British Journal of Nutrition (2004), **92**, 383–389
DOI: 10.1079/BJN20041220

The *trans*-10,*cis*-12 isomer of conjugated linoleic acid reduces hepatic triacylglycerol content without affecting lipogenic enzymes in hamsters

Amaia Zabala[1], Itziar Churruca[1], M. Teresa Macarulla[1], Víctor M. Rodríguez[1], Alfredo Fernández-Quintela[1], J. Alfredo Martínez[2] and María P. Portillo[1]*

[1]*Department of Nutrition and Food Science, University of País Vasco, Paseo de la Universidad 7, 01006 Vitoria, Spain*
[2]*Department of Physiology and Nutrition, University of Navarra, c/ Irunlarrea s/n, 31008 Pamplona, Spain*

(Received 16 January 2004 – Revised 3 May 2004 – Accepted 10 May 2004)

Conjugated linoleic acid (CLA) refers to the positional and geometric dienoic isomers of linoleic acid. The dietary intake of CLA has been associated with changes in lipid metabolism. The aim of the present work was to assess the effects of the two main isomers of CLA on sterol regulatory element binding protein (SREBP)-1a and SREBP-1c mRNA levels, as well as on mRNA levels and the activities of several lipogenic enzymes in liver. For this purpose hamsters were fed an atherogenic diet supplemented with 5 g linoleic acid, *cis*-9,*trans*-11 or *trans*-10,*cis*-12 CLA/kg diet for 6 weeks. The *trans*-10,*cis*-12 isomer intake produced significantly greater liver weight, but also significantly decreased liver fat accumulation. No changes in mRNA levels of SREBP-1a, SREBP-1c and lipogenic enzymes, or in the activities of these enzymes, were observed. There was no effect of feeding *cis*-9,*trans*-11 CLA. These results suggest that increased fat accumulation in liver does not occur on the basis of liver enlargement produced by feeding the *trans*-10,*cis*-12 isomer of CLA in hamsters. The reduction in hepatic triacylglycerol content induced by this isomer was not attributable to changes in lipogenesis.

Conjugated linoleic acid: Lipogenesis: Sterol regulatory element binding protein: Liver: Hamster

Conjugated linoleic acid (CLA) refers to different positional and geometric dienoic isomers of linoleic acid. These fatty acids are a natural food component occurring in the lipid fraction of meat, milk and other dairy products. The *cis*-9,*trans*-11 CLA isomer is the principal dietary form of CLA, but lower levels of *trans*-10,*cis*-12 and other isomers are also present in these food sources (Roche *et al.* 2001).

CLA intake is known to produce changes in lipid metabolism in adipose tissue and liver. A great deal of work has been performed on adipose tissue (Pariza *et al.* 2000; Roche *et al.* 2001). In contrast, data on the effects of CLA on liver are scarcer. In some studies it has been reported that CLA consumption may modify hepatic fatty acid oxidation and synthesis (Park *et al.* 1997; Martin *et al.* 2000; Takahashi *et al.* 2003; Tsuboyama-Kasaoka *et al.* 2003).

The transcription of genes encoding lipogenic enzymes, such as fatty acid synthase and acetyl-CoA carboxylase, is controlled by a family of membrane-bound transcriptional factors known as sterol regulatory element binding proteins (SREBP; Pai *et al.* 1998). Three types of SREBP have been identified. It has been shown that SREBP-1a and SREBP-1c, both derived from a single gene, participate in the regulation of the gene expression of enzymes involved in fatty acid synthesis (Shimano *et al.* 1997; Shimano, 2001; Amemiya-Kudo *et al.* 2002; Horton *et al.* 2002).

Numerous studies have found that SREBP respond to nutrients and to hormones to control lipid metabolism (Shimomura *et al.* 1999; Osborne, 2000). PUFA have been shown to decrease SREBP-1 mRNA levels, as well as the nuclear content of SREBP-1 in liver, thus leading to reduced lipogenic enzyme gene expression (Kim *et al.* 1999; Xu *et al.* 1999, 2002; Yahagi *et al.* 1999).

The aim of the present work was to assess the effects of the two main isomers of CLA, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA, on SREBP-1a and SREBP-1c expression, as well as on the expression and activity of several lipogenic enzymes in livers from hamsters fed an atherogenic diet.

Material and methods

Animals, diets and experimental design

The experiment was conducted with twenty-four 9-week-old male Syrian Golden hamsters (body weight 105 (SEM 1) g) purchased from Harlan Ibérica (Barcelona, Spain) and was 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 groups of eight animals each and, over a period of 6 weeks, fed the semi-purified

Abbreviations: CLA, conjugated linoleic acid; SREBP, sterol regulatory element binding protein.

