5	16.5	(0.86)	14.6	14.4	15.0	14.7	(0.67)
G6PDH (μmol/min/liver)	130	130	123	139	(18.8)	97.0	102	89.1	127	(15.7)
ME (μmol/min/liver)[#]	37.7[a]	38.5[a]	55.6[ab]	57.5[b]	(7.76)	36.0	32.6	44.8	44.3	(6.41)
FAS (μmol/min/liver)	10.1	13.4	11.9	11.0	(2.36)	10.6	9.38	11.2	11.8	(1.85)
G3PDH (μmol/min/liver)	879	1135	1187	1076	(123)	949	846	1063	1040	(144)
Cardiac muscle										
Weight (g)	1.08	1.07	1.06	1.1	(0.04)	1.06	1.03	1.03	1.06	(0.04)
LPL (μmol/min/heart)	305	291	304	315	(29)	357	281	319	302	(42)

[a, b] Mean values within a row and within sedentary or exercise-trained rats with unlike superscipts letters are significantly different ($P<0.05$).
* Values represent mean for $n=5$ or 6 per diet group.
[†] *Cis* 9, *trans* 11 conjugated linoleic acid (CLA).
[‡] *Trans* 10, *cis* 12 CLA.
[§] PSE = pooled standard error for the sedentary rats.
[¶] PSE = pooled standard error for the exercise-trained rats.
[‖] Effect of exercise (E) ($P<0.01$).
[#] Effect of *t*10,*c*12 ($P<0.01$).

treatments (control, *c*9,t11 CLA, *t*10,*c*12 CLA, *c*9,*t*11 and *t*10,*c*12 CLA). The differences between two treatments were tested using the PLSD Fisher test with differences considered significant when $P<0.05$.

3. Results

3.1. Effect of exercise on plasma metabolites and hormones and on tissue weight and enzyme activities

Exercise did not affect plasma metabolite and hormone concentrations except for total cholesterol, insulin, and leptin (Table 2). In fact, exercise tended to decrease total cholesterol ($P<0.1$) and to decrease levels of insulin ($P<0.05$; −23%) and leptin ($P<0.01$; −20%).

In addition, exercise significantly decreased ($P<0.01$) the weight of perirenal (−22%) and epididymal (−25%) AT and of liver (−10%) but did not affect the volume of adipocytes in the two adipose sites (Tables 3–5). A significant effect ($P<0.01$) of exercise was observed on LPL, G6PDH, ME, FAS, and G3PDH activities in perirenal AT, which were decreased from 25% to 51% (Table 3). Similar effects ($P<0.01$) were observed in epididymal AT, with decreases of 31% and 51% (Table 4). Exercise did not affect lipogenic enzyme activities in the liver or LPL activity in cardiac muscle (Table 5).

3.2. Effect of CLA isomers on plasma metabolites, insulin, and leptin in sedentary and exercise-trained rats

The ingestion of *t*10,*c*12 CLA isomer significantly decreased ($P<0.04$) plasma urea, total and free cholesterol, and triglycerides concentrations in sedentary and exercise-trained rats (Table 2). For plasma urea levels, the decrease was significant when exercise-trained rats were considered separately. Conversely, the ingestion of *t*10,*c*12 CLA isomer tended to increase ($P<0.07$) plasma 3-hydroxybutyrate concentrations, with a significant effect (+36%) in sedentary rats (Table 2).

The ingestion of *c*9,*t*11 CLA isomer tended to decrease plasma total cholesterol ($P<0.10$) and leptin ($P<0.07$) concentrations in sedentary and exercise-trained rats, with a significant effect (−14%) on plasma leptin levels in sedentary rats (Table 2). Moreover, the ingestion of *t*10,*c*12 + *c*9,*t*11 CLA isomers significantly decreased plasma leptin (−19%) or total cholesterol (−20%) concentrations in sedentary and exercise-trained rats, respectively (Table 2).

3.3. Effect of CLA isomers on tissue weight and cellularity from sedentary or exercise-trained rats

The ingestion of *t*10,*c*12 and/or *c*9,*t*11 CLA isomers did not affect the weight of AT, liver, and cardiac muscle (either expressed as grams, as in Tables 3–5, or as percentage of body weight or carcass weight [results not shown]), nor did it affect the volume of perirenal and epididymal adipocytes in sedentary and exercise-trained rats (Tables 3 and 4). However, a significant negative *t*10,*c*12 × *c*9,*t*11 CLA interaction ($P<0.05$) was observed in regard to the diameter of epididymal adipocytes, which differed according to whether rats underwent exercise training or remained sedentary (exercise × *t*10,*c*12 × *c*9,*t*11 CLA interaction, $P<0.03$) (Table 4). In fact, the low values of adipocyte volumes observed in the sedentary rats and in the presence of the two CLA isomer mixture were due to the values observed in two rats that had a greater number of smaller adipocytes in both perirenal and epididymal AT, with the weights of these two AT comparable to those of the other animals.

