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trans-10,*cis*-12 Conjugated linoleic acid prevents adiposity but not insulin resistance induced by an atherogenic diet in hamsters☆

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Abstract

While conjugated linoleic acid (CLA) has received a great deal of attention as a supplement that can favourably modify body composition, its potential impact on insulin sensitivity has not received equal attention. The aim of the present work was to analyse the effects of *trans*-10,*cis*-12 CLA isomer on insulin sensitivity in hamsters fed an atherogenic diet. Hamsters were divided into three groups: one group was fed a chow diet (control) and the other two a semipurified atherogenic diet supplemented with 0.5% linoleic acid (LA) or *trans*-10,*cis*-12 CLA, respectively. Serum glucose, FFAs, insulin, leptin and adiponectin were measured using commercial kits. HOMA-IR was calculated using the formula of Matthews et al. PPARγ mRNA was assessed in epididymal adipose tissue by reverse transcription–polymerase chain reaction (RT-PCR). After 6 weeks, atherogenic feeding produced an increase in body fat accumulation as compared with control feeding. The addition of *trans*-10,*cis*-12 CLA to the atherogenic diet avoided this feature. Atherogenic feeding also led to significantly higher serum concentrations of glucose, insulin, FFAs, as well as greater HOMA-IR values. *trans*-10,*cis*-12 CLA did not prevent these effects. No significant differences were found among experimental groups in serum leptin and adiponectin concentrations, nor in PPARγ expression. In summary, although the addition of *trans*-10,*cis*-12 CLA to an atherogenic diet reduces fat accumulation, it does not improve the impairment of insulin action associated with this feeding. The maintenance of insulin resistance in hamsters fed the atherogenic CLA-enriched diet is probably due to the high serum FFA concentration observed in these animals.

Keywords: CLA; HOMA-IR; Atherogenic diet; Hamster

1. Introduction

Conjugated linoleic acid (CLA), in particular the *trans*-10,*cis*-12 isomer, reduces body fat accumulation and, thus, has a potential application in the prevention and management of obesity [1–3]. Because many obese people show insulin resistance, it would be useful to determine the effects of CLA on plasma insulin and glucose levels.

While CLA has received a great deal of attention as a supplement that can favourably modify body composition, its potential impact on insulin sensitivity has not received equal attention. Only a few studies have been performed to test the effects of CLA isomers on insulin action, and the results obtained in various rodent models have been controversial and, in some cases, apparently contradictory.

In C57BL/6J mice a mixture of CLA [4] and *trans*-10,*cis*-12 isomer [5] induced lipodistrophy and insulin resistance. The *trans*-10,*cis*-12 CLA isomer also induced a strong increase in plasma levels of glucose and insulin in *ob*/*ob* C57BL/6J mice, reflecting an insulin-resistant state [6]. Glucose serum concentration was also higher in hamsters fed a CLA mixture than in control animals [7]. In contrast, *trans*-10,*cis*-12 CLA treatment has been shown to enhance glucose tolerance and insulin-stimulated glucose transport in skeletal muscle in insulin-resistant female obese Zucker (*fa*/*fa*) rats [8]. Similarly, improvements in insulin action in Zucker diabetic fatty *fa*/*fa* rats were observed by using CLA mixtures [9–11]. Improved insulin sensitivity

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was also observed in diabetic mice (*db*/*db*) after TG-CLA feeding [12]. Concerning the effects of CLA in humans, decreased insulin sensitivity induced by *trans*-10,*cis*-12 CLA has been shown in insulin-resistant obese men [13].

These results suggest that the effects of CLA on insulin sensitivity depend on species and/or metabolic status. Thus, the present work was designed to analyse the effects of *trans*-10,*cis*-12 CLA intake on fat accumulation in adipose tissue and to determine whether this fatty acid modifies insulin sensitivity in an experimental model of diet-induced insulin resistance. The species selected was the hamster.

