

## ***trans*-10,*cis*-12 Conjugated linoleic acid inhibits lipoprotein lipase but increases the activity of lipogenic enzymes in adipose tissue from hamsters fed an atherogenic diet**

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The aim of the present work was to investigate the effects of *trans*-10,*cis*-12 conjugated linoleic acid (CLA) on the activity and expression of lipogenic enzymes and lipoprotein lipase (LPL), as well as on the expression of transcriptional factors controlling these enzymes, in adipose tissue from hamsters, and to evaluate the involvement of these changes in the body fat-reducing effect of this CLA isomer. Thirty male hamsters were divided into three groups and fed atherogenic diets supplemented with 0 (linoleic group), 5 or 10 g *trans*-10,*cis*-12 CLA/kg diet, for 6 weeks. Body and adipose tissue weights, food intake and serum insulin were measured. Total and heparin-releasable LPL and lipogenic enzyme activities (acetyl-CoA carboxylase (ACC); fatty acid synthase (FAS); glucose-6-phosphate dehydrogenase (G6PDH); and malic enzyme (ME)) were assessed. ACC, FAS, LPL, sterol regulatory element-binding proteins (SREBP-1a), SREBP-1c and PPAR $\gamma$  mRNA levels were also determined by real-time PCR. CLA did not modify food intake, body weight and serum insulin level. CLA feeding reduced adipose tissue weight, LPL activity and expression, and increased lipogenic enzyme activities, despite a significant reduction in ACC and FAS mRNA levels. The expression of the three transcriptional factors analysed (SREBP-1a, SREBP-1c and PPAR $\gamma$ ) was also reduced. These results appear to provide a framework for partially understanding the reduction in body fat induced by CLA. Inhibition of LPL activity seems to be an important mechanism underlying body fat reduction in hamsters. Further research is needed to better characterize the effects of CLA on lipogenesis and the role of these effects in CLA action.

### **Conjugated linoleic acid: Lipogenesis: Lipoprotein lipase: Adipose tissue**

Conjugated linoleic acid (CLA) has been reported as a potent modulator of body composition, especially by reducing the accumulation of body fat into adipose tissue. Although this effect has been observed in several animal models (mouse, rat, hamster, pig, chicken), important differences in their responses to CLA have been described. The vast majority of studies in this area have been done in the mouse, which clearly exhibit an exaggerated response in comparison with other species. Responsiveness of hamster and rat is lower (Roche *et al.* 2001; Belury, 2002; Evans *et al.* 2002; Kim *et al.* 2002).

There is now strong evidence showing that the *trans*-10,*cis*-12 isomer is mostly responsible for this adipose mass-lowering effect of CLA (Park *et al.* 1999; Pariza *et al.* 2000, 2001; Evans *et al.* 2002; Martin & Valeille, 2002; Navarro *et al.* 2003), suggesting that *trans*-10,*cis*-12 CLA is likely to be the biologically active isomer in fat management.

The mechanisms by which *trans*-10,*cis*-12 CLA exerts this effect have not been completely clarified. A negative energy balance due to decreased energy intake and/or increased

energy expenditure may play a role (Pariza *et al.* 2001; Roche *et al.* 2001, 2002; Ealey *et al.* 2002; Takahashi *et al.* 2002). Moreover, direct effects on adipose tissue metabolic pathways, such as lipolysis,  $\beta$ -oxidation of fatty acids, triacylglycerol synthesis, fatty acid uptake and pre-adipocyte differentiation may also be involved (Park *et al.* 1997, 2004; Choi *et al.* 2000; Martin *et al.* 2000; Tsuboyama-Kasaoka *et al.* 2000; Lin *et al.* 2001; Pariza *et al.* 2001; Roche *et al.* 2001; Xu *et al.* 2003; Macarulla *et al.* 2005; Simón *et al.* 2005).

The aim of the present work was to investigate the effects of *trans*-10,*cis*-12 CLA intake on the activity and expression of several lipogenic enzymes and lipoprotein lipase (LPL) in adipose tissue from hamsters, and to evaluate the potential involvement of these changes in the body fat-reducing effect of this CLA isomer. In order to gain more insight concerning CLA mechanism of action, the effects on the expression of transcriptional factors that regulate the activity of these enzymes (sterol regulatory element-binding proteins (SREBP-1a), SREBP-1c and PPAR $\gamma$ ) was also studied.

**Abbreviations:** ACC, acetyl-CoA carboxylase; CLA, conjugated linoleic acid; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; HR-LPL, heparin-releasable lipoprotein lipase; LPL, lipoprotein lipase; ME, malic enzyme; SREBP, sterol regulatory element-binding protein.

