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ScienceDirect
Growth Hormone & IGF Research 18 (2008) 361–368
www.elsevier.com/locate/ghir

Dietary conjugated linoleic acid increases PPARγ gene expression in adipose tissue of obese rat, and improves insulin resistance

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Received 19 August 2007; revised 28 December 2007; accepted 7 January 2008
Available online 4 March 2008

Abstract

Conjugated linoleic acid (CLA) is a class of positional, geometric, conjugated dienoic isomers of linoleic acid. Dietary CLA supplementation has resulted in a dramatic decrease in body fat mass in mice. However, some but not all studies in mice and humans have found that CLA promoted insulin resistance, and there were conflicting reports on the effects of CLA on peroxisomal proliferator-activated receptor-γ (PPARγ) activation and expression. The objective of present study was to investigate the effect of CLA on insulin resistance and its molecular mechanisms. Fifty male Wistar rats were randomly designed to the control, high-fat and high-fat with CLA (0.75, 1.50, and 3.00 g in per 100 g diet) groups. The effect of CLA on insulin sensitivity and the mechanism of resisting diabetes by CLA were investigated by RT-PCR assay. The results showed that supplementation with CLA significantly reduced body weight gain and white fat pad weight in the rats, the levels of plasma free fatty acids (FFA), triglycerides (TGs), cholesterin (TC), leptin, insulin and blood glucose concentration in the obese rats of CLA group were also decreased compared to the rats in the high-fat group. Dietary CLA increased the mRNA expression of PPARγ, fatty acid binding proteins (aP2), fatty acid transporter protein (FATP), acyl-CoA synthetase (ACS) and adiponectin in the adipose tissues of obese rats. The results suggest that CLA may ameliorate insulin resistance by activating PPARγ, and increasing the expression of PPARγ target genes such as ap2, FATP, FAT, and adiponectin in the white adipose tissue.

Keywords: Adiponectin; Peroxisome proliferators-activated receptor-γ; Fatty acid binding proteins; Fatty acid transporter protein; Acyl-CoA synthetase; Insulin resistance; Conjugated linoleic acid

1. Introduction

Type 2 diabetes is a common cause of morbidity and mortality that is characterized by insulin resistance often in association with central obesity [1], in parallel with the global epidemic of type 2 diabetes with the significant health and socioeconomic burdens [2]. Thus, there is a need to identify effective dietary strategies to attenuate the effect of type 2 diabetes, which is a heterogeneous disease characterized by target-tissue insulin resistance that cannot be overcome by β-cell hypersecretion [3].

Along with the increase in incidence of type 2 diabetes, there is a growing interest in finding molecules capability of fighting type 2 diabetes. CLA is a class of geometric and positional conjugated dienoic isomers. The major dietary sources of CLA are ruminant meat, dairy products, and partially hydrogenated vegetable oils. The predominant isomers found in foods and

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doi:10.1016/j.ghir.2008.01.001

commercial preparations are *cis*-9, *trans*-11 isomer and *trans*-10, *cis*-12 isomer. In the rumens of ovines and bovines, microbial bioconversion mainly produces the *c*9, *t*11-CLA isomer, whereas commercial preparations, sold as dietary supplements, contain *c*9, *t*11-CLA and *t*10, *c*12-CLA isomers in approximately equal amounts. CLA is a potent cancer preventative agent in animal models of chemical-induced carcinogenesis [4,5] and in vitro cell culture [6–8]. However, it has received considerable attention for its antiobesity and antidiabetes actions in animals [9]. A number of studies have found that CLA decreases body fat [10,11] and improves insulin sensitivity [12–15], but others study results disagree, and found that CLA exacerbates glucose tolerance and induces insulin resistance [16–18]. However, in one study treatment of lep^{db}/lep^{db} mice with CLA for five weeks improved glucose tolerance but plasma insulin was raised, treatment for eleven weeks improved glucose tolerance and reduced plasma insulin concentration [13]. Therefore, it is possible that long-term treatment with CLA can improve insulin sensitivity. However, there is a relative paucity of data regarding the mechanisms.