* **Corresponding author:** Dr María Puy Portillo, fax +34 945 013014, email knppobam@vc.ehu.es

atherogenic diets supplemented with 5 g linoleic acid, *cis*-9,*trans*-11 or *trans*-10,*cis*-12 CLA/kg diet (Table 1). The experimental diets were freshly prepared once per week, gassed with N_2 and stored at 0–4°C to avoid rancidity. DL-Methionine and cholesterol were supplied by Sigma (St Louis, MO, USA), wheat starch and cellulose by Vencasser (Bilbao, Spain), conjugated linoleic isomers by Natural Lipids Ltd (Hovdebygda, Norway), sucrose and sunflower oil were obtained from local markets, and palm oil was a generous gift from Agra-Unilever (Leioa, Spain). Vitamin and mineral mixes were formulated according to AIN-93 guidelines (Reeves *et al.* 1993) and supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). All animals had free access to food and water. Food intake and body weight were measured daily.

At the end of the experimental period animals were killed under anaesthesia (diethyl ether) and blood was collected by cardiac puncture. Adipose tissue from different locations (perirenal, epididymal and subcutaneous) and liver were dissected, weighed 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.

Hepatic triacylglycerol content

Total lipids were extracted from liver following the method described by Folch *et al.* (1957). The lipid extract was dissolved in isopropanol. Triacylglycerol was measured using a commercial kit (Biosystems, Barcelona, Spain).

Extraction and analysis of RNA and semiquantification by RT–PCR

Total RNA was isolated from 100 mg liver using Ultraspec-II (Biotecx Laboratories, Houston, TX, USA) according to the manufacturer's instructions. RNA (1·5 μg) was used to synthesize first-strand complementary DNA (cDNA) after 60 min at 37°C treatment with RNase-free DNase I (Roche, Mannheim, Germany). The RT–PCR was carried out as described by Corbalan *et al.* (1999). Specific sense and antisense primers used for SREBP-1a, SREBP-1c, fatty acid synthase and acetyl-CoA carboxylase mRNA quantification were those described by Field *et al.* (2003); primers used to amplify β-actin were designed using the Oligo 6.0 Primer Analysis Software for Windows (National Biosciences, Annapolis, MN, USA) (Table 2). cDNA was amplified using the variables shown in Table 2. A first step of denaturation (95°C for 30 s) and a final extension step (72°C for 7 min) were applied for all primers. Amplifications were linear under these conditions and carried out in a Gene Amp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). Our preliminary experiments demonstrated that the cycle numbers were well below the plateau phase for PCR products. Linearity of the PCR reactions were tested by amplification of 200 ng first strand cDNA per reaction from twenty to forty cycles. The amplified products were resolved in an agarose (12 g/kg) gel (MS-8, Pronadisa, Madrid, Spain) and stained with ethidium bromide. PCR band intensity was semiquantified by scanning densitometric analysis using the Gel Doc 1000 UV fluorescent gel documentation system and Molecular Analyst software for quantification of images (Bio-Rad Laboratories, Hercules, CA, USA). The values were normalized using β-actin as an endogenous internal standard.

Lipogenic activities

Liver samples (0·5 g) were homogenized in 5 ml buffer (pH 7·6) containing 150 mM-KCl, 1 mM-$MgCl_2$, 10 mM-*N*-acetyl-cysteine and 0·5 mM-dithiothreitol.

After centrifugation at 100 000 ***g*** for 40 min at 4°C, the supernatant fraction was used for quantification of enzyme activities. Glucose-6-phosphate dehydrogenase was determined from the rate of glucose-6-phosphate-dependent reduction of $NADP^+$ (Kuby & Noltmann, 1966), malic enzyme from the rate of malate-dependent $NADP^+$ reduction (Hsu & Lardy, 1969) and fatty acid synthase from the rate of malonyl-CoA dependent NADPH

Table 1. Composition of experimental diets (g/kg)*

Ingredients	Linoleic acid	*cis*-9,*trans*-11 CLA	*trans*-10,*cis*-12 CLA
Casein†	200	200	200
DL-Methionine	4	4	4
Sucrose	404	404	404
Wheat starch	200	200	200
Palm oil	100	100	100
Sunflower oil‡	8·2	–	–
cis-9,*trans*-11 CLA§	–	5·6	–
trans-10,*cis*-12 CLA§	–	–	5·6
Cholesterol	1	1	1
Cellulose	30	30	30
Mineral mix‖	40	40	40
Vitamin mix‖	11	11	11
Choline chloride salt	4	4	4

CLA, conjugated linoleic acid.
* For details of suppliers of ingredients, see p. 384.
† 900 g casein/kg.
‡ 617 g linoleic acid/kg (Rodríguez *et al.* 2002).
§ 900 g CLA/kg.
‖ Formulated according to the American Institute of Nutrition (AIN-93; Reeves *et al.* 1993).