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## Materials and methods

### Animals, diets and experimental design

The experiment was conducted with thirty 9-week-old male Syrian Golden hamsters purchased from Harlan Ibérica (Barcelona, Spain) and took place in accordance with the institution's guide for the care and use of laboratory animals. The hamsters were individually housed in polycarbonate metabolic cages (Techniplast Gazzada, Guguggiate, Italy) and placed in an air-conditioned room ( $22 \pm 2^\circ\text{C}$ ) with a 12 h light–dark cycle. After a 6 d adaptation period, hamsters were randomly divided into three dietary groups of ten animals each for feeding varied doses of *trans*-10,*cis*-12 CLA as NEFA (0, 0.5 and 1.0 g/100 g diet) in a semi-purified atherogenic diet consisting of 200 g/kg casein (Sigma, St Louis, MO, USA), 4 g/kg L-methionine (Sigma), 200 g/kg wheat starch (Vencasser, Bilbao, Spain), 405 g/kg sucrose (local market), 100 g/kg palm oil (Agra-Unilever, Leioa, Spain), 30 g/kg cellulose (Vencasser), 4 g/kg choline-HCl (Sigma) and 1 g/kg cholesterol (Sigma). *trans*-10,*cis*-12 CLA (95 %) was supplied by Natural Lipids Ltd (Hovdebygd, Norway). Vitamin (11 g/kg) and mineral (40 g/kg) mixes were formulated according to AIN-93 guidelines (Reeves *et al.* 1993) and supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). The experimental diets were freshly prepared once per week, gassed with, and stored at  $0-4^\circ\text{C}$  to avoid rancidity. Fatty acid composition of experimental diets was analysed by GC as we previously described (Zabala *et al.* 2006), and the results are presented in Table 1. All animals had free access to food and water. Food intake and body weight were measured daily.

At the end of the experimental period (6 weeks) animals were killed under anaesthesia (diethyl ether) and blood was collected by cardiac puncture. Adipose tissues from different anatomical locations (epididymal, perirenal and subcutaneous) were dissected, weighed, sliced and immediately frozen. Serum was obtained from blood samples after centrifugation (1000 g for 10 min at  $4^\circ\text{C}$ ). Samples were stored at  $-80^\circ\text{C}$  until analysis.

**Table 1.** Fatty acid composition (g/100 g total fatty acids) of experimental diets\*

(Mean values with their standard errors)

	Linoleic group		0.5 % CLA group		1 % CLA group	
	Mean	SEM	Mean	SEM	Mean	SEM
12:0	0.67	0.06	0.81	0.01	0.77	0.02
14:0	1.23	0.04	1.38	0.03	1.37	0.05
16:0	40.22	0.98	43.78	0.09	43.01	0.33
16:1 $n$ -7	0.17	0.02	0.16	0.01	0.17	0.03
18:0	0.16	0.07	0.18	0.03	0.14	0.08
18:1 $n$ -9	35.65	0.40	36.94	0.14	38.11	0.21
18:1 $n$ -7	0.76	0.005	0.77	0.002	0.77	0.03
18:2 $n$ -6	13.15	0.20	09.69	0.04	9.83	0.05
18:3 $n$ -3	0.19	0.03	0.17	0.003	0.18	0.01
20:0	0.40	0.05	0.38	0.004	0.36	0.02
<i>cis</i> -9, <i>trans</i> -11 CLA	0.00		0.039	0.002	0.039	0.005
<i>trans</i> -10, <i>cis</i> -12 CLA	0.00		0.74	0.05	1.18	0.14

CLA, conjugated linoleic acid.

\*Only fatty acids detected higher than 0.10 g/100 g total fatty acids are listed. For details of diets and procedures, see this page.

### Serum parameters

Serum insulin was measured by RIA, using a commercial kit (Linco, St Charles, MO, USA).

### Lipogenic activities

Samples of epididymal adipose tissue (1.0 g) were homogenized in 2.5 ml buffer (pH 7.6) containing 150 mM-KCl, 1 mM-MgCl<sub>2</sub>, 10 mM-N-acetyl-cysteine and 0.5 mM-dithiothreitol.

After centrifugation at 100 000 g for 40 min at  $4^\circ\text{C}$ , the supernatant fraction was used for quantification of enzyme activities. Acetyl-CoA carboxylase (ACC; EC 6.4.1.2), fatty acid synthase (FAS; EC 2.3.1.85), glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) and malic enzyme (ME; EC 1.1.1.40) activities were measured as described earlier (Zabala *et al.* 2004). Enzyme activities were expressed either as nmol HCO<sub>3</sub><sup>-</sup> incorporated (ACC), or as nmol NADPH consumed (FAS) or as nmol NADPH produced (G6PDH and ME), per min per mg protein and per g tissue.