2. Material and methods

2.1. Animals, diets and experimental design

Twenty-four, 9-week-old, male Syrian Golden hamsters were purchased from Harlan Ibérica (Barcelona, Spain). They 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 day–night rhythm. After a 6-day adaptation period, the hamsters were randomly divided into three groups of eight animals each and fed the experimental diets for 6 weeks. One group (control group) was fed a commercial chow diet (Panlab, Barcelona, Spain). The other two groups were given semi-purified atherogenic diets consisting of 200 g/kg casein and 4 g/kg L-methionine (Sigma, St. Louis, MO, USA), 200 g/kg wheat starch (Vencasser, Bilbao, Spain), 404 g/kg sucrose (local market), 100 g/kg palm oil (Agra-Unilever, Leioa, Spain), 30 g/kg cellulose (Vencasser) and 1 g/kg cholesterol (Sigma). Linoleic acid (LA diet) or *trans*-10,*cis*-12 CLA (CLA diet) (Natural Lipids, Hovdebygda, Norway) was supplemented at a level of 0.5% to the atherogenic diets. Vitamin and mineral mixes were formulated according to AIN-93 guidelines [14] and supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). All animals had free access to food and water.

2.2. Tissue removal and serum sampling

At the end of the experimental period the hamsters were fasted for 12 h, and blood samples were collected under inhalation anaesthesia (diethyl ether) by cardiac puncture. Adipose tissues from different anatomical regions (perirenal, epididymal and gluteal subcutaneous) were dissected and weighed. Serum was obtained from blood samples after centrifugation (1000×*g* for 10 min at 4°C). All samples were stored at −80°C until analysis.

2.3. Serum analysis

Serum glucose and free fatty acids (FFAs) were measured by spectrophotometry using commercial kits (BioSystems, Barcelona, Spain). Insulin, adiponectin and leptin were assessed by RIA using commercial kits (Linco, St. Charles, MO, USA). The homeostatic model assessment for insulin resistance (HOMA-IR) was used because it is a valuable method that shows a strong relationship with euglycemic–hyperinsulinemic clamp [15]. It was calculated from insulin and glucose values using the formula of Matthews et al. [16]:

$$\text{HOMA} - \text{IR} = \frac{\text{Fasting glucose (mmol/L)} \times \text{fasting insulin (mU/L)}}{22.5}$$

2.4. Extraction of total RNA and semiquantification by reverse transcription–polymerase chain reaction

Total RNA was extracted from 100 mg of epididymal adipose tissue using Trizol (Invitogen, Carlsbad, CA, USA), according to the manufacturer's instructions. One and a half micrograms of RNA 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 reverse transcription–polymerase chain reaction (RT-PCR) was carried out as previously published [17]. Specific sense and antisense primers used to amplify PPARγ cDNA (GenBank AF156665) were 5′ -ATTCTGGCCCACCAAC-TTCGG-3′ (sense, 222–242) and 5′ -TGGAAGCCTGA-TGCTTTATCCCCA-3′ (antisense, 537–560); primers for β-actin cDNA (GenBank J00691) were 5′ -TCTACAATG-AGCTGCGTGTG-3′ (sense, 1599–1618) and 5′ -GGTCA-GGATCTTCATGAGGT-3′ (antisense, 2357–2376). Primers used for PPARγ were those designed previously [18]. Specific primers used to amplify β-actin were designed using Oligo 6.0 Primer Analysis Software for Windows (National Biosciences, Plymouth, MN, USA). cDNAs were amplified for 31 cycles, using the following parameters: 94°C for 45 s, 55°C for 30 s and 72°C for 90 s (PPARγ) or 95°C for 30s, 59°C for 30 s and 72°C for 30 s (β-actin). A first step of denaturation (94°C for 3 min for PPARγ and 95°C for 5 min for β-actin) and a final extension step of denaturation (72°C for 10 min for PPARγ and 72°C for 7 min for β-actin) 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. The linearity of the PCR reactions was tested by amplification of 200 ng of first-strand cDNA per reaction from 20 to 40 cycles. The amplified products were

Table 1
Initial and final body weights, and food and energy intakes of hamsters fed the experimental diets for 6 weeks

	Control	LA	CLA
Initial body weight (g)	105±1	105±1	105±1
Final body weight (g)	119±3	120±3	117±1
Food intake (g/day)	7.9±0.5[a]	5.6±0.4[b]	5.6±0.1[b]
Energy intake (kJ/day)	99.3±6.2	97.6±6.2	96.2±1.7

Values are means±S.E.M., $n=8$.
[a,b] Values in the same row with different subscript are significantly different at $P<.05$.