Table 2. Primers for PCR amplification gene products and specific conditions used for the amplification of each gene studied

Primers...	Sense primer	Antisense primer	Length (bp)	GenBank accession no.
SREBP-1a	5′-GGCTGTGGAACAGGCACTG-3′	5′-AGCTGGAGCATGTCTTCGATG-3′	78	Field *et al.* (2003)
SREBP-1c	5′-GCGGACGCAGTCTGGG-3′	5′-ATGAGCTGGAGCATGTCTTCAAA-3′	95	Field *et al.* (2003)
FAS	5′-AGCCCCTCAAGTGCACAGTG-3′	5′-TGCCAATGTGTTTTCCCTGA-3′	81	AF356086
ACC	5′-ACACTGGCTGGCTGGACAG-3′	5′-CACACAACTCCCAACATGGTG-3′	76	AF356089
β-Actin	5′-TCTACAATGAGCTGCGTGTG-3′	5′-GGTCAGGATCTTCATGAGGT-3′	300	J00691
Conditions...	Annealing	Elongation	No. of cycles	
SREBP-1a	60°C – 60 s	60°C – 60 s	29	
SREBP-1c	60°C – 60 s	60°C – 60 s	30	
FAS	60°C – 60 s	60°C – 60 s	30	
ACC	60°C – 60 s	60°C – 60 s	33	
β-Actin	59°C – 30 s	72°C – 30 s	32	

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

oxidation (Lynen, 1969). NADPH was measured by reading absorbance at 340 nm. Acetyl-CoA carboxylase was measured from the acetyl-CoA-dependent incorporation of $^{14}HCO_3^-$ into acid-stable product (Chang *et al.* 1967; Chakrabarty & Leveille, 1969). All enzyme assays were conducted at 37°C. Soluble protein in the supernatant fraction was determined using bovine serum albumin as standard (Bradford, 1976). Glucose-6-phosphate dehydrogenase and malic enzyme activities were expressed as nmol NADPH produced/min per mg protein, fatty acid synthase activity as nmol NADPH consumed/min per mg protein and acetyl-CoA carboxylase activity as nmol bicarbonate incorporated/min per mg protein.

Serum variables

Serum glucose and insulin were measured by spectrophotometry and RIA respectively using commercial kits (Bio-Systems, Barcelona, Spain; Linco, St Charles, MO, USA).

Statistical analysis

Results are presented as mean values with their standard errors. Statistical analysis was performed using SPSS 8.0 (SPSS Inc., Chicago, IL, USA). Data were analysed by one-way ANOVA followed by Newman–Keuls *post hoc* test. Statistical significance was at $P<0.05$.

Results

Body weight, food intake, adipose tissue and liver weights, and hepatic triacylglycerol content

No differences in final body weight and total food intake were found among the experimental groups. There was a significant effect of *trans*-10,*cis*-12 CLA on liver weight and composition: the addition of this CLA isomer to the diet led to significantly greater liver weight, whether expressed as absolute value or as percentage of total body weight ($P<0.05$). This effect was not due to increased fat accumulation; on the contrary, hamsters from the *trans*-10,*cis*-12 CLA group had significantly lower triacylglycerol content (−34 % *v.* linoleic group; $P<0.01$) (Table 3). No changes in these variables were induced by *cis*-9,*trans*-11 CLA intake.

The *trans*-10,*cis*-12 CLA isomer, but not the *cis*-9,*trans*-11 isomer, also reduced fat accumulation in adipose tissue (−36 % *v.* linoleic group; $P<0.001$). The results in Table 3 show the weight of white adipose tissue (a pool of perirenal, epididymal and subcutaneous depots).