### Lipoprotein lipase activity

For total LPL activity determination, homogenates of epididymal adipose tissue (250 mg) were prepared in 750  $\mu\text{l}$  10 mM-HEPES buffer (pH 7.5) containing 1 mM-dithiothreitol, 1 mM-EDTA, 250 mM-sucrose and 2 g/l heparin and used as enzyme source. Soluble protein in homogenates was determined using bovine serum albumin as standard (Bradford, 1976). For heparin-releasable LPL (HR-LPL) activity determination enzyme source was obtained by incubating 250 mg epididymal adipose tissue in 750  $\mu\text{l}$  10 mM-HEPES buffer (described earlier) at  $37^\circ\text{C}$  for 30 min.

Enzyme activity was assessed following the method described by Nilsson-Ehle & Schotz (1976) with modifications. Briefly, samples (20  $\mu\text{l}$ ) of tissue homogenate (total LPL) or incubation medium (HR-LPL) were incubated with 20  $\mu\text{l}$  2.5 M-NaCl or 20  $\mu\text{l}$  HEPES buffer for 30 min at  $37^\circ\text{C}$  under gentle agitation with 160  $\mu\text{l}$  emulsified substrate mixture (pH 7.5) consisting of 27.5 mM-piperazine-1,4-bis(ethanesulfonic acid), 55.5 mM-MgCl<sub>2</sub>, 0.5 g/l fatty acid-free bovine serum albumin (Sigma), 6.4 mCi/l [<sup>3</sup>H]triolein (Amersham Biosciences, Buckinghamshire, UK), 6.6 mM-triolein (cold) and 33 ml/l hamster inactivated serum as a source of apo C-II. Oleate released by LPL was then separated from intact triolein using a liquid partition system (Belfrage & Vaughan, 1969). A solution containing [<sup>14</sup>C]oleic acid (3.3  $\mu\text{Ci}$ ; Amersham Biosciences) and 2 mM-oleic acid (cold) was used to calculate oleate recovery. The radioactivity was determined in a Tribarb counter (Beckman Coulter, Fullerton, CA, USA).

LPL (total and HR-LPL) activity was calculated by subtracting non-LPL lipolytic activity in the presence of NaCl from the total lipolytic activity determined without NaCl and expressed either as nmol oleate released per min per g tissue or as nmol oleate released per min per total tissue.

### Extraction and analysis of RNA and semi-quantification by reverse transcription-PCR

Total RNA was isolated from 100 mg adipose tissue using Trizol (Invitrogen, Carlsbad, CA, USA) according to the

manufacturer's instructions. RNA samples were treated with RNase-free DNase I (Takara, Otsu, Japan) to remove any genomic DNA. The yield and quality of the RNA were assessed by measuring absorbance at 260, 270, 280 and 310 nm and by electrophoresis on 1.3% agarose gels. Total RNA (1.5 µg) was used to synthesize first-strand cDNA using iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) that blends polydT and random hexamer primers.

Relative ACC, FAS, SREBP-1a, SREBP-1c, LPL and PPARγ mRNA levels were quantified using real-time PCR with an ABI Prism 7000HT Sequence Detection System (Applied Biosystems). A volume (0.1 µl) of each cDNA was added to PCR reagent mixture, SYBR® Green Master Mix (Applied Biosystems) with the upstream and downstream primers (300 nm each). Information concerning specific sense and antisense primers are described in Table 2. The PCR parameters were as follows: initial 2 min at 50°C; denaturation at 95°C for 10 min followed by forty cycles of denaturation at 95°C for 15 s; combined annealing and extension at 60°C for 1 min. All samples were normalized to the values of β-actin and the results expressed as relative fold changes of threshold cycle (Ct) value relative to the linoleic group using the  $2^{-\Delta\Delta Ct}$  method (Livak & Schmittgen, 2001).

#### Statistical analysis

Results are presented as means with their standard errors. Statistical analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA). Data were analysed by one-way ANOVA followed by Newman-Keuls *post-hoc* test. Statistical significance was set at the  $P < 0.05$  level.

## Results

#### Body weight, food intake, adipose tissue weights and serum insulin

No differences in food intake were found among the three experimental groups. Although no significant differences in final body weight were found between hamsters fed the *trans*-10,*cis*-12 CLA diets and hamsters fed the linoleic diet, animals fed 1% CLA showed significantly higher final body weight (+4.2%) than those fed 0.5% CLA ( $P < 0.05$ ; Table 3). We previously described that this effect was due to increased skeletal muscle mass (Zabala *et al.* 2006). CLA feeding significantly reduced adipose tissue weights in epididymal, perirenal and subcutaneous locations. A dose-dependent effect was not observed (Fig. 1).

