British Journal of Nutrition (2007), **97**, 1074–1082
doi: 10.1017/S0007114507682932
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Effects of *trans*-10, *cis*-12 conjugated linoleic acid on the expression of uncoupling proteins in hamsters fed an atherogenic diet

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(Received 6 September 2006 – Revised 29 November 2006 – Accepted 3 January 2007)

It is known that conjugated linoleic acid (CLA) feeding decreases body adiposity but the mechanisms involved are not clear. The aim of this study was to analyse whether alterations in uncoupling protein (UCP) expression in white and brown adipose tissues (WAT and BAT, respectively) and in skeletal muscle may be responsible for the effect of *trans*-10, *cis*-12 CLA on the size of body fat depots in hamsters. Animals were divided into three groups and fed an atherogenic diet with different amounts of *trans*-10, *cis*-12 CLA (0 control, 0·5, or 1 g/100 g diet) for 6 weeks. CLA feeding reduced adipose depot weights, but had no effect on body weight. Leptin mRNA expression decreased in both subcutaneous and perirenal WAT depots, in accordance with lower adiposity, whereas resistin mRNA expression was not changed. Animals fed CLA had lower UCP1 mRNA levels in BAT (both doses of CLA) and in perirenal WAT (the low dose), and lower UCP3 mRNA levels in subcutaneous WAT (the high dose). UCP2 mRNA expression in WAT was not significantly affected by CLA feeding. Animals fed the high dose of CLA showed increased UCP3 and carnitine palmitoyl transferase-I (CPT-I) mRNA expression levels in skeletal muscle. In summary, induction of UCP1 or UCP2 in WAT and BAT is not likely to be responsible for the fat-reduction action of CLA, but the increased expression of UCP3 in skeletal muscle, together with a higher expression of CPT-I, may explain the previously reported effects of dietary CLA in lowering adiposity and increasing fatty acid oxidation by skeletal muscle.

Conjugated linoleic acid: Uncoupling proteins: Atherogenic diet: Hamster

CLA (conjugated linoleic acid) is the acronym describing a group of octadecadienoic acids (18:2) which are isomers of the essential fatty acid, linoleic acid (C18:2 *n*-6), whose double bonds are not separated by a methylene group but are conjugated. The CLA chemically produced for commercialisation and used in dietetic complements or foods is usually a relatively rich (about 55 and 90 %) CLA mixture containing about equal proportions of two isomers, *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11 CLA (Gaullier *et al.* 2002) with a minor contribution from other isomers.

Interest in CLA arose, initially, in its anticarcinogenic action (Pariza *et al.* 1979) but there is an increasing amount of specific scientific literature on the biological effects and properties of CLA (for current citations of the published scientific literature on CLA, see http://www.wisc.edu/fri/clarefs.htm). In particular, CLA has been shown to decrease body fat in various animal models including mice, rats, hamsters and pigs, as well as in man (Park *et al.* 1997; Azain *et al.* 2000; Gavino *et al.* 2000; Kritchevsky *et al.* 2000; Tsuboyama-Kasaoka *et al.* 2000; Cherian *et al.* 2002; Navarro *et al.* 2003; Ostrowska *et al.* 2003; Pariza, 2004; Terpstra, 2004). Although in most of the published studies concerning CLA, mixtures with different isomer proportions have been used (Navarro *et al.* 2006), there is strong evidence indicating that the biologically active isomer showing anti-obesity effects is *trans*-10, *cis*-12 (Evans *et al.* 2002*a*; Martin & Valeille, 2002).

Several mechanisms of action have been proposed to explain the fat-lowering effect of CLA: decreased energy intake (Kamphuis *et al.* 2003); increased energy expenditure (West *et al.* 2000); increased lipolysis and fatty acid oxidation (Evans *et al.* 2002*b*; Macarulla *et al.* 2005); decreased TAG synthesis and fatty acid uptake (Evans *et al.* 2000); decreased pre-adipocyte differentiation (Brodie *et al.* 1999) and increased apoptosis (Tsuboyama-Kasaoka *et al.* 2000), but evidence supporting these mechanisms is not equally strong. Concerning energy expenditure, several works in the literature have been devoted to analyse the effects of CLA on the expression of uncoupling proteins (UCP) which are transmembrane proteins found in the inner mitochondrial membrane that dissipate energy as heat and consequently increase energy expenditure. However, the results obtained are still scarce and not very consistent, and show species-specific differences (Tsuboyama-Kasaoka *et al.* 2000; West *et al.* 2000; Ryder

Abbreviations: BAT, brown adipose tissue; CLA, conjugated linoleic acid; CPT-I, carnitine palmitoyl transferase I; UCP, uncoupling protein; WAT, white adipose tissue.