Liver gene expression and activities of lipogenic enzymes

mRNA levels of SREBP-1a and SREBP-1c were not affected by CLA feeding (Fig. 1). Likewise, mRNA

Table 3. Adipose tissue and liver weights, and hepatic triacylglycerol content in hamsters fed on the experimental diets for 6 weeks*

(Mean values with their standard errors for eight hamsters per group)

Experimental groups...	Linoleic acid		*cis*-9,*trans*-11 CLA		*trans*-10,*cis*-12 CLA		Statistical significance of effect (ANOVA)
	Mean	SEM	Mean	SEM	Mean	SEM	
Adipose tissue weight†							
g	6·80[a]	0·43	6·07[a]	0·26	4·36[b]	0·25	$P<0.001$
% body weight	5·64[a]	0·30	5·06[a]	0·20	3·72[b]	0·19	$P<0.001$
Liver weight							
g	4·62[b]	0·16	4·48[b]	0·19	5·10[a]	0·11	$P<0.05$
% body weight	3·84[b]	0·11	3·74[b]	0·13	4·37[a]	0·08	$P<0.01$
Hepatic triacylglycerol content							
mg/g	7·21[a]	0·60	6·50[a]	0·56	4·79[b]	0·48	$P<0.01$
mg/liver	33·3[a]	2·9	29·1[ab]	2·7	24·2[b]	2·1	$P<0.05$

CLA, conjugated linoleic acid.
[a,b] Mean values within the same row with unlike superscript letters were significantly different ($P<0.05$).
* For details of diets and procedures, see Table 1 and pp. 383–384.
† A pool of perirenal, epididymal and subcutaneous adipose tissues.

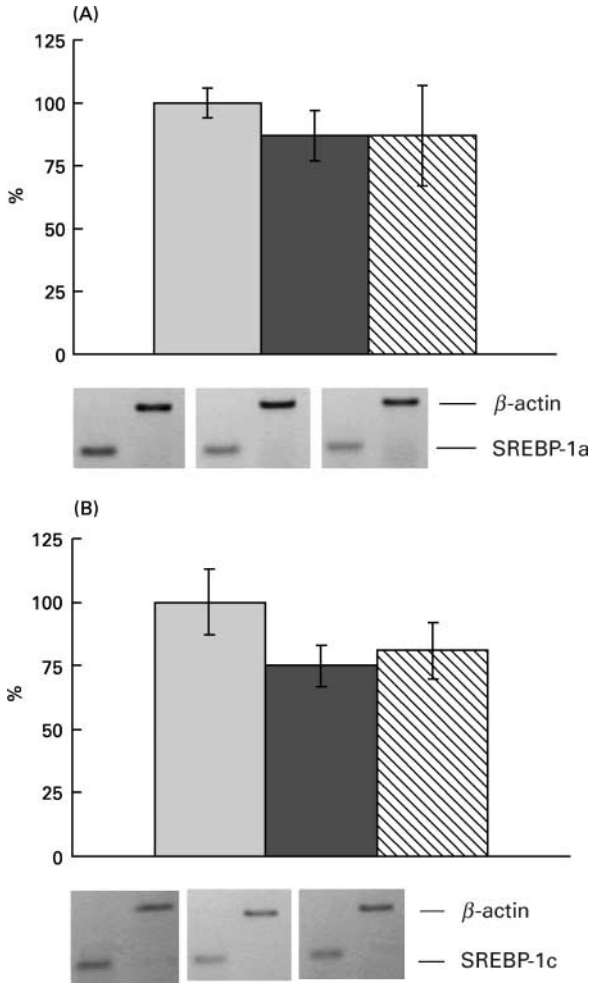

Fig. 1. RT–PCR analysis of sterol regulatory element binding protein (SREBP)-1a mRNA (A) and SREBP-1c mRNA (B) in the liver of hamsters fed the experimental diets. ▫, Linoleic acid; ■, *cis*-9,*trans*-11 conjugated linoleic acid; ▨, *trans*-10,*cis*-12 conjugated linoleic acid. For details of diets and procedures, see Table 1 and pp. 383–384. The results are shown as values relative to mRNA levels in the linoleic acid group.