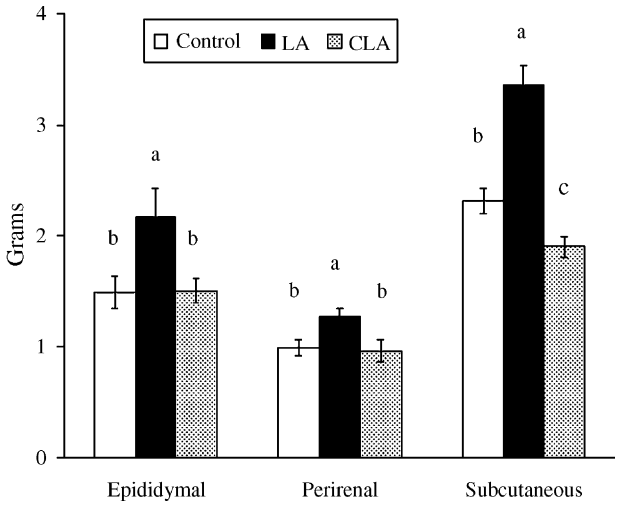

Fig. 1. Weights of white adipose tissues from different anatomical locations of hamsters fed the experimental diets for 6 weeks. Data not sharing a common letter are significantly different at $P<.05$.

resolved in a 1.2% agarose gel (MS-8, Pronadisa, Madrid, Spain) with ethidium bromide. Polymerase chain reaction 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). Levels of mRNA were expressed as the ratio of signal intensity for PPARγ relative to that for β-actin, as published elsewhere.

2.5. Statistical analysis

Results are presented as mean±S.E.M. Statistical analysis was performed using SPSS 8.0 (SPSS, Chicago, IL, USA). Data were analysed by one-way ANOVA followed by Newman–Keuls post hoc test. Correlations were analysed by Pearson's test. Significance was assessed at the $P<.05$ level.

3. Results

No differences in either energy intake or final body weight were found among the three experimental groups (Table 1). Feeding an atherogenic diet (LA group) increased body fat accumulation: adipose tissue weights from perirenal, epididymal and subcutaneous regions were significantly greater than in control animals ($P<.01$). This effect was more marked in subcutaneous (+45.5%) and epididymal (+45.6%) depots than in perirenal (+28.3%) tissue. The addition of *trans*-10,*cis*-12 CLA to the atherogenic diet avoided this effect. In fact, subcutaneous adipose tissue weight was even significantly lower than in control animals ($P<.05$) (Fig. 1).

Table 2
Serum parameters of hamsters fed the experimental diets for 6 weeks

	Control	LA	CLA
Glucose (mmol/L)	6.9±0.5[b]	9.1±0.9[a]	9.0±0.6[a]
Insulin (pmol/L)	60±10[b]	176±25[a]	184±16[a]
HOMA-IR	2.8±0.6[b]	10.1±1.6[a]	10.5±1.4[a]
FFA (mmol/L)	0.52±0.04[b]	0.97±0.06[a]	0.96±0.06[a]
Adiponectin (ng/ml)	1.80±0.04	1.97±0.07	1.78±0.05
Leptin (ng/ml)	6.7±0.7	8.4±0.9	6.3±1.1

Values are means±S.E.M., $n=8$.
[a,b] Values in the same row with different subscript are significantly different at $P<.05$.

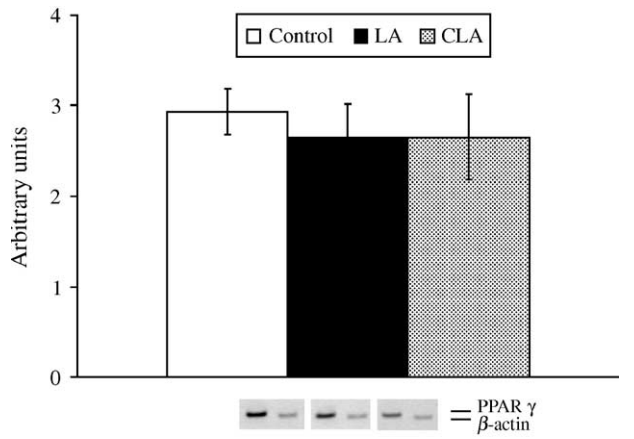

Fig. 2. RT-PCR analysis of PPARγ mRNA in epididymal adipose tissue of hamsters fed the experimental diets for 6 weeks.