3.4. Effect of CLA isomers on lipogenic enzyme and/or lipoprotein lipase activities in adipose tissue, cardiac muscle, and liver from sedentary and exercise-trained rats

Similar trends were observed when activities were expressed per gram of tissue, per total weight of tissue, or per adipocyte for AT. Consequently only those expressed per total weight of tissue, i.e., representing the whole lipogenic potential of each tissue, are presented in Tables 3–5.

The ingestion of $t10,c12$ CLA isomer significantly increased ($P<0.01$) LPL, G6PDH, ME, and G3PDH activities in perirenal AT from sedentary and exercise-trained rats (Table 3). Moreover, significant negative interactions ($P<0.05$) were observed between exercise and $t10,c12$ CLA on G6PDH, ME, and G3PDH activities (Table 3). Similar trends ($P<0.08$) were observed in epididymal AT (Table 4) with negative interactions between exercise and $t10,c12$ CLA for LPL ($P<0.06$), ME ($P<0.06$), and G3PDH ($P<0.09$) activities (Table 4). The ingestion of $t10,c12$ CLA significantly increased ($P<0.01$) ME activity in rat liver from sedentary and exercise-trained rats (Table 5).

The ingestion of $c9,t11$ CLA isomer significantly increased ($P<0.03$) FAS activity in perirenal AT from sedentary and exercise-trained rats (Table 3), but had no significant effect in epididymal AT (Table 4). A significant negative interaction ($P<0.01$) between exercise and $c9,t11$ CLA was observed on FAS activity in perirenal AT (Table 3).

The ingestion of the two CLA isomers significantly increased ($P<0.05$) LPL, G6PDH, ME, FAS, and G3PDH activities (from 43% to 213%) in perirenal AT from sedentary rats (Table 3). Similar effects ($P<0.05$) were observed for ME (an increase of ~97%) in perirenal and epididymal AT, and for G6PDH (+74%) in perirenal AT, from exercise-trained rats (Tables 3 and 4).

The ingestion of CLA mixture significantly increased ($P<0.05, +53\%$) ME activity in liver from sedentary rats (Table 5).

The ingestion of the CLA isomers had no effect on LPL activity in the heart in either sedentary or exercise-trained rats (Table 5).

4. Discussion

This study shows, in adult male Wistar rats, that exercise for 6 days per week for 6 weeks resulted in a significant decrease of lipogenic enzyme activities in perirenal and epididymal AT, which was consistent with the decreases in the weight of these AT. These results corroborate previous studies showing that exercise lessened fat gain and in vivo lipogenic capacity of rat AT [20,21]. Insulin could play a role in mediating these effects, since it was decreased by exercise. Moreover, the decrease of plasma leptin level with exercise is in agreement with previous studies in rats [22,23] and is consistent with the decrease in AT weight. Furthermore, exercise did not significantly affect heart LPL activity and hepatic tissue lipogenic enzyme activity, in agreement with several previous studies [21,24].

Most of the studies on the metabolic effects of CLA in growing animals suggest that mixtures of CLA are potent in reducing the fat body mass in different species including Sprague-Dawley rats [25], mice [2,3], and pigs [7,26]. Among the identified mechanisms, a greater channeling of fatty acids toward β-oxidation [2], decreased AT fatty acid uptake [2,27] and increased AT lipolysis [2,27], and an overall increased metabolic rate [3] are responsible for body fat reduction. The in vitro study in cultured 3T3-L1 adipocytes showed that these effects were specifically due to the $t10,c12$ CLA isomer [11].

By contrast, we observed in this study that ingestion of CLA isomers for 6 weeks by adult Wistar rats increased the activity of lipogenic enzymes involved in NADPH generation for de novo lipogenesis (G6PDH, EM), in fatty acid esterification (G3PDH), and in fatty acid uptake (LPL) in epididymal and more markedly in perirenal AT. However, the ingestion of CLA isomers did not affect AT weight or the volume of adipocytes, in agreement with the study by Demaree et al. [28] in pigs. This could be related to the fact that the same dose of the $t10,c12$ CLA isomer increased plasma NEFA concentration (+34%) arising from lipomobilisation in post-absorptive adult Wistar rats [29], whereas it decreased the other plasma lipids. These data thus suggest that CLA ingestion in adult Wistar rats could stimulate AT turnover. These effects were due specifically to the ingestion of $t10,c12$ CLA isomer except for FAS activity, which was increased only in perirenal AT and only by the ingestion of $c9,t11$ CLA. These effects of $t10,c12$ CLA were slightly greater in sedentary rats than in exercise-trained rats (negative interaction between the effects of exercise and $t10,c12$ CLA on lipogenic enzymes), which could be related to the higher lipogenesis in sedentary rats. Another study [30] showed, in weaned pigs, that ingestion of a CLA mixture (34% $c9,t11$ CLA + 36% $t10,c12$ CLA + other CLA isomers) for 5 weeks increased G6PDH and ME activities in back and omental AT, probably indirectly by increasing the glucose availability for de novo fatty acid synthesis. However, in our study, plasma glucose concentration was not affected by CLA ingestion. In addition, we observed that the lipogenic capacity was more important in the liver than in AT of adult rat, in agreement with studies by Barakat et al. [25] and Roberts et al. [31], and that the ingestion of CLA, especially the $t10,c12$ isomer, increased liver ME activity. However, the weight of liver was not affected by the ingestion of CLA isomers in rats, contrary to studies by Tsuboyama-Kasaoka et al. [12] and Chardigny et al. [32], who observed liver steatosis in mice fed $t10,c12$ CLA.