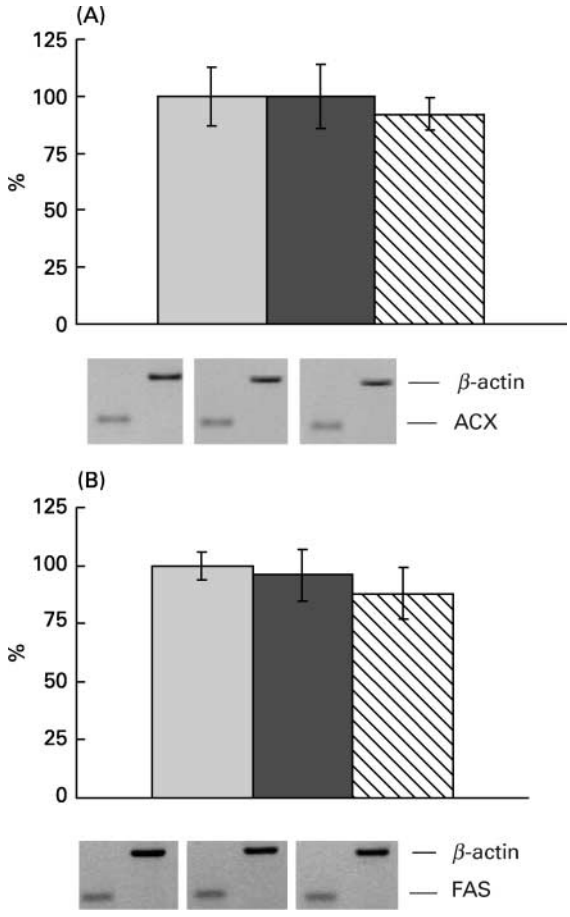

Fig. 2. RT–PCR analysis of acetyl-CoA carboxylase (ACC) mRNA (A) and fatty acid synthase (FAS) mRNA (B) in the liver of hamsters fed the experimental diets. ▫, Linoleic acid; ■, *cis*-9,*trans*-11 conjugated linoleic acid; ▨, *trans*-10,*cis*-12 conjugated linoleic acid. For details of diets and procedures, see Table 1 and pp. 383–384. The results are shown as values relative to mRNA levels in the linoleic acid group.

levels of acetyl-CoA carboxylase and fatty acid synthase were not modified by dietary CLA isomers (Fig. 2). We found no changes in the SREBP-regulated enzyme activities (acetyl-CoA carboxylase and fatty acid synthase) or in glucose-6-phosphate dehydrogenase and malic enzyme activities (enzymes that provide NADPH for reductive biosynthesis) (Table 4).

Serum variables

Neither serum glucose concentration (9·56 (SEM 0·91) mmol/l in linoleic acid group, 9·25 (SEM 1·27) mmol/l in *cis*-9,*trans*-11 CLA group and 9·35 (SEM 0·59) mmol/l in *trans*-10,*cis*-12 CLA group, $P > 0{\cdot}05$) nor serum insulin concentration (176 (SEM 25) pmol/l in linoleic acid group, 144 (SEM 12) pmol/l in *cis*-9,*trans*-11 CLA group and 184 (SEM 16) pmol/l in *trans*-10,*cis*-12 CLA group, $P > 0{\cdot}05$) were significantly modified by CLA isomer intake.

Discussion

Several studies in mice have shown that feeding CLA leads to an increase in liver weight (DeLany *et al.* 1999; Martin *et al.* 2000; Hamura *et al.* 2001; Miner *et al.* 2001; Ealey *et al.* 2002; Terpstra *et al.* 2002; Warren *et al.* 2003). In some of these trials, hepatic triacylglycerol content was measured and it was concluded that hepatomegaly was due to lipid accumulation (Belury & Kempa-Steczko, 1997; West *et al.* 1998; Tsuboyama-Kasaoka *et al.* 2000; Takahashi *et al.* 2003). In contrast, the studies performed on rats showed no effects on liver weight induced by CLA (Chin *et al.* 1994; Sakono *et al.* 1999; Azain *et al.* 2000; Martin *et al.* 2000; Stangl, 2000; Clément *et al.* 2002; Wang *et al.* 2003).

These findings suggest the existence of a species-specific response of liver to CLA feeding (Moya-Camarena & Belury, 1999). Differences among species have also been described in terms of CLA-induced reductions in body-fat gain; thus, species responsiveness is of the following order: mice > hamster > rats (Kim *et al.* 2002). In order

Table 4. Hepatic lipogenic enzyme activities in hamsters fed on the experimental diets for 6 weeks*
(Mean values with their standard errors for eight hamsters per group)

Experimental groups...	Linoleic acid		*cis*-9,*trans*-11 CLA		*trans*-10,*cis*-12 CLA		Statistical significance of effect (ANOVA)
	Mean	SEM	Mean	SEM	Mean	SEM	
Glucose 6-phosphate dehydrogenase†	59·6	2·2	63·4	2·2	67·5	4·3	NS
Malic enzyme†	207	6	195	3	192	9	NS
Fatty acid synthase‡	150	8	174	16	165	11	NS
Acetyl-CoA carboxylase§	13·3	1·5	15·5	1·5	17·3	1·4	NS

CLA, conjugated linoleic acid.
* For details of diets and procedures, see Table 1 and pp. 383–384.
† nmol NADPH formed/mg protein per min.
‡ nmol NADPH consumed/mg protein per min.
§ nmol HCO_3^- incorporated/mg protein per min.

to gain insight into these differences among species, the present work was carried out using the hamster as experimental model.