Atherogenic feeding led to significantly greater serum concentrations of glucose, insulin and FFAs, as well as HOMA-IR values ($P<.05$). The inclusion of *trans*-10, *cis*-12 CLA in the atherogenic diet did not prevent these effects. No significant differences in serum adiponectin and leptin concentrations were found among experimental groups (Table 2).

Neither atherogenic feeding nor CLA intake induced changes in PPARγ mRNA levels in epididymal adipose tissue (Fig. 2).

Significant correlations were found between serum leptin concentration and adipose tissue weights from perirenal and epididymal locations ($P=.018$, $r=.533$; $P=.050$, $r=.433$ respectively), and between HOMA-IR and serum FFA concentration, ($P=.005$, $r=.616$).

4. Discussion

The results obtained in various rodent models concerning the effects of CLA on insulin sensitivity have been controversial. Data in the literature show that CLA improves insulin sensitivity in Zucker *fa*/*fa* rats, an animal model that shows either severe insulin resistance or type 2 diabetes [8–11], and in diabetic mice [12]. In contrast, CLA induces lipoatrophic insulin resistance in C57BL/6J mice, an animal model not showing previous glucose metabolic disturbances [4–6]. These results suggest that the effects of CLA on insulin sensitivity depend on species and/or metabolic status.

In order to gain more insight into this issue, the present study was performed in a different experimental model.

Hamsters were selected as species, and CLA was included in a diet, rich in palm oil and sucrose, which induces insulin resistance.

The atherogenic diet produced significantly higher glucose and insulin serum concentrations in LA group, which were not associated with changes in energy intake, as compared with the controls. Consequently, HOMA-IR values were also significantly increased, suggesting the development of insulin resistance. These features are in good accordance with other published works. Thus, high-fat and high-sucrose diets have been shown to increase adiposity and to impair whole-body insulin action [19–21]. Although the addition of *trans*-10,*cis*-12 CLA to the atherogenic diet did not modify energy intake nor final body weight, it reduced fat depot sizes; thus, a good insulin sensitivity, similar to that in control animals, could be expected in the group fed atherogenic diet plus CLA. However, HOMA-IR values in this group revealed impaired insulin sensitivity.

One of the reasons that can justify the impairment of insulin action in LA and *trans*-10,*cis*-12 CLA groups is the increased serum FFA concentrations observed in both groups, as compared with the controls. In fact, a positive and significant correlation ($P=.005$) was found between serum FFAs and HOMA-IR. Although the mechanisms underlying the development of insulin resistance are multifactorial and only partly understood, increased availability of FFAs is of particular importance for liver and skeletal muscle insulin resistance [22,23]. Elevated FFAs stimulate hepatic gluconeogenesis by inhibiting the ability of insulin to suppress hepatic glucose production and reduce muscle glucose uptake and oxidation. Moreover, although FFAs are normally stored as triacylglycerols for future energy needs, in the face of excess delivery of FFAs, intracellular triacylglycerols begin to accumulate in other organs and tissues such as skeletal muscle, liver and pancreas, leading to lipotoxicity [24,25]. The FFA-lowering effect of thiazolidinediones (TDZs), a class of antidiabetic agents, gives further support to the role of FFAs in insulin resistance [26,27].

Because HOMA-IR is a parameter that essentially explores the ability of insulin to restrain hepatic glucose production in the fasting state, but does not provide good information related to peripheral glucose disposal [15], in the present work liver insulin resistance, associated with a high flux of FFAs from the adipose tissue to the liver, can be suspected in LA and CLA groups. Other mechanisms mentioned above which explain peripheral insulin resistance cannot be discarded.

High serum FFA concentrations in hamsters fed the LA diet are probably due to their greater adipose tissue size, because of the high fat intake. It has been described that FFA levels increase when the adipose tissue mass becomes enlarged [28,29]. Although *trans*-10,*cis*-12 CLA intake led to a significant reduction in adipose depot weights, it is surprising that hamsters fed this CLA isomer showed serum FFA concentrations and HOMA-IR values as high as those found in the LA group. Several papers have proposed that *trans*-10,*cis*-12 CLA can stimulate lipid mobilization [30–32]; nevertheless, this is a controversial issue. In the present work lipolysis was not evaluated, but it could be hypothesized that increased triglyceride mobilization in *trans*-10,*cis*-12 group could underlie, at least in part, the high FFA level observed.