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et al. 2001; Ealey *et al.* 2002; Roche *et al.* 2002; Rodriguez *et al.* 2002; Takahashi *et al.* 2002; Choi *et al.* 2004).

We have previously reported that the addition of *trans*-10, *cis*-12 CLA (supplemented as 0·5 g/100 g diet) for 6 weeks to an atherogenic diet (high-fat, high-sucrose) can prevent body fat accumulation induced by this diet in hamsters (Navarro *et al.* 2003) by the inhibition of adipogenesis (Simon *et al.* 2005, 2006) and the increase in liver and muscle fatty acid oxidation (Macarulla *et al.* 2005; Zabala *et al.* 2006). In order to attain more insight into the effect of CLA on energy metabolism, we analysed the effect of two different doses of *trans*-10, *cis*-12 CLA (0·5 and 1 g/100 g diet) on the expression of UCP1, 2 and 3 in brown and white adipose tissues (BAT and WAT, respectively) and in skeletal muscle, which play a crucial role in regulating WAT mass, in hamsters fed an atherogenic diet. Because the influence of *trans*-10, *cis*-12 CLA on glucose homeostasis remains a matter of concern, we also analysed its effects on leptin and resistin expression in adipose tissues and GLUT4 and carnitine palmitoyl transferase-I (CPT-I) expression in skeletal muscle.

Materials and methods

Animals, diets and experimental design

The experiment was conducted with thirty 9-week-old, male Syrian Golden hamsters purchased from Harlan Iberica (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 ± 2°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 (control), 0·5 and 1·0 g/100 g diet) in a semi-purified atherogenic diet consisting of (g/kg): 200 casein (Sigma, St. Louis, MO, USA), 4 L-methionine (Sigma), 200 wheat starch (Vencasser, Bilbao, Spain), 405 sucrose (local market), 100 palm oil (Agra-Unilever, Leioa, Spain), 30 cellulose (Vencasser), 4 choline-HCl (Sigma) and 1 cholesterol (Sigma). *Trans*-10, *cis*-12 CLA was supplied by Natural Lipids Ltd. (Hovdebygda, 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 a week, gassed with N_2, and stored at 0°C to 4°C to avoid rancidity.

At the end of the experimental period (6 weeks) animals were sacrificed under anaesthesia (diethyl ether) and blood was collected by cardiac puncture. Perirenal and subcutaneous WAT depots, interscapular BAT and gastrocnemius muscles were dissected, weighed, sliced and immediately frozen. Serum was obtained from blood samples after centrifugation (1000 ***g*** for 10 min at 4°C). Samples were stored at −80°C until analysis.

Serum leptin concentration

Serum leptin concentration was measured with a mouse leptin enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MIN, USA).

Total RNA preparations

Adipose tissue and muscle samples were homogenised in Tripure reagent (Roche, Barcelona, Spain) with a Teflon/glass homogeniser (10 to 15 strokes) and total RNA was isolated according to the instructions of the manufacturer. The yield and purity of total RNA was measured spectrophotometrically. The ratio of absorption at 260 and 280 nm (A260/A280) was between 1·5 and 1·8 for all preparations. Integrity of the RNA extracted was further verified by ethidium bromide staining, after electrophoresis in 1 % agarose gels.

Northern blot analysis

Total RNA (25 μg), denatured with formamide/formaldehyde, was fractionated by agarose gel electrophoresis as previously described (Oliver *et al.* 2000). The RNA was then transferred onto a Hybond Nylon membrane in 20 × SSC (saline sodium citrate buffer: 1 × SSC in 150 mM NaCl, 15 mM sodium citrate, pH 7·0) by capillary blotting for 16 h, and fixed with UV light (Oliver *et al.* 2000).