Concerning serum insulin, no significant differences were observed among the three experimental groups: 567 (SEM 96), 539 (SEM 70) and 546 (SEM 42) pmol/l for the linoleic group, the 0.5% CLA group and the 1% CLA group, respectively.

#### Enzyme activities and enzyme and transcriptional factor expression in epididymal adipose tissue

CLA feeding significantly reduced the activity (nmol/min per g tissue) of both total LPL and HR-LPL (Fig. 2). Data concerning total tissue capacity (nmol/min per tissue) were 233 (SEM 38) in the linoleic group, 126 (SEM 17) in the 0.5% CLA group ( $P < 0.05$ ) and 81 (SEM 10) in the 1% CLA group ( $P < 0.01$ ) for total LPL, and 40 (SEM 6) in the linoleic group, 23 (SEM 3) in the 0.5% CLA group ( $P < 0.05$ ) and 20 (SEM 2) in the 1% CLA group ( $P < 0.01$ ) for HR-LPL. The same effect was observed on LPL mRNA (Fig. 3). Concerning PPARγ expression, both CLA doses induced significant reductions (Fig. 3).

With regard to the activity of the four lipogenic enzymes analysed, significantly greater values were found in hamsters fed the *trans*-10,*cis*-12 CLA when compared with those fed the linoleic diet. Because similar trends were observed when activities were expressed per mg protein or per g tissue, only those expressed per g tissue are presented (Fig. 4). No differences were found between both doses of CLA.

In order to assess the whole lipogenic potential of epididymal adipose tissue, enzyme activities were also calculated per total tissue weight (nmol/min per tissue): 3170 (SEM 258) in the linoleic group, 9225 (SEM 941) in the 0.5% CLA group and 10 135 (SEM 728) in the 1% CLA group ( $P < 0.001$ ) for G6PDH; 334 (SEM 39) in the linoleic group, 1014 (SEM 50) in the 0.5% CLA group and 1144 (SEM 101) in the 1% CLA group ( $P < 0.001$ ) for ME; 207 (SEM 23) in the linoleic group, 496 (SEM 47) in the 0.5% CLA group and 469 (SEM 41) in the 1% CLA group ( $P < 0.001$ ) for FAS; 101 (SEM 9) in the linoleic group, 123 (SEM 4) in the 0.5% CLA group and 137 (SEM 23) in the 1% CLA group ( $P < 0.001$ ) for ACC.

mRNA of ACC, FAS and the transcriptional factors, SREBP-1a and SREBP-1c, were significantly reduced by CLA feeding (Fig. 5).

## Discussion

*trans*-10,*cis*-12 CLA intake produced a significant decrease in adipose tissue weights. The magnitude of this reduction was

**Table 2.** Primers for PCR amplification gene products of each gene studied

Primers	Sense primer	Antisense primer	GenBank accession no.
SREBP-1a	5'-GGCTGTGGAACAGGCACTG-3'	5'-AGCTGGAGCATGTCTTCGATG-3'	Field <i>et al.</i> (2003)
SREBP-1c	5'-GCGGACGCACTCTGGG-3'	5'-ATGAGCTGGAGCATGTCTTCAA-3'	Field <i>et al.</i> (2003)
FAS	5'-AGCCCTCAAGTGCACAGTG-3'	5'-TGCCAATGTGTTTTCCCTGA-3'	AF356086
ACC	5'-ACACTGGCTGGCTGGACAG-3'	5'-CACACAACCTCCCAACATGGTG-3'	AF356089
LPL	5'-CAGCTGGGCTAACTTTGAG-3'	5'-CCTCTGCAATCACACGAA-3'	AF356087
PPARγ	5'-ATTCTGGCCCACTTCGG-3'	5'-TGGAAGCCTGATGCTTTATCCCA-3'	AF156665
β-Actin	5'-TCTACAATGAGTCTGCGTGTG-3'	5'-GGTCAGGATCTTCATGAGGT-3'	J00691

ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; LPL, lipoprotein lipase; SREBP, sterol regulatory element-binding protein.

**Table 3.** Final body weight, food intake and serum parameters of hamsters fed on the experimental diets for 6 weeks\* (Mean values with their standard errors for ten hamsters per group)

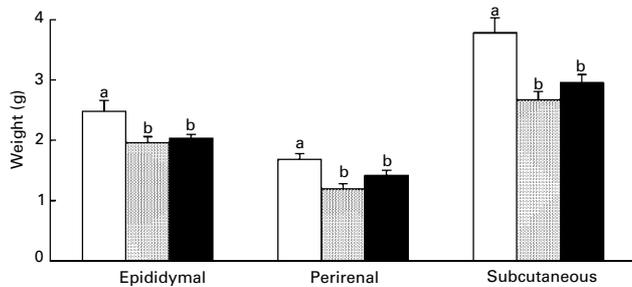
	Linoleic group		0.5% CLA group		1% CLA group		Statistical significance of effect (ANOVA)
	Mean	SEM	Mean	SEM	Mean	SEM	
Initial body weight (g)	105	1	105	1	106	1	NS
Final body weight (g)	121 <sup>ab</sup>	3	119 <sup>b</sup>	1	124 <sup>a</sup>	2	$P < 0.05$
Food intake (g/d)	6.03	0.14	5.66	0.08	5.73	0.12	NS

\* For details of diets and procedures, see Table 1 and p. 1113.