The present results showed that feeding the *trans*-10,*cis*-12 CLA isomer led to greater liver weight. This effect was similar to that found in mice, but in hamsters the effect was not due to increased fat accumulation; on the contrary, the liver triacylglycerol content in animals fed this CLA isomer was significantly lower than that in the linoleic acid group. Moreover, a significant increase in the hepatocyte number was found (23 % $P<0{\cdot}01$; MT Macarulla, A Fernández-Quintela, A Zabala, V Navarro, E Echevarria, I Charruca, VM Rodríguez and MP Portillo, unpublished results). Tsuboyama-Kasaoka *et al.* (2003) proposed that liver fat accumulation in mice was probably a result of an important fat mobilization from adipose tissue. Thus, when they added 10 g CLA mixture/kg diet, which provided 4 % total fat, animals showed lipodystrophy. This situation led to a great fatty acid delivery into liver, and probably not all fatty acids could be oxidized. In the present study, 5 g *trans*-10,*cis*-12 CLA/kg diet led to a significant, but not massive, reduction in adipose tissue size (−36 % *v.* linoleic acid group) and thus, the net flux of NEFA to liver can be assumed to be lower. All these results demonstrate that the response of liver to CLA is different in the hamster compared with other rodent species, such as the mouse and the rat.

It is well known that lipogenesis and fatty acid oxidation are metabolic pathways controlling liver lipid content. We previously studied the effects of CLA feeding on mitochondrial–peroxisomal fatty acid oxidation and we found that *trans*-10,*cis*-12 CLA, but not *cis*-9,*trans*-11 CLA, induced a significant increase in the activity of hepatic carnitine palmitoyltransferase-I (43·0 %, $P<0{\cdot}01$) (Zabala *et al.* 2003) and acyl-CoA oxidase (157·3 %, $P<0{\cdot}01$), a rate-limiting enzyme in peroxisomal oxidation (MT Macarulla, A Fernández-Quintela, A Zabala, V Navarro, E Echevarria, I Charruca, VM Rodríguez and MP Portillo, unpublished results). Thus, increased fatty acid oxidation is probably responsible, at least in part, for the reduced hepatic triacylglycerol content.

Hepatic fatty acid oxidation and synthesis are regulated reciprocally under various nutritional and physiological conditions. Thus, in the present experimental design, where the activity of enzymes regulating fatty acid oxidation was significantly increased, a decreased lipogenesis could be expected. In order to analyse the effects of CLA feeding on this metabolic pathway, relative mRNA levels and activity of lipogenic enzymes, as well as relative mRNA levels of the SREBP involved in its regulation, were assessed.

Although the predominant form of SREBP-1 in hamster, mouse and human liver is SREBP-1c (Kim *et al.* 1999; Shimano, 2001), in the present study both SREBP-1a and SREBP-1c were studied because it has been reported that some nutritional factors can affect these two transcripts differently. Thus, Horton *et al.* (1998) showed that 24 h fasting decreased SREBP-1c mRNA expression by 60 %, but not SREBP-1a mRNA, and Kim *et al.* (1999) observed greater reduction in SREBP-1c than in SREBP-1a when mice were fed a high-fish-oil diet.

In the present study no changes in the expression of SREBP-1a, SREBP-1c and lipogenic enzymes (acetyl-CoA carboxylase and fatty acid synthase) induced by both CLA isomers were found. Despite this lack of effect, the possibility that mRNA levels of transcription factors and enzymes did not parallel enzyme activities should not be discarded. Thus, activities of acetyl-CoA carboxylase, fatty acid synthase and enzymes that provide NADPH for reductive biosynthesis (glucose-6-phosphate dehydrogenase and malic enzyme) were also assessed, and no effects of CLA isomers were found. Finally, serum concentrations of glucose and insulin, two important regulators of SREBP and lipogenic enzyme expression and activity, remained unchanged. These results suggest that changes in lipogenesis are not on the basis of the *trans*-10,*cis*-12 effects on liver composition. This is in accordance with the results published by Tsuboyama-Kasaoka *et al.* (2003). They found that lipogenesis was not affected when a CLA mixture (10 g/kg diet) accounted for 3 % total dietary fat, but was significantly increased when the same amount of the CLA mixture accounted for 25 % total dietary fat. In the present experimental design CLA isomers represented about 5 % total dietary fat. Together these results suggest that the effect of CLA on hepatic lipogenesis depends on the CLA:total fat ratio.