The mRNA for UCP1, UCP2, UCP3 leptin and resistin and the 18S rRNA (used as a control to check the loading and transfer of RNA during blotting) were detected by a chemiluminescence-based procedure, using specific antisense oligonucleotide probes which were synthesised commercially (TIB MOLBIOL, Berlin, Germany), labelled at both ends with a single digoxigenin ligand. The probes were: for UCP1, 5′-GAAGACCACTGTACAGTTTCGGCAACCCTTCTG-3′; for UCP2, 5′-GGCAGAGTTCATGTATCTCGTCTTGACCAC-3′; for UCP3, 5′-GACTCCTTCTTCCCTGGCGATGGTTCT-GTAGG-3′; for leptin, 5′- GGTCTGAGGCAGGGAGCAGC-TCTTGGAGAAGGC-3′; for resistin, 5′-CCCACGAGCCAC-AGGCAGAGCCACAGGAGCAGC-3′ and for 18S, 5′-CGC-CTGCTGCCTTCCTTGGATGTGGTAGCCG-3′. Prehybridisation was at 42°C for 15 min in DIG-Easy Hyb (Roche, Barcelona, Spain). Hybridisation was at 42°C overnight in DIG-Easy Hyb containing the oligonucleotide probe (34 ng/ml for the specific mRNA and 70 pg/ml for 18S rRNA). Then, hybridised membranes were washed twice for 15 min at room temperature with 2 × SSC–0·1 % SDS (sodium dodecyl sulphate) followed by two 15 min washes at 48°C with 0·1 × SSC–0·1 % SDS. After 1 h blocking at room temperature with Blocking reagent (Roche), the membranes were incubated first with antidigoxigenin-alkaline phosphatase conjugate (Roche) and then with the chemiluminescent substrate CDP-Star (Roche). Finally, membranes were exposed to Hyperfilm ECL (Amersham Biosciences, Barcelona, Spain). Bands in films were analysed by scanner photodensitometry and quantified using the KODAK 1D Image Analysis Software 3.5 (Kodak, Mering, Germany). Blots were stripped and reprobed sequentially for the other specific mRNA. Finally, blots were stripped and reprobed for 18S rRNA as previously described (Oliver *et al.* 2000). Levels of mRNA were expressed as the ratio of their specific signal intensity relative to that for 18S rRNA.

Reverse transcriptase–polymerase chain reaction analysis

Levels of CPT-I, GLUT4, mRNA and 18S rRNA (used as a control) were semi-quantified by a RT–PCR assay (Ribot *et al.* 2001). In brief, 1 μg total RNA was denatured at 65°C for

10 min and reverse-transcribed in the presence of 50 pmol random primers, using MuLV reverse transcriptase (PerkinElmer, Wellesley, MA, USA) at 42°C for 40 min in a PerkinElmer 9700 Thermal Cycler. After the reaction, the RT medium (4 %) was added to a PCR mix containing Taq DNA polymerase (Promega, Lyon, France) and 20 pmol (for CPT-I and GLUT4 mRNA) or 1 pmol (for 18S rRNA) primers. Specific sense and antisense primers used were designed with specific primer analysis software Primer3 (Whitehead Institute for Biomedical Research, Cambridge, MA, USA) and were: for CPT-I, sense primer 5′-GGCTATCTGTGTTCGCCTTC-3′ and antisense primer 5′-TGGGGGCAGTGTTGTTCT-3′; for GLUT4, sense primer 5′-GGCATGGGTTTCCAGTATGT-3′ and antisense primer 5′-GCCCCTCAGTCATTCTCATC-3′ and for 18S rRNA, sense primer 5′-GGACCAGAGCGAAAGC-ATTTGCC-3′ and antisense primer 5′-TCAATCTCGGGTGGC-TGAACGC-3′. The specificity of the sequences and primer binding sites were analysed by the ENTREZ and BLAST database utilities (National Center for Biotechnology Information, Bethesda, MD, USA). The reaction mixture was first heated to 95°C for 2 min to denature the cDNA. This was followed by cycles of denaturation (twenty four for CPT-I, twenty six for GLUT4 and fourteen for 18S rRNA) at 95°C for 15 s, annealing at 60°C (for CPT-I and 18S rRNA) or 61°C (for GLUT4) for 15 s and extension at 72°C for 30 s, with an additional extension at 72°C for 7 min after the last cycle. The PCR products were separated in 3 % agarose gel (MS-8; Pronadisa, Madrid, Spain) in 0·5 × Tris-borate EDTA buffer, stained with ethidium bromide and visualised using an image recording system (GeneSnap/Chemigenius; Syngene, Cambridge, UK). The densities of the target bands were then quantified using an image processing and analysing program (GeneTools; Syngene). Levels of mRNA were expressed as the ratio of signal intensity for specific mRNA relative to that for 18S rRNA. Linearity of the RT–PCR was tested using different amounts of total RNA (results not shown).

Statistical analysis

Data are expressed as means with their standard errors. Statistical significance was assessed by one-way ANOVA followed by the least significant difference *post hoc* comparison or by Student's *t* test. The minimum significance level was set at $P<0·05$. The analyses were performed with SPSS for Windows (SPSS, Chicago, IL, USA).

Results

Trans-10, *cis*-12 CLA feeding, both the low and the high dose, resulted in significantly lower weight of the subcutaneous and perirenal WAT depots, without modifying interscapular BAT weight (Table 1). These effects on adiposity were not dependent on the dose. In addition, animals fed 1 % CLA showed significantly greater gastrocnemius muscles than control animals and those fed 0·5 % CLA. As previously reported (Zabala *et al.* 2006), CLA treatment did not affect final body weight of animals compared with their controls, but animals fed 1 % CLA showed significantly greater final body weight than those fed 0·5 % CLA ($P<0·05$). No significant differences in food intake were found between the three experimental groups.