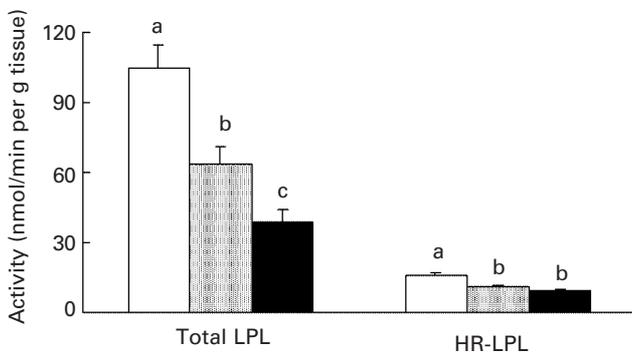
<sup>a,b,c</sup> Mean values within the same row with unlike superscript letters were significantly different ( $P < 0.05$ ).

similar when CLA was added to the diet at either 0.5% or 1%, perhaps because 0.5% CLA is sufficient to maximally induce body fat reduction in the hamster.

The present work was designed to shed light on some of the potential mechanisms underlying the decrease of white adipose tissue size, produced by the *trans*-10,*cis*-12 CLA



**Fig. 1.** Epididymal, perirenal and subcutaneous adipose tissue weights from hamsters (ten per group) fed on the experimental diets for 6 weeks. For details of diets and procedures, see Table 1 and p. 1113. Values are means with their standard errors depicted by vertical bars. <sup>a,b</sup> Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). □, Linoleic group; ▨, 0.5% conjugated linoleic acid (CLA) group; ■, 1% CLA group. Decrease in adipose tissue weight: epididymal (21.0% in 0.5% CLA,  $P < 0.05$ ; 18.1% in 1% CLA,  $P < 0.05$ ), perirenal (29.2% in 0.5% CLA,  $P < 0.05$ ; 15.5% in 1% CLA,  $P < 0.05$ ) and subcutaneous (29.4% in 0.5% CLA,  $P < 0.001$ ; 22.0% in 1% CLA,  $P < 0.001$ ).

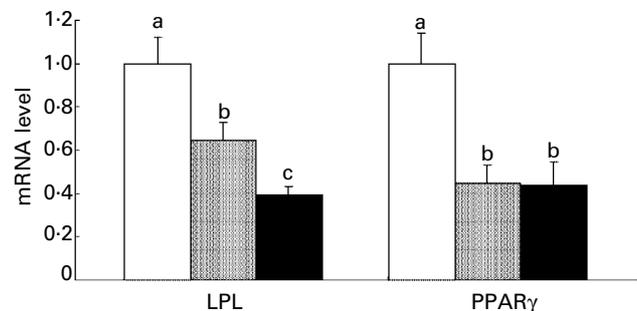


**Fig. 2.** Total lipoprotein lipase (LPL) and heparin-releasable LPL (HR-LPL) activities in epididymal adipose tissue from hamsters (ten per group) fed on the experimental diets for 6 weeks. For details of diets and procedures, see Table 1 and p. 1113. Values are means with their standard errors depicted by vertical bars. <sup>a,b,c</sup> Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). □, Linoleic group; ▨, 0.5% conjugated linoleic acid (CLA) group; ■, 1% CLA group. Decrease in total and HR-LPL activities: total LPL (40.0% in 0.5% CLA,  $P < 0.01$ ; 62.8% in 1% CLA,  $P < 0.001$ ) and HR-LPL (30.1% in 0.5% CLA,  $P < 0.05$ ; 39.7% in 1% CLA,  $P < 0.01$ ).