Changes in circulating concentrations of hormones and metabolites associated with energy homeostasis, as well as altered response to homeostatic signals involved in the regulation of lipid metabolism, have also been postulated as the basis for the CLA effects in growing animals [2,27,33].

In our study, we observed that ingestion of CLA (*c*9,*t*11 CLA and/or *t*10,*c*12 CLA) decreased plasma lipid concentrations (cholesterol and triglycerides), in agreement with studies in rabbits by Corino et al. [34] and studies in hamsters by Gavino et al. [9]. It is likely that increased adipose LPL could explain in part the CLA-induced plasma triglyceride reduction, since it may increase the uptake of plasma very–low density lipoprotein-triglycerides. It is also likely that the CLA-dependent increase in hepatic fatty acid oxidation, as observed in mice by Belury et al. [35] and Peters et al. [36], may decrease very low density lipoprotein production and hence lower serum lipid levels. Moreover, Sakono et al. [37] showed that dietary CLA decreased cholesterol and triacylglycerols secretion and increased ketone body production by perfused rat liver, in agreement with our results showing an increase in plasma 3-hydroxybutyrate concentrations with *t*10,*c*12 CLA. The ingestion of *c*9,*t*11 CLA also decreased plasma leptin concentrations in exercise-trained and more markedly in sedentary rats. These results are consistent with the findings of other investigators, who observed that feeding a mixture of CLA reduced leptinemia in rats [38], mice [4,12], and humans [39]. William et al. [40] observed that leptin directly inhibited de novo fatty acid synthesis in the adipocytes of rats. Thus, it might be that the decrease of leptinemia could partly explain the increase of AT lipogenic enzymes activities observed in our study. CLA did not alter significantly plasma concentrations of glucose or insulin in the present study, in agreement with studies in mice [3], pigs [27], and adult humans [39].

Previous work examining the effect of dietary CLA on body composition and lipid metabolism was carried out using weaning or adolescent animals that were still growing. Therefore, the differences between our study and previous studies could be related to age differences of the animals. CLA may affect lipid metabolism of growing animals differently from adults. Indeed, Zambell et al. [41] showed no effect of CLA ingestion on lipolytic rates and free fatty acid release from AT in adult women in contrast with previous studies in growing animals [2,11]. Moreover, previous reports documented differences between species in lipogenic enzyme responses to CLA ingestion, which did not significantly affect the FAS activity in liver and AT of growing Sprague-Dawley rats, unlike the effect observed in mice [26]. The difference in the composition of the CLA isomers and the form of CLA (triglycerides vs free fatty acids) used could be other factors accounting for the differing results among these studies. Indeed, most of the published animal experiments were conducted with commercial supplements containing a mixture of CLA isomers and in the form of free fatty acid [2–5], in contrast to our study, in which CLA used was either *t*10,*c*12 CLA, *c*9,*t*11 CLA or the two isomers in similar amounts, and in the form of triglycerides. Moreover, dietary fat, level of CLA supplementation, and different durations of CLA supplementation may all have contributed to the differences between our results and those reported in the majority of previous studies.

In conclusion, this study shows for the first time that the ingestion of CLA isomers, especially *t*10,*c*12 CLA, increased AT and liver lipogenic enzyme activities, decreased plasma lipid concentrations and increased plasma ketone bodies in adult Wistar rat in post-prandial conditions, without affecting adipose and hepatic tissue weights or adipose cellularity. Further studies are needed to test the hypothesis that AT turnover is increased by CLA for a complete day–night cycle in adult rats fed ad libitum.

Acknowledgments

We thank Jeannette Fléchet, Martine Tourret and Carole Delavaud for laboratory technical assistance, as well as Christine Cubizolles and her team (Comparative Nutrition Experimental Unit) for animal management. This work was supported by a grant from the European commission (QLRT-1999-00076) for studies on "CLA in functional foods: a potential benefit for overweight middle-aged Europeans" (FunCLA Programme).

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