Leptin mRNA expression levels varied depending on the adipose tissue depot ($P<0·05$). Representative northern blots for leptin expression in adipose tissues are shown comparing the patterns of control hamsters (Fig. 1). As a general feature, leptin expression was higher in subcutaneous WAT than in perirenal WAT and interscapular BAT. A similar pattern for the resistin mRNA expression was observed in the adipose depots analysed, but the differences did not attain statistical significance.

In accordance with lower body fat mass, animals fed CLA (both the low and the high dose) displayed significantly lower leptin mRNA expression levels in both subcutaneous and perirenal WAT depots, and again, no dose-dependent effect was observed (Table 2). A trend to lower circulating leptin was also observed as an effect of CLA (722 (SEM 182) pg/ml in controls, 292 (SEM 91) pg/ml in 0·5 % CLA-treated animals, and 373 (SEM 59) pg/ml in 1 % CLA-treated animals), although differences were not statistically significant ($P=0·057$). Resistin mRNA expression levels in both WAT depots studied tended to decrease by CLA feeding (Table 2) but did not attain statistical significance. No changes were

Table 1. Effects of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) supplementation on body weight, food intake, and the weights of subcutaneous and perirenal white adipose tissues and gastrocnemious muscle*†

(Values are means with their standard errors for ten animals per group)

	Control		0·5 % CLA		1 % CLA		
	Mean	SEM	Mean	SEM	Mean	SEM	ANOVA
Body weight (g)	121	3[ab]	119	1[b]	124	2[a]	$P<0·05$
Food intake (g/d)	6·03	0·14	5·66	0·08	5·73	0·12	
Adipose tissues (g)							
sWAT	3·78	0·25[a]	2·67	0·14[b]	2·95	0·14[b]	$P<0·001$
pWAT	1·68	0·10[a]	1·19	0·09[b]	1·42	0·08[b]	$P<0·05$
BAT	0·34	0·01	0·31	0·01	0·29	0·02	
Gastrocnemious muscle (mg)	598	18[a]	599	19[a]	654	13[b]	$P<0·05$

sWAT, subcutaneous white adipose tissue; pWAT, perirenal white adipose tissue; BAT, interscapular brown adipose tissue.

* 9-week-old, male Syrian Golden hamsters were fed with a semi-purified atherogenic diet supplemented with different doses of *trans*-10, *cis*-12 CLA as NEFA (0 (control), 0·5 and 1·0 g/100 g diet) for 6 weeks.

† Data on body weight, food intake and the weight of gastrocnemius muscle have been previously published (Zabala *et al.* 2006).

[a,b] Values in the same row not sharing a common letter were statistically different. Significant differences were tested by one way ANOVA and least significant difference *post hoc* comparisons ($P<0·05$).

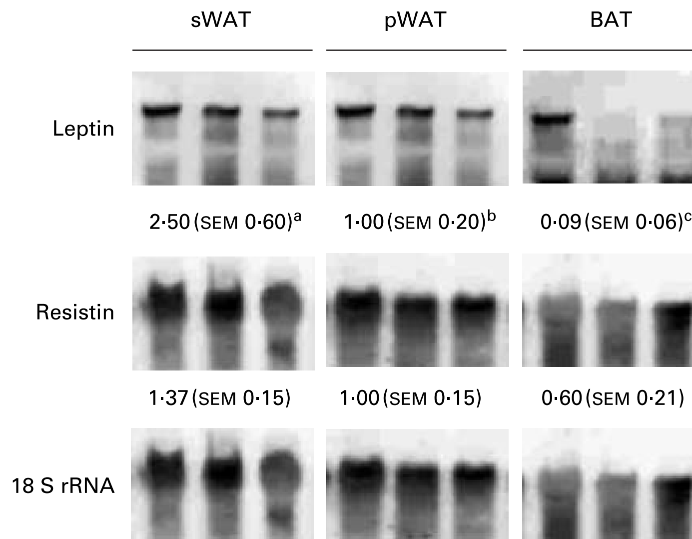

Fig. 1. Representative northern blots comparing the relative mRNA levels of leptin and resistin between subcutaneous white adipose tissue (sWAT), perirenal white adipose tissue (pWAT) and interscapular brown adipose tissue (BAT) in control male hamsters fed an atherogenic diet. Total RNA (25 μg) was used for specific determination of the mRNA, using 18S rRNA as a control for quantity of RNA. Data represent means with their standard errors for 3–5 animals per group and are expressed relative to the mean value of the pWAT, which was set to 1. Significant differences were tested by one-way ANOVA and least significant difference *post hoc* comparisons.[a,b,c] Values in the same row not sharing a common letter were statistically different ($P<0{\cdot}05$).

found concerning leptin and resistin expression in BAT as an effect of CLA feeding (data not shown), although it should be pointed out that in this tissue the expression levels of these genes were very low compared with WAT (see Fig. 1, corresponding to control animals).