Besides FFAs, white adipose tissue secretes a variety of adipocytokines involved in insulin-sensitivity regulation. This is true of leptin and adiponectin. Leptin reduces ectopic fat accumulation in nonadipose tissues, enhancing insulin-mediated stimulation of glucose disposal [21,33]. Moreover, adiponectin promotes fatty acid oxidation in muscle and inhibits liver glucose production [34–36]. Thus, leptin and adiponectin may work hand in hand to sensitise peripheral tissues to insulin.

trans-10,*cis*-12 CLA has been found to decrease leptin expression [37,38]. In the present work no significant differences were found in serum leptin levels among experimental groups, despite differences in adipose tissue weights. Nevertheless, a trend toward higher levels in LA group was observed, and, indeed, positive and significant correlations were found between serum leptin and adipose tissue weights. It should be remembered that, in the present work, serum was obtained in 12-h fasted animals. Thus, potential changes in serum leptin levels associated with changes in adipose tissue size and/or CLA feeding may have been masked by the well-known lowering effect induced by fasting.

Studies concerning the effects of CLA on adiponectin expression and serum concentrations are scarce. Only two works have been published and contradictory results have been found. Thus, whereas Warren et al. [39] observed a decrease in adiponectin expression in female mice C57BL/6J fed a diet containing 0.5% of CLA mixture, Nagao et al. [11] found an increase in adiponectin expression and serum concentrations in diabetic Zucker *fa*/*fa* rats fed a diet containing 1% of CLA mixture. In the present work no changes in serum adiponectin levels were induced by *trans*-10,*cis*-12 CLA intake, suggesting that insulin resistance observed in CLA group was not related to this adipocytokine.

PPARγ is a transcriptional factor, highly expressed in adipose tissue compared with muscle or liver [40], that stimulates the differentiation of preadipocytes into mature adipocytes (adipogenesis), as well as the storage of fatty acids in mature adipocytes by acting at several steps: release of fatty acids from the lipoprotein triacylglycerols (lipoprotein lipase), intracellular fatty acid transport (aP2), activation of fatty acids (acyl-CoA synthase) and fatty acid esterification. A stimulating effect on the insulin-dependent glucose transporter GLUT4 has also been described. Activation of PPARγ controls the expression of products secreted by the adipocyte, reducing leptin expression and activating adiponectin expression. By modulating the

secretion of these proteins and channelling fatty acids into adipose tissue PPARγ potentiates insulin sensitivity [41,42].

Because PPARγ regulates lipid accumulation and insulin action, its potential involvement in the effects of the *trans*-10,*cis*-12 CLA isomer has been studied but remains controversial. In vitro studies performed in 3T3-L1 mature adipocytes have revealed contradictory effects of CLA on PPARγ mRNA expression. Thus, whereas Kang et al. [43] and Grandlund et al. [44] reported a reduction in PPARγ mRNA levels induced by *trans*-10,*cis*-12 CLA, Choi et al. [45] did not observe any effect. Data concerning in vivo studies are scarcer and have been performed by using CLA mixtures. Takahashi et al. [46] reported a reduced PPARγ expression in adipose tissue from C57BL/6J mice but no changes in ICR mice, when a CLA mixture (2%) was added to the diet. In contrast, Kang et al. [43] found a reduced PPARγ expression in epididymal adipose tissue from ICR mice fed 0.5% of CLA mixture. In the present work no effects of *trans*-10,*cis*-12 CLA intake on PPARγ mRNA levels were observed in epididymal adipose tissue. These data suggest that the reduction in adipose tissue weight as well as the maintenance of insulin resistance produced by this fatty acid was not mediated by changes in PPARγ expression. Several authors have reported that CLA isomers are weak agonists of PPARγ [5,9,47], and it could be speculated that these fatty acids might act as partial agonists, thereby antagonizing PPARγ activity by competing with endogenous ligands with higher efficacy.

