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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 $P<.05$.