UCP1 mRNA expression was measured in BAT and WAT depots and significant expression was found in BAT and also in the perirenal WAT (see Fig. 2, corresponding to control animals). In both depots, CLA feeding resulted in lower UCP1 mRNA levels, the decrease being significant ($P<0{\cdot}05$) with both doses in BAT and with the lower dose in perirenal WAT (Table 3). UCP2 mRNA expression in both WAT depots studied was not significantly affected by CLA feeding, although a tendency to lower levels was observed in both depots (Table 3). No significant UCP2 mRNA expression was detected in BAT (see Fig. 2, corresponding to control animals).

The effect of CLA on UCP3 expression was tissue-specific. Animals fed the high dose of CLA showed decreased UCP3 mRNA expression levels in the subcutaneous WAT depot (Table 3), but increased expression in the skeletal muscle ($P<0{\cdot}05$; Fig. 3). No statistically significant effect was observed in the perirenal WAT depot or BAT. The increased UCP3 mRNA levels in the skeletal muscle with the high dose of CLA were also accompanied by increased CPT-I mRNA expression and a tendency to lower GLUT4 mRNA expression in this tissue (Fig. 3). No significant changes were observed with the low dose of CLA on UCP3, CPT-I and GLUT4 expression in gastrocnemious muscle (data not shown).

Discussion

It is increasingly known that CLA isomers have different effects which may even be opposing (Evans *et al.* 2002*a*,

Table 2. Effects of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) supplementation on the relative leptin and resistin mRNA levels in subcutaneous and perirenal white adipose tissues*

(Values are means with their standard errors for six to nine animals per group and are expressed relative to the mean value of the control group, which was set to 100).

	Control		0·5 % CLA		1 % CLA		
	Mean	SEM	Mean	SEM	Mean	SEM	ANOVA
sWAT							
mRNA Leptin	100	7·3[a]	54·6	4·4[b]	66·8	6·4[b]	$P<0{\cdot}001$
mRNA Resistin	100	9·8	74·4	6·0	86·2	6·1	
pWAT							
mRNA Leptin	100	12[a]	67·8	3·4[b]	69·3	10[b]	$P<0{\cdot}05$
mRNA Resistin	100	15	72·6	8·5	86·3	16	

sWAT, subcutaneous white adipose tissue; pWAT, perirenal white adipose tissue.

* 9-week-old, male Syrian Golden hamsters were fed with a semi-purified atherogenic diet supplemented with different doses of *trans*-10, *cis*-12 CLA as NEFA (0 (control), 0·5 and 1·0 g/100 g diet) for 6 weeks. mRNA levels were analysed by northern blot. Total RNA (25 μg) was used for specific determination of the mRNA, using 18S rRNA as a control for quantity of RNA.

[a,b] Values in the same row not sharing a common letter were statistically different. Significant differences were tested by one way ANOVA and least significant difference *post hoc* comparisons ($P<0{\cdot}05$).

