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Effects of conjugated linoleic acid on skeletal muscle triacylglycerol metabolism in hamsters

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Abstract

Objective: The present work evaluated the effects of conjugated linoleic acid (CLA) on various aspects of triacylglycerol metabolism in skeletal muscle to determine the potential involvement of this tissue in the effect of CLA to decrease body fat.

Methods: Animals were randomized to three groups that were fed atherogenic diets that provided different amounts of *trans*-10,*cis*-12 CLA (0%, 0.5%, or 1%) for 6 wk. Muscle triacylglycerol, protein, water, glycogen, and DNA contents and fatty acid profile in triacylglycerols were analyzed. Lipoprotein lipase and carnitine palmitoyltransferase-I (CPT-I) activities were assessed. Triacylglycerol, glucose, and insulin concentrations were evaluated in serum.

Results: The high dose of CLA increased food efficiency and gastrocnemius muscle weight. CLA feeding resulted in decreased muscle triacylglycerol content without changes in protein, water, glycogen, and DNA contents or in cell size (protein/DNA ratio) and produced decreased lipoprotein lipase activity and increased CPT-I activity. No differences were found between CLA doses. CLA feeding led to the saturation of stored triacylglycerols.

Conclusions: Decreased fatty acid uptake and increased fatty acid oxidation can contribute to the decreased muscle triacylglycerol content observed in hamsters fed the CLA diets. The increase in muscle fatty acid β-oxidation might ultimately prevent storage of triacylglycerols in adipose tissue. Nevertheless, the lack of matching of lipoprotein lipase and CPT-I modifications makes it difficult to ensure that skeletal muscle is responsible, at least in part, for the effect of CLA on decreasing body fat; thus, further research is needed.

Keywords: Conjugated linoleic acid; Muscle composition; Lipoprotein lipase; Carnitine palmitoyltransferase-I; Hamster

Introduction

In recent years numerous studies have demonstrated the body fat lowering effect of conjugated linoleic acid (CLA) in different animal species [1,2]. Although most studies have been performed by using CLA mixtures with different isomer proportions, there is now strong evidence showing that antiobesity effects are attributable to the *trans*-10,*cis*-12 isomer [3,4]. Various mechanisms of action have been proposed as a result of studies performed in cell cultures or animal models; nevertheless, a general consensus has not been reached [5].

It has been suggested that CLA can modify energy metabolism. Concerning energy intake, although some studies have shown that CLA feeding decreases food or energy intake [6–8], the decreases are small and thus cannot explain the marked decrease in fat deposition. Results of other studies have suggested that CLA increases energy expenditure [9–11]. Nevertheless, most of the proposed mecha-

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nisms take place in adipose tissue. Thus, decreased lipid synthesis, increased lipolysis, decreased activity of steroyl-coenzyme A desaturase, decreased preadipocyte proliferation and differentiation, and increased apoptosis have been reported in the literature [5].

Aside from adipose tissue, other organs and tissues, which play an important role in body lipid metabolism and in turn are involved in the regulation of body fat accumulation, can be affected by CLA feeding. Several investigators have found increased liver fatty acid oxidation [12–15]. Moreover, Pariza et al. [1] and Yotsumoto et al. [16] established that CLA decreases apolipoprotein-B secretion in cultured HepG2 cells, and this effect can result in decreased production of very low-density lipoprotein. Although skeletal muscle plays a pivotal role in triacylglycerol metabolism, less attention has been paid to this tissue when compared with other organs and tissues.

The present work evaluated the effects of *trans*-10,*cis*-12 CLA feeding on various aspects of triacylglycerol metabolism in skeletal muscle to determine the potential involvement of this tissue in the body fat lowering effect of CLA.

Materials and methods

Animals, diets, and experimental design

Although the vast majority of studies concerning CLA have been done in the mouse, this animal species exhibits an exaggerated response in comparison with other rodent species, making it difficult to extrapolate obtained results. Thus, in the present study, the hamster was chosen as an animal model.

The experiment was conducted with 9-wk-old, male Syrian Golden hamsters (105 ± 1 g) purchased from Harlan Ibérica (Barcelona, Spain) and was in accord with the institution's guideline for the care and use of laboratory animals. 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/12-h dark cycle. After a 6-d adaptation period, hamsters were randomized into three dietary groups (n = 10) for feeding of different doses of *trans*-10,*cis*-12 CLA (0 [control], 0.5, and 1.0 g/100 g of diet) in a semipurified atherogenic diet consisting of 200 g/kg of casein (Sigma, St. Louis, MO, USA), 4 g/kg of L-methionine (Sigma), 200 g/kg of wheat starch (Vencasser, Bilbao, Spain), 405 g/kg of sucrose (local market), 100 g/kg of palm oil (Agra-Unilever, Leioa, Spain), 30 g/kg of cellulose (Vencasser), 4 g/kg of choline-HCl (Sigma), and 1 g/kg of cholesterol (Sigma). The *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 [17] and supplied by ICN Pharmaceuticals (Costa Mesa, CA, USA). The experimental diets were freshly prepared once a week, gassed with nitrogen, and stored at 0°C to 4°C to avoid rancidity. All animals had free access to food and water. Food intake and body weight were measured daily.

At the end of the experimental period (6 wk), animals were killed under anaesthesia (diethyl ether) and blood was collected by cardiac puncture. Adipose tissues (epididymal, perirenal, and subcutaneous) were dissected out and weighed. Both gastrocnemius muscles were dissected out just to evaluate the influence of CLA feeding on skeletal muscle weight. Thigh muscles were dissected out and used to evaluate composition, DNA content, and enzyme activities. Serum was obtained from blood samples after centrifugation (1000*g* for 10 min at 4°C). All muscles and serum samples were frozen and stored at −80°C until analysis.

Serum measurements

Serum triacylglycerols and glucose were measured by spectrophotometry and insulin by radioimmunoassay by using commercial kits (BioSystems, Barcelona, Spain; Linco, St. Charles, MO, USA).

Muscle composition and cell size estimation

Muscle water content was measured gravimetrically by drying samples at 105°C until it reached constant weight, and glycogen was measured as previously described [18].

Total lipids were extracted from muscle samples according to the method described by Folch et al. [19]. Lipid extract was dissolved in isopropanol. Triacylglycerol content was measured with a commercial kit (BioSystems).

Thin-layer chromatography on silica gel plates, using hexane:diethyl ether:acetic acid (70:30:1 by volume) as a developing solvent, was used to separate triacylglycerols. For fatty acid methyl ester preparation, triacylglycerols were trans-esterified with 0.5 mol/L of sodium methoxide as previously reported [20]. The completion of methylation process was checked by thin-layer chromatography.

Samples were transferred to gas chromatographic vials, dried under N_2, and dissolved in heptane containing 50 ppm of butylated hydroxytoluene as a stabilizer. Fatty acid methyl esters were analyzed with a Hewlett Packard HP6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector, split/splitless injection port, an HP Chemstation software data system, and an HP7673 autosampler (Hewlett Packard). The analytical column was a fused silica capillary SP-2380 column (100 m × 0.32 mm inner diameter, 0.20 μm film thickness) from Supelco (Bellafonte, PA, USA). The oven temperature was initially programmed at 150°C (hold 4 min) and raised to 220°C at a rate of 4°C/min (hold 25 min). Injection (1 μL) was run in split (40:1) mode. Helium was the carrier gas at constant flow (1 mL/min) and make-up gas