In summary, when liver is studied, the responsiveness of hamsters to CLA feeding is different from that elicited by mice and rats. The *trans*-10,*cis*-12 CLA isomer, but not the the *cis*-9,*trans*-11 CLA, produces an increase in liver weight, but a decrease in hepatic triacylglycerol content. The reduced fat deposition in liver is not apparently due

to changes in lipogenesis and should be ascribed to an increase in fatty acid oxidation.

Acknowledgements

This study was supported by grants from the Ministerio de Ciencia y Tecnología (BFI2002-00273), the Government of the Basque Country (Biogune, Programa Etortek) and University of the País Vasco (00101.125-E-14788/2002). V. Navarro has a doctoral fellowship from the Spanish Government (Ministerio de Ciencia y Tecnología). Palm oil was a generous gift from Agra-Unilever Foods España S.A. (Leioa, Spain).

References

Amemiya-Kudo M, Shimano H, Hasty AH, *et al.* (2002) Transcriptional activities of nuclear SREBP-1a, -1c, and -2 to different target promoters of lipogenic and cholesterolgenic genes. *J Lipid Res* **43**, 1220–1235.

Azain MJ, Hausman DB, Sisk MB, Flatt WP & Jewell DE (2000) Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. *J Nutr* **130**, 1548–1554.

Belury MA & Kempa-Steczko A (1997) Conjugated linoleic acid modulates hepatic lipid composition in mice. *Lipids* **32**, 199–204.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**, 248–254.

Chakrabarty K & Leveille GA (1969) Acetyl-CoA carboxylase and fatty acid synthase activities in liver and adipose tissue of meal-fed rats. *Proc Sci Exp Biol Med* **131**, 1051–1054.

Chang HC, Seidman I, Teebor G & Lane DH (1967) Liver acetyl-CoA carboxylase and fatty acid synthase: relative activities in the normal state and in hereditary obesity. *Biochim Biophys Res Comm* **28**, 682–686.

Chin SF, Storkson JM, Albright KJ, Cook ME & Pariza MW (1994) Conjugated linoleic acid is a growth factor for rats as shown by enhanced weight gain and improved feed efficiency. *J Nutr* **124**, 2344–2349.

Clément L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief S, Staels B & Bernard P (2002) Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. *J Lipid Res* **43**, 1400–1409.

Corbalan MS, Margareto J, Martínez JA & Martí A (1999) High-fat feeding reduced muscle uncoupling protein 3 expression in rats. *J Physiol Pharmacol* **55**, 67–72.

DeLany JP, Blohm F, Truett AA, Scimeca JA & West DB (1999) Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. *Am J Physiol* **276**, R1172–R1179.

Ealey KN, El-Sohemy A & Archer MC (2002) Effects of dietary conjugated linoleic acid on the expression of uncoupling proteins in mice and rats. *Lipids* **37**, 853–861.

Field FJ, Born E & Mathur SN (2003) Fatty acid flux suppress fatty acid synthesis in hamsters intestine independently of SREBP1 expression. *J Lipid Res* **44**, 1199–1208.

Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from the animal tissues. *J Biol Chem* **226**, 497–509.

Hamura M, Yamatoya H & Kudo S (2001) Glycerides rich in conjugated linoleic acid (CLA) improve blood glucose control in diabetic C57BLKS-lepr^{db}/lepr^{db} mice. *J Oleo Sci* **50**, 889–894.

Horton JD, Bashmakov Y, Shimomura I & Shimano H (1998) Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci USA* **95**, 5987–5992.

Horton JD, Goldstein JL & Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* **109**, 1125–1131.

Hsu RY & Lardy HA (1969) Malic enzyme. *Methods Enzymol* **17**, 230–235.

Kim HJ, Takahashi M & Ezaki O (1999) Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J Biol Chem* **274**, 25892–25898.

Kim MR, Park Y, Albright KJ & Pariza MW (2002) Differential responses of hamsters and rats fed high-fat or low-fat diets supplemented with conjugated linoleic acid. *Nutr Res* **22**, 715–722.

Kuby SA & Noltmann EA (1966) Glucose 6-phosphate dehydrogenase from brewers yeast. *Methods Enzymol* **9**, 116–117.

Lynen F (1969) Yeast fatty acid synthase. *Methods Enzymol* **14**, 14–17.

Martin JC, Grégoire S, Siess MH, Genby M, Chardigny JM, Berdeaux O, Juanéda P & Sébédio JL (2000) Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats. *Lipids* **35**, 91–98.