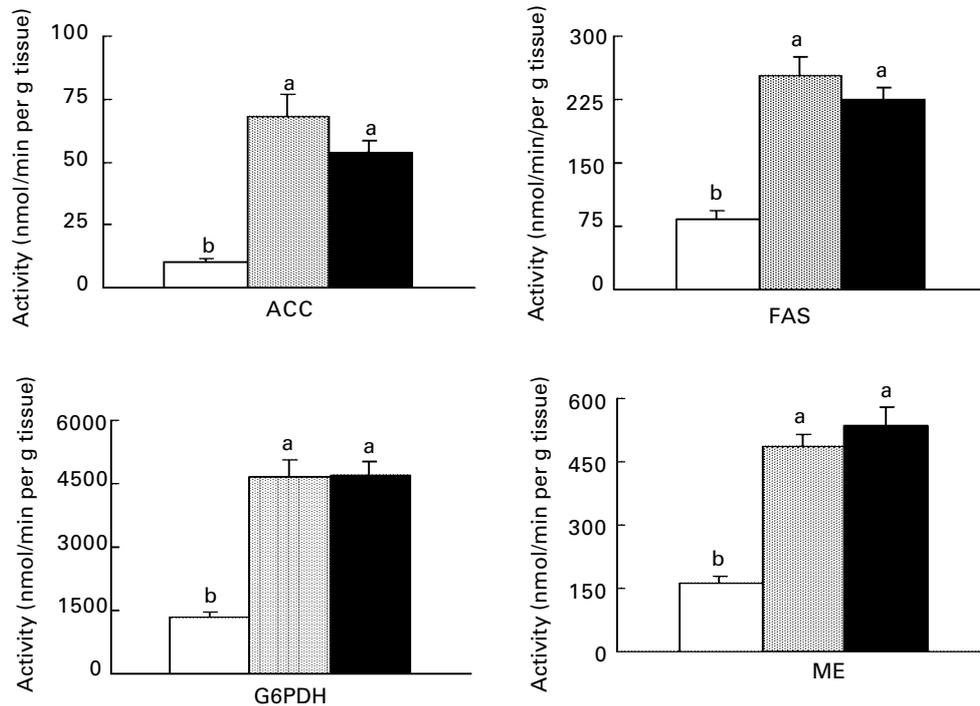
isomer. For this purpose mRNA levels, as well as the activities of LPL and several enzymes involved in lipogenesis, were examined.

The effects of CLA on LPL activity have been analysed mainly in cultured cells. Reduced LPL activity in 3T3-L1 adipocytes after supplementation has been reported in several papers (Park *et al.* 1997, 1999, 2004; Park & Pariza, 2001). Lin *et al.* (2001) measured the activity of both intracellular LPL and HR-LPL in 3T3-L1 adipocytes and observed that *trans*-10,*cis*-12 CLA only inhibited HR-LPL but not intracellular LPL activity. They concluded that CLA acted at the post-translation stage.

To the best of our knowledge, there are only two *in vivo* studies in which the effect of CLA on adipose tissue LPL activity was measured. Xu *et al.* (2003) reported a significant reduction in both HR-LPL and intracellular LPL activities in adipose tissue from mice fed a 0.5% CLA mixture-supplemented diet for a very short experimental period (4 d), and Faulconnier *et al.* (2004) found increased LPL activity in perirenal adipose tissue from adult rats, but not in the epididymal depot after 6 weeks of *trans*-10,*cis*-12 CLA feeding. Because the results obtained in cultured cells cannot always be extrapolated to *in vivo* situations, and due to the fact that different sensitivity to CLA has been reported in different rodent species, the present study provides new useful information.

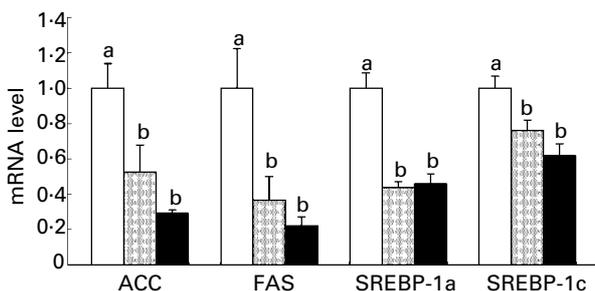


**Fig. 3.** Lipoprotein lipase (LPL) and PPAR $\gamma$  mRNA levels measured by real-time PCR, in epididymal adipose tissue from hamsters (ten per group) fed on the experimental diets for 6 weeks. For details of diets and procedures, see Table 1 and p. 1113. Values are expressed relative to the linoleic group and results are given as means with their standard errors depicted by vertical bars. <sup>a,b</sup> Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). □, Linoleic group; ▨, 0.5% conjugated linoleic acid (CLA) group; ■, 1% CLA group. Decrease in LPL and PPAR $\gamma$  mRNA levels: LPL (35.8% in 0.5% CLA,  $P < 0.05$ ; 60.4% in 1% CLA,  $P < 0.001$ ) and PPAR $\gamma$  (55.4% in 0.5% CLA,  $P < 0.01$ ; 56.3% in 1% CLA,  $P < 0.01$ ).