In summary, although the addition of *trans*-10,*cis*-12 CLA to an atherogenic diet reduces fat accumulation in perirenal, epididymal and subcutaneous adipose tissues, it does not improve the impairment of insulin action associated with this diet. This can be considered as a negative aspect for the use of CLA in the prevention and management of obesity. The maintenance of insulin resistance in hamsters fed the atherogenic CLA-enriched diet is probably due to the high serum FFA concentration observed in these animals.

References

[1] Roche HM, Noone E, Nugent A, Gibney MJ. Conjugated linoleic acid: a novel therapeutic nutrient? Nutr Res Rev 2001;14:173–87.

[2] Belury MA. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr 2002;22:505–31.

[3] Evans M, Brown J, McIntosh M. Isomer-specific effects of conjugated linoleic acid (CLA) on adiposity and lipid metabolism. J Nutr Biochem 2002;13:508–16.

[4] Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 2000;49:1534–42.

[5] Clément L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief S, et al. Dietary *trans*-10,*cis*-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res 2002;43:1400–9.

[6] Roche HM, Noone E, Sewter C, McBennett S, Savage D, Gibney MJ, et al. Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1c and LXRalpha. Diabetes 2002;51:2037–44.

[7] Bouthegourd JC, Even PC, Gripois D, Tiffon B, Blouquit MF, Roseau S, et al. A CLA mixture prevents body triglyceride accumulation without affecting energy expenditure in Syrian hamsters. J Nutr 2002;132:2682–9.

[8] Henriksen EJ, Teachey MK, Taylor ZC, Jacob S, Ptock A, Krämer K, et al. Isomer-specific actions of conjugated linoleic acid on muscle glucose transport in the obese Zucker rat. Am J Physiol 2003;285:E98–E105.

[9] Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty *fa*/*fa* rat. Biochem Biophys Res Commun 1998;244:678–82.

[10] Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatsiaris T, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes 2001;50:1149–57.

[11] Nagao K, Inoue N, Wang YM, Yanagita T. Conjugated linoleic acid enhances plasma adiponectin level and alleviates hyperinsulinemia and hypertension in Zucker diabetic fatty (*fa*/*fa*) rats. Biochem Biophys Res Commun 2003;310:562–6.

[12] Hamura M, Yamatoya H, Kudo S. Glycerides rich in conjugated linoleic acid (CLA) improve blood glucose control in diabetic C57BLKS-Lepr^{db}/lepr^{db} mice. J Oleo Sci 2001;50:889–94.

[13] Risérus U, Arner P, Brismar K, Vessby B. Treatment with dietary *trans*-10,*cis*-12 conjugated linoleic acid causes isomer specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care 2002;25:1516–21.

[14] Reeves PG, Nielsen FH, Fahey Jr GC. 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 1993;123:1939–51.

[15] Bonora E, Targher G, Alberiche M, Bonadonna RC, Saggiani F, Zenere MB, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. Diabetes Care 2000;23:57–63.

[16] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.

[17] Corbalán MS, Margareto J, Martínez JA, Martí A. High-fat feeding reduced muscle uncoupling protein 3 expression in rats. J Physiol Biochem 1999;55:67–72.

[18] Miyahara T, Schrum L, Rippe R, Xiong S, Yee Jr HF, Motomura K, et al. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000;275:35715–22.

[19] Boyd JJ, Contreras I, Kern M, Tapscott EB, Downes DL, Frisell WR, et al. Effect of high-fat-sucrose diet on in vivo insulin receptor kinase activation. Am J Physiol 1990;259:E111–6.

[20] Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ, Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. Diabetes 1991;40:1397–403.

[21] Yaspelkis III BB, Singh MK, Krisan AD, Collins DE, Kwong CC, Bernard JR, et al. Chronic leptin treatment enhances insulin-stimulated glucose disposal in skeletal muscle of high-fat fed rodents. Life Sci 2004;74:1801–16.

[22] Mason TM, Goh T, Tchipashvili V, Sandhu H, Gupta N, Lewis GF, et al. Prolonged elevation of plasma free fatty acids desensitizes the insulin secretory response to glucose in vivo in rats. Diabetes 1999; 48:524–30.

[23] Wyne KL. Free fatty acids and type 2 diabetes mellitus. Am J Med 2003;115(Suppl 8A):29S–36S.

[24] Wolfe RR. Metabolic interactions between glucose and fatty acids in humans. Am J Clin Nutr 1998;67:519S–26S.