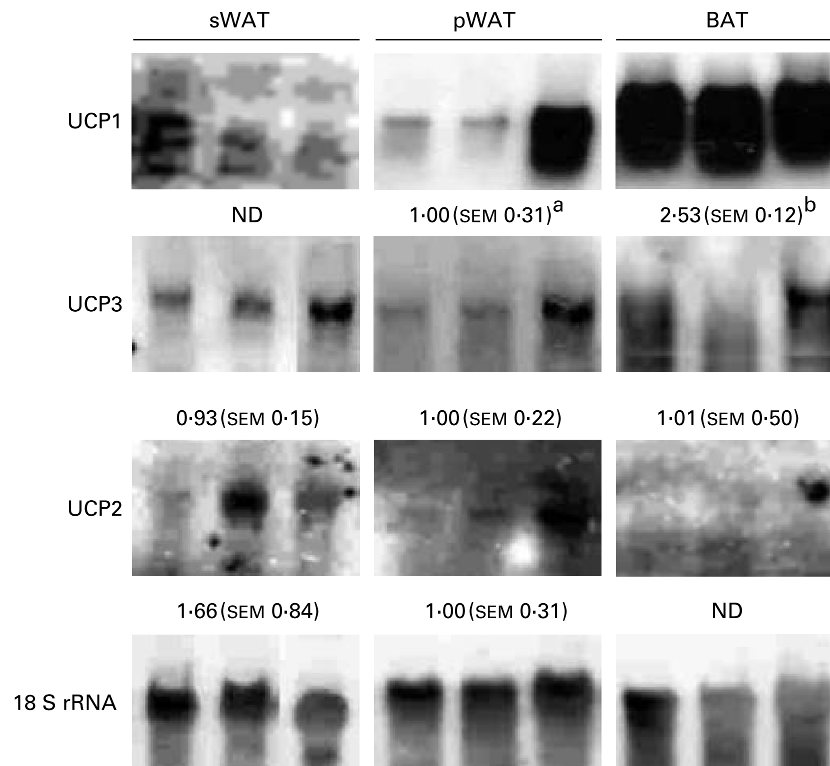

Fig. 2. Representative northern blots comparing the relative mRNA levels of uncoupling proteins (UCP) 1, 2 and 3 between subcutaneous white adipose tissue (sWAT), perirenal white adipose tissue (pWAT) and interscapular brown adipose tissue (BAT) in control male hamsters fed an atherogenic diet. Total RNA (25 μg) was used for specific determination of the mRNA, using 18S rRNA as a control for quantity of RNA. Data represent means with their standard errors for 3–5 animals per group and are expressed relative to the mean value of the pWAT, which was set to 1. Significant differences were tested by one-way ANOVA and least significant difference *post hoc* comparisons.[a,b] Values in the same row not sharing a common letter were statistically different ($P < 0·05$). ND, not detected.

Table 3. Effects of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) supplementation on the relative uncoupling proteins (UCP) 1, 2 and 3 mRNA levels in adipose tissues*

(Values are means with their standard errors for six to nine animals per group and are expressed relative to the mean value of the control group, which was set to 100)

	Control		0·5 % CLA		1 % CLA		
	Mean	SEM	Mean	SEM	Mean	SEM	ANOVA
sWAT							
mRNA UCP1	ND		ND		ND		
mRNA UCP2	100	14	85·0	11	65·0	10	
mRNA UCP3	100	8·9[a]	93·1	3·7[ab]	69·2	11[b]	$P < 0·05$
pWAT							
mRNA UCP1	100	26[a]	31·4	15[b]	51·8	16[ab]	$P < 0·05$
mRNA UCP2	100	31	45·5	14	40·3	11	
mRNA UCP3	100	22	104	3·1	113	9·4	
BAT							
mRNA UCP1	100	20[a]	55·3	7·5[b]	55·6	10[b]	$P < 0·05$
mRNA UCP2	ND		ND		ND		
mRNA UCP3	100	35	146	23	76·7	17	

sWAT, subcutaneous white adipose tissue; pWAT, perirenal white adipose tissue; BAT, interscapular brown adipose tissue; ND, not detected by northern blot.

* 9-week-old, male Syrian Golden hamsters were fed with a semi-purified atherogenic diet supplemented with different doses of *trans*-10, *cis*-12 CLA as NEFA (0 (control), 0·5 and 1·0 g/100 g diet) for 6 weeks. mRNA levels were analysed by northern blot. Total RNA (25 μg) was used for specific determination of the mRNA, using 18S rRNA as a control for quantity of RNA.

[a,b] Values in the same row not sharing a common letter were statistically different. Significant differences were tested by one way ANOVA and least significant difference *post hoc* comparisons ($P < 0·05$).

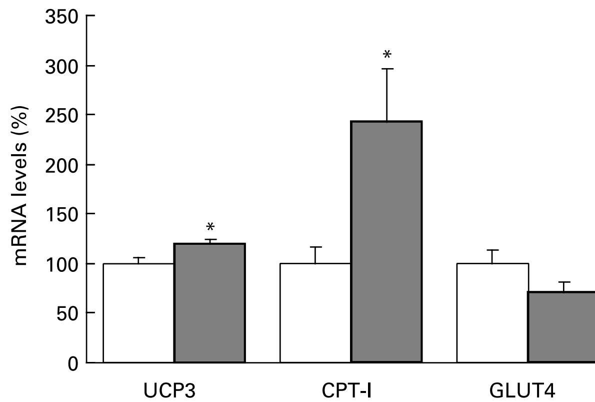

Fig. 3. Effects of *trans*-10, *cis*-12 conjugated linoleic acid (CLA) supplementation on the relative uncoupling protein 3 (UCP3), carnitine palmitoyl transferase I (CPT-I) and GLUT4 in gastrocnemious muscle. Male Syrian Golden hamsters (9-weeks-old) were fed with a semi-purified atherogenic diet supplemented with 1·0 g *trans*-10, *cis*-12 CLA as NEFA/100 g diet (■), or without supplementation (control; □), for 6 weeks. UCP3 mRNA levels were analysed by Northern blot, using 25 μg total RNA and 18S rRNA as a control for quantity of RNA. CPT-I and GLUT4 mRNA levels were analysed by RT–PCR and normalised to the expression of 18S rRNA. Data represent means and their standard errors for 6–10 animals per group and are expressed relative to the mean value of the control group, which was set to 100. Significant differences were tested by Student's *t* test one. Mean values for the CLA supplemented diet were significantly different from the controls: $^{*}P < 0·05$.