Miner JL, Cederberg CA, Nielsen MK, Chen X & Baile CA (2001) Conjugated linoleic acid (CLA), body fat, and apoptosis. *Obes Res* **9**, 129–134.

Moya-Camarena SY & Belury MA (1999) Species differences in the metabolism and regulation of gene expresión by conjugated linoleic acid. *Nutr Rev* **57**, 336–340.

Osborne TF (2000) Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem* **275**, 32379–32382.

Pai JT, Guryev O, Brown MS & Goldstein JL (1998) Differential stimulation of cholesterol and unsaturated fatty acid biosynthesis in cells expressing individual nuclear sterol regulatory element-binding proteins. *J Biol Chem* **273**, 26138–26148.

Pariza MW, Park Y & Cook ME (2000) Mechanisms of action of conjugated linoleic acid: evidence and speculation. *Proc Soc Exp Biol Med* **223**, 8–13.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME & Pariza MW (1997) Effect of conjugated linoleic acid on body composition in mice. *Lipids* **32**, 853–858.

Reeves PG, Nielsen FH & Fahey GC Jr (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition *ad hoc* writing committee on the reformulation of the AIN-76 rodent diet. *J Nutr* **123**, 1939–1951.

Roche HM, Noone E, Nugent A & Gibney MJ (2001) Conjugated linoleic acid: a novel therapeutic nutrient? *Nutr Res Rev* **14**, 173–187.

Rodríguez VM, Picó C, Portillo MP, Macarulla MT & Palou A (2002) Dietary fat source regulated *ob* gene expression in white adipose tissue of rats under hyperphagic feeding. *Br J Nutr* **87**, 427–434.

Sakono M, Miyanaga F, Kawahara S, Yamauchi K, Fukuda N, Watanabe K, Iwata T & Sugano M (1999) Dietary conjugated linoleic acid reciprocally modifies ketogenesis and lipid secretion by the rat liver. *Lipids* **34**, 997–1000.

Shimano H (2001) Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. *Prog Lipid Res* **40**, 439–452.

Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS & Goldstein JL (1997) Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* **99**, 846–854.

Shimomura I, Bashmakov Y & Horton JD (1999) Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* **274**, 30028–30032.

Stangl GI (2000) High dietary levels of conjugated linoleic acid mixture alter hepatic glycerophospholipid class profile and cholesterol-carrying serum lipoproteins of rats. *J Nutr Biochem* **11**, 184–191.

Takahashi Y, Kushiro M, Shinohara K & Ide T (2003) Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. *Biochim Biophys Acta* **1631**, 265–273.

Terpstra AH, Beynen AC, Everts H, Kocsis S, Katan MB & Zock PL (2002) The decrease in body fat in mice fed conjugated linoleic acid is due to increases in energy expenditure and energy loss in the excreta. *J Nutr* **132**, 940–945.

Tsuboyama-Kasaoka N, Miyazaki H, Kasaoka S & Ezaki O (2003) Increasing the amount of fat in a conjugated linoleic acid-supplemented diet reduces hipodystrophy in mice. *J Nutr* **133**, 1793–1799.

Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S & Ezaki O (2000) Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. *Diabetes* **49**, 1534–1542.

Wang YM, Rahman SM, Nagao K, Han SY, Buang Y, Cha JY & Yanagita T (2003) Conjugated linoleic acid reduces hepatic microsomal triacylglycerol transfer protein activity and hepatic triacylglycerol mass in obese rats. *J Oleo Sci* **52**, 129–134.

Warren JM, Simon VA, Bartolini G, Erickson KL, Mackey BE & Kelley DS (2003) *trans*-10,*cis*-12 CLA increases liver and decreases adipose tissue lipids in mice: possible roles of specific lipid metabolism genes. *Lipids* **38**, 497–504.

West DB, DeLany JP, Camet P, Blohm FY, Truett AA & Scimeca J (1998) Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. *Am J Physiol* **275**, R667–R672.

Xu J, Cho H, O'Malley S, Park JH & Clarke SD (2002) Dietary polyunsaturated fats regulate rat liver sterol regulatory element binding proteins-1 and -2 in three distinct stages and by different mechanisms. *J Nutr* **132**, 3333–3339.

Xu J, Nakamura MT, Cho HP & Clarke SD (1999) Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem* **274**, 23577–23583.

Yahagi N, Shimano H, Hasty AH, *et al.* (1999) A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J Biol Chem* **274**, 35840–35844.

Zabala A, Chávarri M, Macarulla MT, Navarro V, Fernández A, Rodríguez V & Portillo MP (2003) Effect of conjugated linoleic acid on hepatic oxidation of fatty acids. *Nutrition* **19**, 1053.