[25] Smith SA. Central role of adipocyte in the insulin-sensitising and cardiovascular risk modifying actions of the thiazolidinediones. Biochimie 2003;85:1219–30.

[26] Hevener AL, Reichart D, Janez A, Olefsky J. Thiazolidinedione treatment prevents free fatty acid-induced insulin resistance in male Wistar rats. Diabetes 2001;50:2316–22.
[27] Racette SB, Davis AO, McGill JB, Klein S. Thiazolidinediones enhance insulin-mediated suppression of fatty acid flux in type 2 diabetes mellitus. Metabolism 2002;51:169–74.
[28] Jéquier E, Tappy L. Regulation of body weight in humans. Physiol Rev 1999;79:451–80.
[29] Kadowaki T, Hara K, Yamauchi T, Terauchi Y, Tobe K, Nagai R. Molecular mechanism of insulin resistance and obesity. Exp Biol Med 2003;228:1111–7.
[30] Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids 1997;32:853–8.
[31] Sujkowska D, Auchter C, Franklin D, Ellis N, Lea-Currie Y, Sen A. Effects of conjugated linoleic acid on lipolysis and leptin secretion from cultured human adipocytes. Obes Res 2001;9:147 [abstract P28].
[32] Evans M, Lin X, Odle J, McIntosh M. *trans*-10,*cis*-12 Conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes. J Nutr 2002;132:450–5.
[33] Iwai H, Ohno Y, Aoki N. The effect of leptin, tumor necrosis factor-alpha (TNF-alpha), and nitric oxide (NO) production on insulin resistance in Otsuka long-Evans fatty rats. Endocr J 2003;50: 673–80.
[34] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947–53.
[35] Tsao TS, Lodish HF, Fruebis J. ACRP30, a new hormone controlling fat and glucose metabolism. Eur J Pharmacol 2002;440:213–21.
[36] Pajvani UB, Scherer PE. Adiponectin: systemic contributor to insulin sensitivity. Curr Diabetes Rep 2003;3:207–13.
[37] Kang K, Pariza MW. *trans*-10,*cis*-12-Conjugated linoleic acid reduces leptin secretion from 3T3-L1 adipocytes. Biochem Biophys Res Commun 2001;287:377–82.
[38] Rodríguez E, Ribot J, Palou A. *trans*-10,*cis*-12, but not *cis*-9,*trans*-11 CLA isomer, inhibits brown adipocyte thermogenic capacity. Am J Physiol 2002;282:R1789–97.
[39] Warren JM, Simon VA, Bartolini G, Erickson KL, Mackey BE, Kelley DS. *trans*-10,*cis*-12 CLA increases liver and decreases adipose tissue lipids in mice: possible roles of specific lipid metabolism genes. Lipids 2003;38:497–504.
[40] Kersten S. Peroxisome proliferator activated receptors and obesity. Eur J Pharmacol 2002;440:223–34.
[41] Khan SA, Vanden Heuvel JP. Role of nuclear receptors in the regulation of gene expression by dietary fatty acids (Review). J Nutr Biochem 2003;14:554–67.
[42] Ferré P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 2004;53:S43–S50.
[43] Kang K, Liu W, Albright KJ, Park Y, Pariza MW. *trans*-10,*cis*-12 CLA inhibits differentiation of 3T3-L1 adipocytes and decreases PPARγ expression. Biochem Biophys Res Commun 2003;303:795–9.
[44] Grandlund L, Juvet LK, Pedersen JI, Nebb HI. *trans*10,*cis*12-Conjugated linoleic acid prevents triacylglycerol accumulation in adipocytes by acting as a PPARgamma modulator. J Lipid Res 2003;44:1441–52.
[45] Choi Y, Kim YC, Han YB, Park Y, Pariza MW, Ntambi JM. The *trans*-10,*cis*-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. J Nutr 2000;130:1920–4.
[46] Takahashi Y, Kushiro M, Shinohara K, Ide T. Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. Comp Biochem Physiol B Biochem Mol Biol 2002;133:395–404.
[47] Belury MA, Moya-Camarena SY, Lu M, Shi L, Leesnitzer LM, Blanchard SG. Conjugated linoleic acid is an activator and ligand for peroxisome proliferator-activated receptor-gamma (PPARγ). Nutr Res 2002;22:817–24.