**Fig. 4.** Lipogenic enzyme activities in epididymal adipose tissue from hamsters (ten per group) fed on the experimental diets for 6 weeks. For details of diets and procedures, see Table 1 and p. 1113. Values are means with their standard errors depicted by vertical bars. <sup>a,b</sup>Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). □, Linoleic group; ▨, 0.5% conjugated linoleic acid (CLA) group; ■, 1% CLA group. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; G6PDH, glucose-6-phosphate dehydrogenase; ME, malic enzyme. Increase in lipogenic enzyme activities: G6PDH (249.0% in 0.5% CLA,  $P < 0.001$ ; 252.2% in 1% CLA,  $P < 0.001$ ), ME (201.9% in 0.5% CLA,  $P < 0.001$ ; 233.5% in 1% CLA,  $P < 0.001$ ), FAS (206.0% in 0.5% CLA,  $P < 0.001$ ; 170.0% in 1% CLA,  $P < 0.001$ ) and ACC (583.8% in 0.5% CLA,  $P < 0.001$ ; 445.4% in 1% CLA,  $P < 0.001$ ).

The results obtained in hamsters in the present work are thus in good accordance with those found in mice but not in rats. The reduction in LPL activity resulted in diminished adipose tissue size and probably explains, at least in part, the body fat-lowering action of this CLA isomer.



**Fig. 5.** Acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), sterol regulatory element-binding protein-1a (SREBP-1a) and SREBP-1c mRNA levels, measured by real-time PCR, in epididymal adipose tissue from hamsters (ten per group) fed on the experimental diets for 6 weeks. For details of diets and procedures, see Table 1 and p. 1113. Values are expressed relative to the linoleic group and results are given as means with their standard errors depicted by vertical bars. <sup>a,b</sup>Mean values with unlike superscript letters were significantly different ( $P < 0.05$ ). □, Linoleic group; ▨, 0.5% conjugated linoleic acid (CLA) group; ■, 1% CLA group. Decrease in ACC, FAS, SREBP-1a and SREBP-1c mRNA levels: ACC (47.6% in 0.5% CLA,  $P < 0.05$ ; 70.8% in 1% CLA,  $P < 0.001$ ), FAS (63.4% in 0.5% CLA,  $P < 0.05$ ; 77.9% in 1% CLA,  $P < 0.001$ ), SREBP-1a (56.3% in 0.5% CLA,  $P < 0.001$ ; 54.2% in 1% CLA,  $P < 0.001$ ), SREBP-1c (24.0% in 0.5% CLA,  $P < 0.05$ ; 38.2% in 1% CLA,  $P < 0.01$ ).

The activity of LPL can be regulated at multiple levels including transcription, translation, processing and transport from adipocytes to endothelial cells (Fielding & Frayn, 1998). Due to the fact that both total and HR-LPL activities were reduced in the CLA-fed groups when compared with the linoleic group, changes in secretion from adipocytes and transport until the capillary endothelial cells does not seem likely. Moreover, the reduction in mRNA levels leads one to the conclusion that the regulation of LPL by CLA takes place at the transcriptional level, which is in good accordance with other works carried out in mice. Tsuboyama-Kasaoka *et al.* (2000) reported a significant reduction in LPL mRNA level in C57BL/6J mice fed a 1% CLA mixture-supplemented diet for 8 months, and Kang *et al.* (2003) observed the same effect in ICR mice fed a 0.5% CLA mixture-supplemented diet for 4 weeks. In these studies LPL activity was not measured.

PPAR $\gamma$  is a transcriptional factor, highly expressed in adipose tissue compared with muscle or liver (Kersten, 2002), that plays an important role in the regulation of lipid metabolism. Its activation promotes lipid storage by stimulation of LPL (Khan & Vanden Heuvel, 2003). Thus, in order to obtain more insight concerning the reduction in LPL activity produced by CLA feeding, the expression of this nuclear receptor was assessed in the present work and a significant reduction was observed. These results suggest that the reduction in mRNA LPL induced by CLA feeding can be mediated by the reduced PPAR $\gamma$  expression.

The involvement of PPAR $\gamma$  in the body fat-lowering effect of CLA is a controversial issue. *In vitro* studies performed in

3T3-L1 adipocytes and pre-adipocytes have revealed contradictory results concerning the effects of CLA on PPAR $\gamma$  mRNA expression. Thus, whereas Brodie *et al.* (1999), Brown *et al.* (2003), Grandlund *et al.* (2003) and Kang *et al.* (2003) reported a reduction in PPAR $\gamma$  mRNA levels induced by *trans*-10,*cis*-12 CLA, Choi *et al.* (2000) did not observe any effect. Evans *et al.* (2001) reported that the effects of *trans*-10,*cis*-12 CLA on pre-adipocytes depend on the time period of treatment. Data from *in vivo* studies are scarcer. Kang *et al.* (2003) found a reduced PPAR $\gamma$  expression in epididymal adipose tissue from ICR mice fed a 0.5% CLA mixture and Takahashi *et al.* (2002) reported the same effect in C57BL/6J mice fed 2% CLA for 21 d, but not in ICR mice.