Rodriguez *et al.* 2002). Thus, the present study refers specifically to the effects of a single CLA isomer, the one particularly used as a main component in commercial preparations for human consumption (*trans*-10, *cis*-12-CLA) and not a mixture as was tested in the vast majority of previously reported *in vivo* studies (Navarro *et al.* 2006).

Regarding body composition, CLA has been demonstrated to reduce the amount of fat (Pariza, 2004). Among the different species studied, the mouse has been shown to be the most sensitive to CLA, while the effects of CLA on rats has been generally less marked. We have previously shown that hamsters show an intermediate response between mice and rats (Navarro *et al.* 2003); thus, we considered it interesting to analyse the effects of CLA feeding in this species.

One of the potential mechanisms accounting for a decrease in fat content could be through an increase in thermogenesis, which in small mammals is mediated by the UCP and, particularly, BAT (Palou *et al.* 1998; Cannon & Nedergaard, 2004). Nevertheless, the analysis of the effects of CLA on UCP is scarce in the literature and has led to contradictory results. The vast majority of the studies have been performed in mice. One of the most frequently reported effects in this animal model is increased UCP2 expression in WAT (Tsuboyama-Kasaoka *et al.* 2000; Ealey *et al.* 2002; Roche *et al.* 2002; Warren *et al.* 2003) and BAT (West *et al.* 2000; Ealey *et al.* 2002; Roche *et al.* 2002; Takahashi *et al.* 2002), although other authors have found no significant changes in WAT (Takahashi *et al.* 2002). The effects on UCP1 in BAT have also been considered: some authors have found decreased mRNA levels after CLA feeding (Ealey *et al.* 2002; Takahashi *et al.* 2002) but others found no significant changes (West *et al.* 2000). With regard to UCP3, increased expression in skeletal muscle has been reported by Roche *et al.* (2002) but this UCP remains unchanged in other studies (Ealey *et al.* 2002; Takahashi *et al.* 2002). In Sprague-Dawley rats, no changes in the expression of UCP were observed by using either *trans*-10, *cis*-12 or a CLA mixture (Ealey *et al.* 2002).

The different results published concerning the effects of CLA on UCP expression in BAT can be attributed, at least partially, to the different effects produced by both main isomers of CLA. Most of the *in vivo* studies have used mixtures containing several CLA isomers, mainly *cis*-9, *trans*-11 and *trans*-10, *cis*-12 in equal concentration. By using cultured brown adipocytes, we have described opposite effects of both CLA isomers on thermogenic capacity (Rodriguez *et al.* 2002): *trans*-10, *cis*-12 CLA inhibits both UCP1 and UCP2 mRNA expression, while the *cis*-9, *trans*-11 isomer increases UCP1 expression.

Our results show a decrease rather than an increase in UCP1 expression in BAT, while UCP2 expression was not detected, and the effects on UCP3 if any were not statistically significant. We also measured UCP expression in subcutaneous and perirenal WAT depots. We found no significant effects on UCP2 expression by the *trans*-10, *cis*-12 CLA treatment. Of interest, we found significant UCP1 expression in the perirenal WAT, but not in the subcutaneous depot, and its expression was also significantly reduced by CLA feeding, as in BAT. CLA feeding also reduced UCP3 expression in subcutaneous WAT, without affecting its expression in the perirenal depot. Although we do not have a direct measure of core temperature, these results suggest that CLA effects in hamsters are not mediated through adipose tissue thermogenesis.

The effect of CLA increasing UCP3 mRNA expression in skeletal muscle differed from that described for adipose tissues. Tissue-specific responses in UCP3 regulation have also been reported under other experimental conditions such as triiodothyronine treatment and retinoid treatments, and fasting (Gong *et al.* 1997; Felipe *et al.* 2003).

UCP3 has been proposed to be more important as a regulator of fatty acid utilisation than as an uncoupler involved in thermogenesis (Samec *et al.* 1998). Thus, UCP3 in skeletal muscle seems to be primarily associated with the regulation of lipids as fuel and increased fatty acid oxidation (Wang *et al.* 2003; Bezaire *et al.* 2005). Here we show that not only UCP3 but also CPT-I expression were up regulated by the high dose of CLA. We have previously reported increased CPT-I activity in skeletal muscle of hamsters fed either 0·5 % or 1 % *trans*-10, *cis*-12 CLA (Zabala *et al.* 2006). Thus, considering that skeletal muscle plays the largest role in fatty acid oxidation in the body due to its relative whole size, it can be proposed that increased fatty acid oxidation in skeletal muscle is one of the mechanisms that contributes to the decrease in adiposity induced by *trans*-10, *cis*-12 CLA. On the other hand, although no significant changes were found in skeletal muscle concerning the expression of GLUT4, an important determinant of the muscle capacity for glucose transport activated by insulin (Henriksen *et al.* 1990), a tendency to lower expression levels (30 % decrease compared with controls) as an effect of CLA treatment may indicate some preferential use of fatty acids instead of glucose as fuel.