PPARγ are ligand-activated nuclear hormone receptors that heterodimerize with the retinoid X receptor and act to control the expression of genes involved in glucose and lipid metabolism [19,20]. PPARγ is the master adipogenic transcription factor, and induces glucose and fatty acid uptake by directly or indirectly enhancing the transcription of genes encoding proteins such as aP2 [21], insulin-dependent glucose transporter 4 (GLUT4) [22], FATP, and ACS. Since a report showed that a mixture of CLA isomers can activate PPARγ response elements in vitro and improved glucose tolerance and insulin resistance in Zucker diabetic fatty (fa/fa) rats [23]. We hypothesized that CLA might be a partial agonist of PPARγ, or alternatively may interfere with the expression of PPARγ and PPARγ target genes, to improve insulin resistance.

In the current study, we investigated the effect of CLA on insulin sensitivity by increasing dose and prolonging treatment, further characterize the effects of CLA on glucose and lipid metabolism in insulin resistance rats fed with high-fat diet, and to explore the mechanism of resisting diabetes by CLA.

2. Materials and methods

2.1. Experimental protocols

In this experiment, 50 male Wistar rats were obtained at 4 months old (Shanghai Slaccas, Inc.) and were individually housed in a temperature-controlled (20–22 °C) animal room with a 12 h light: dark cycle and had free access to drinking water. After a 7 d acclimation period, the animals were randomly divided into five groups of 10, balanced for body weights. The experimental diets shown in Table 1 were fed. Diets were based on the AIN-93 recommendations. Care and treatment of rats followed the recommended guidelines of the National Research Council (1985). CLA was obtained from Qing-Dao AoHai Biological Product Limited Company and the mixture of CLA isomers contained primarily 38.2% *cis*9, *trans*11 CLA, 42.3% *trans*10, *cis*12 CLA and 19.6% other CLA isomers determined by gas chromatography. The rats were weighed two times each week, and food intake was also measured at these times. At the end of the experimental period at week 12, 12 h after the last feeding, rats were anaesthetized with ethylether, the blood was taken from the abdominal aorta, and serum was obtained by centrifugation at 700 g for 15 min at 4 °C and stored at −80 °C until the measurement of plasma insulin, leptin, TGs, TC, and FFA. The epididymal adipose tissues and nephritic adipose tissues were collected, weighed, the body fat content[(epididymal adipose tissues + nephritic adipose tissues)/body

Table 1
Diet compositions

Ingredient	Basic diet	High-fat	High-fat + 3%CLA	High-fat + 1.5%CLA	High-fat + 0.75%CLA
Casein	20.0[a]	20.0	20.0	20.0	20.0
Cornstarch	63.0	53.0	53.0	53.0	53.0
Sodium carboxymethycellulose	1[b]	1	1	1	1
Sucrose	6	6	6	6	6
Fat (lard)	5.00[c]	15.00	12.00	13.50	14.25
CLA	0	0	3.0	1.5	0.75
Mineral mix,[d] AIN-93G	3.5[c]	3.5	3.5	3.5	3.5
Vitamin mix,[e] AIN-93G	1.5[b]	1.5	1.5	1.5	1.5

[a] Refers to AOAC and AIN-93 purified diets for laboratory rats, the percent protein of casein is 90%.
[b] Refers to the AOAC method.
[c] Refers to refined composition for mice and rats of Oriental Yeast 1991.
[d] Milligrams provided by 1 kg of mineral mixture: $CaHPO_4$ 500.0 g; NaCI 74.0 g; $K_3C_6H_5O_7 \cdot H_2O$ 220.0 g; K_2SO_4 52.0 g; MgO 24.0 g; $MnCO_3$ 3.5 g; Fe-citrate 6.0 g; $ZnCO_3$ 1.6 g; $CuCO_3 \cdot Cu(OH)_2 \cdot H_2O$ 0.3 g; KIO_3 0.01 g; $Na_2SeO_3 \cdot 5H_2O$ 0.01 g; $CrK(SO_4)_2 \cdot 12H_2O$ 0.55 g; sucrose 118.97 g.
[e] Provided by 1 kg of vitamin mixture: vitamin B1 600 mg; vitamin B2 600 mg; vitamin B6 700 mg; nicotinic acid 3000 mg; panthotenate 1600 mg; folic acid 200 mg; vitamin B_{12} 1 mg; biotin 20 mg; vitamin E 5000IU; vitamin A 400,000IU; vitamin K 5 mg; vitamin D_3 2.5 mg; sucrose 899.095 g.