In a previous work, we reported increased liver fatty acid oxidation in hamsters fed *trans*-10,*cis*-12 CLA (Macarulla *et al.* 2005). Moreover, several studies performed in HepG2 cells have shown decreased VLDL secretion induced by CLA (Yotsumoto *et al.* 1999; Pal *et al.* 2005; Storkson *et al.* 2005). Thus, in addition to a direct effect of *trans*-10,*cis*-12 CLA on PPAR $\gamma$ , the potential involvement of decreased availability of VLDL substrate in the reduction of LPL activity should not be discarded. This fact suggests that some of the effects induced by CLA in adipose tissue could be a consequence of changes in liver triacylglycerol metabolism.

With regard to the effects of CLA on adipose tissue lipogenesis, conflicting results have been reported when either enzyme activities or expression were assessed. West *et al.* (2000) observed increased fatty acid synthesis, despite a significant reduction in adipose tissue weight, in growing mice fed a 1% CLA mixture. Increased lipogenic enzyme activities, without changes in adipose tissue size, were reported in adult rats fed 1% *trans*-10,*cis*-12 CLA for 6 weeks by Faulconnier *et al.* (2004). Further, Kang *et al.* (2004) found an increase in FAS mRNA induced by 0.2% *trans*-10,*cis*-12 CLA feeding for 4 weeks in mice. In contrast, Azain *et al.* (2000) did not find changes in FAS activity induced by CLA feeding in rats. Other authors found reduced expression of FAS and ACC in mice (Tsuboyama-Kasaoka *et al.* 2000; Clement *et al.* 2002). With regard to non-ruminant species, Corino *et al.* (2002) described decreased ACC activity, without changes in ME and G6PDH activities in adipose tissue from rabbits fed a CLA mixture. Unfortunately, no works in the literature evaluating both lipogenic enzyme activities and mRNA in the same experiment are available. Thus, we performed both determinations in the same tissue in order to bring some new facts to light on this issue.

In the present work, *trans*-10,*cis*-12 CLA feeding significantly reduced the mRNA expression of the two specific lipogenic enzymes measured (ACC, FAS). These results are in good accordance with those published by Tsuboyama-Kasaoka *et al.* (2000) and Clement *et al.* (2002) but not with those reported by Kang *et al.* (2004) in mice. Nevertheless, when comparisons are made among different animal species, it should be taken into account that they differ in the major site of fatty acid synthesis. Whereas, in the rat adipose tissue accounts for over 50% of total fatty acid synthesis (Waterman *et al.* 1975), in the mouse the most important lipogenic organ is the liver (Mourot *et al.* 1994).

SREBP-1a and SREBP-1c are potent activators of enzymes involved in fatty acid synthesis, and have been shown to be affected by nutritional factors (Horton *et al.* 2002).

The reduction that we found in the mRNA expression of these transcriptional factors, induced by *trans*-10,*cis*-12, paralleled changes observed in ACC and FAS.

The activities of the four lipogenic enzymes were surprisingly increased. This effect is in line with that found by West *et al.* (2000) in mice and Faulconnier *et al.* (2004) in rats, but disagrees with that reported by Azain *et al.* (2000) in rats. Obviously, this effect is not one of the potential mechanisms by which CLA reduces adipose tissue size. In our opinion, CLA acts directly by inhibiting the expression of lipogenic enzymes, an effect that is in good accordance with its body fat-lowering effect. On the contrary, the increase in lipogenic enzyme activities could represent a compensatory mechanism as a response to the reduced fatty acid uptake from serum triacylglycerol-rich lipoproteins, due to LPL inhibition. Moreover *trans*-10,*cis*-12 CLA has been reported to reduce fatty acid transport in inguinal fat pads from rats (Sauer *et al.* 2004). The high availability of glucose-6-phosphate, due to the high content of sucrose in the diet, could allow this compensatory phenomenon acting on enzyme activation.

In summary, the results here reported appear to provide a framework for partially understanding the reduction in body fat induced by *trans*-10,*cis*-12 CLA intake. Inhibition of LPL activity seems to be a mechanism underlying body fat reduction in hamsters. The possibility of a reduction of this enzyme activity as a consequence of reduced triacylglycerol availability cannot be discarded. Further research is needed to characterize better the effects of CLA on lipogenesis, under different experimental conditions, and the role of these effects in CLA body fat-lowering action.

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