Different results have been published concerning the effect of CLA on muscle GLUT4 expression. Tsuboyama-Kasaoka *et al.* (2000) found increased GLUT4 expression levels in gastrocnemius muscle of C57BL/6J mice fed CLA, while other authors, Henriksen *et al.* (2003), in diabetic Zucker rats, and Takahashi *et al.* (2002), in two different strains of mice, did not find significant effects. We also determined GLUT4 mRNA expression levels in muscle in a reduced group of three animals fed a standard diet, instead of the atherogenic diet, and levels were significantly lower than those of animals fed the atherogenic diet free of CLA (37·8 (SEM 18·0) %). Thus, it should be mentioned that the effect of CLA on GLUT4 mRNA expression in the muscle would tend to normalise the increased expression caused by feeding the atherogenic diet used in the present experimental design.

UCP3 expression in muscle is stimulated by the elevation of circulating NEFA. However, it is not likely that the up-regulation of UCP3 found in this study is explained by this mechanism because we previously found no changes in the concentration of circulating NEFA in hamsters as an effect of 0·5 % CLA feeding in the same conditions as in this study (Simon *et al.* 2006). This is in agreement with the concept that regulation of UCP3 expression by fat could be more related to actual increases in the rate of fat oxidation than to high NEFA levels *per se*. In fact, studies in human subjects under high fat diets showed increased fat oxidation and UCP3 expression in muscle that were not accompanied by increases in circulating NEFA levels (Schrauwen *et al.* 1997, 2001).

We also determined leptin mRNA expression levels in adipose tissue from different anatomical localisations. We found that, in hamsters, leptin is expressed at higher levels in the subcutaneous than in the perirenal WAT; in the latter, lower leptin expression can be related to significant expression of UCP1, thus indicating differences between both fat depots. In both depots, leptin mRNA expression decreased as an effect of CLA feeding, independently of the dose. This decrease can be related to the decrease in the size of these fat depots. Leptin expression in BAT was not affected by CLA feeding, neither was the size of this depot. A trend to lower circulating leptin levels was also observed after CLA feeding. A decrease in blood leptin levels as an effect of CLA treatment has been previously described in C57BL76J mice (Tsuboyama-Kasaoka *et al.* 2000; Yamasaki *et al.* 2000; Rahman *et al.* 2001). It is known that leptin can enhance insulin-mediated stimulation of glucosal disposal (Kamohara *et al.* 1997; Cusin *et al.* 1998). Thus, the decrease in leptin expression as an effect of CLA could be one of the mechanisms that contribute to the development of insulin resistance, as previously suggested (Tsuboyama-Kasaoka *et al.* 2000).

We have previously described that although *trans*-10, *cis*-12-CLA feeding in hamsters prevents adiposity, it cannot prevent insulin resistance induced by feeding an atherogenic diet. Since no changes were found either in blood glucose and insulin levels or in the homeostatic model assessment for insulin resistance index with both doses of CLA used (Simon *et al.* 2006; Zabala *et al.* 2006), then the potential involvement of the decreased leptin production, as well as the tendency to decreased GLUT4 transporter expression in gastrocnemious muscle, in the maintenance of impaired insulin sensitivity cannot be ruled out. In spite of the effect of CLA on the size of adipose tissue depots, we did not find significant differences between the three experimental groups concerning resistin expression, which in rodents is related to the development of insulin resistance (Kusminski *et al.* 2005).

In summary, our results do not sustain increased thermogenesis in the adipose tissue, mediated by UCP, as a clear determinant of *trans*-10, *cis*-12 CLA body-fat lowering effect. Rather, skeletal muscle UCP3 and CPT-I up-regulation seems to be related to increased fatty acid oxidation and, thus, decreased availability of this lipid species for TAG accumulation.

Acknowledgements

This work was supported by the Spanish Government (grants G03/028, BFI2003-04 439, and AGL 2004-07 496/ALI to A. P. and BFI2002-0273 to M. P. P.) and the University of the Basque Country (grant GIU03/18 to M. P. P.). The group of Bioquímica, Biología Molecular, Nutrición y Biotecnología (Nutrigenómica) of the UIB is a member of the European Research Network of Excellence NuGO (The European Nutrigenomics Organization, EU Contract: FP6-506360).

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