· Original Article in English ·

Effects of 11β-hydroxysteroid dehydrogenase inhibitor on body weight and glucose tolerance in Sprague-Dawley rats fed with a high-fat diet

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Abstract: Objective Many studies have shown that glucocorticoids play a crucial role in the development of obesity and insulin resistance. This study investigated the therapeutic effects of long-term inhibition of glucocorticoid activity on obesity and insulin resistance. **Methods** Four-week-old male Sprague-Dawley (SD) rats were randomly fed with a high-fat diet (fat content accounting for 20% of total calorie) (control group, $n = 8$) or with a high-fat diet along with glycyrrhetic acid (GE, 800 mg/L), an inhibitor of 11 β-hydroxysteroid dehydrogenase (11β-HSD) for 24 weeks (GE-treated group, $n = 9$). The body weights and the amount of food intake were monitored weekly and daily, respectively. After 24 weeks of GE treatment, oral glucose tolerance tests were performed. Blood glucose was measured by glucose oxidase method. The levels of plasma glucocorticoids, insulin and leptin were measured with radioimmunoassay. The levels of serum cholesterol and triglyceride were determined with an automatic measuring analyzer. **Results** The food intake amount decreased significantly in the GE-treated group from 6 weeks and body weight gain was markedly less from 8 weeks after GE administration compared with the control group. After 24 weeks of treatment, the plasma levels of leptin and insulin in GE- treated rats were significantly reduced compared with the control group. The serum levels of cholesterol and triglyceride decreased markedly compared with the control group and the levels of blood glucose were significantly lower 15, 30, 60 and 120 minutes after oral glucose load in the GE-treated group compared with the control group. **Conclusions** Long-term GE treatment may contribute to resisting diet-induced obesity and insulin resistance.

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Key words: 11β-hydroxysteroid dehydrogenase inhibitor; Glucocorticoids; Obesity; Glucose tolerance; Rats

11β-羟甾类脱氢酶抑制剂对高脂喂养SD大鼠体重及糖耐量的影响

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[**摘　要**] **目的**　糖皮质激素在肥胖及胰岛素抵抗发病机制中起着至关重要的作用。该研究旨在探讨长期抑制糖皮质激素活性对肥胖及胰岛素抵抗的防治作用。**方法**　采用4周龄雄性SD大鼠为动物模型,在给于高脂饲料喂养的同时,予含11β-羟甾类脱氢酶(11β-hydroxysteroid dehydrogenase,11β-HSD)抑制剂(glycyrrhetic acid, GE) 800 mg/L的水长期饮用至24周,并以单纯高脂饲料喂养组作为对照组,监测两组大鼠食物摄入量及体重变化。在GE应用24周后,进行口服葡萄糖耐量试验,并采用放射免疫方法检测血浆糖皮质激素、瘦素及胰岛素的水平,采用全自动生化分析仪检测血清胆固醇及甘油三脂的含量。**结果**　随着GE治疗时间的延长,大鼠的食物摄入量较对照组逐渐减少,至6周时达到统计学意义,同时伴有相应的体重增长减慢,至8周时,GE组体重明显低于对照组。治疗24周时,血浆糖皮质激素水平较对照组无明显降低,但血浆瘦素及胰岛素水平均明显低于对照组;血清胆固醇及甘油三脂的含量也明显低于对照组;口服葡萄糖耐量试验结果显示GE组在15, 30, 60和120分钟时的血糖水平明显低于对照组。**结论**　长期应用11β-羟甾类脱氢酶抑制剂能够抵抗高脂饮食诱导的肥胖及胰岛素抵抗。

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[**关　键　词**] 11β-羟甾类脱氢酶抑制剂;糖皮质激素;肥胖症;糖耐量;大鼠

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Glucocorticoids are crucial factors in the regulation of food intake and body adipose, affecting energy metabolism at both the central and peripheral levels [1,2]. More and more studies have shown that glucocorticoids play a significant role in the development of obesity and insulin resistance [3], as is clearly evident in patients with Cushing's syndrome. In an animal experiment, it has been observed that adrenalectomy improved several changes, including increased body weight and high levels of plasma insulin [4], which was reversed by glucocorticoid replacement [5]. Glucocorticoid action is regulated by negative feedback via the hypothalamic-pituitary-adrenal (HPA) and intracellular prereceptor glucocorticoid metabolism [6]. Since patients with obesity and obesity-related metabolic disorders do not exhibit increased circulating glucocorticoid levels, it has been suggested that obesity and metabolic syndrome may result from increased intracellular glucocorticoid tone [7,8]. The main regulators of intracellular glucocorticoid levels are 11-hydroxysteroid dehydrogenase (HSD) enzymes, which are known to have two isozymes (11β-HSD1 and 11β-HSD2). In humans, an increased 11β-HSD1 activity or expression has been noted in adipose tissue from human obesity [9,10]. Similarly in animals, the transgenic mice over-expressing 11β-HSD in adipose tissue develop central obesity, insulin resistance and hypertension [11,12]. Improvement of insulin sensitivity and glucose tolerance in 11β-HSD1 null mice [13] suggests that inhibition of 11β-HSD may have a therapeutic effect on reducing body weight and ameliorating metabolic syndrome. Therefore, the present study was designed to investigate the effects of long-term intervention in the 11β-HSD system on body weight regulation and glucose tolerance. Glycyrrhetic acid (GE), an inhibitor of 11β-hydroxysteroid dehydrogenase, was administered to four-week-old male rats fed with a high fat diet for 24 weeks.

Materials and methods

Animals

Four-week-old (after weaning) male Sprague-Dawley (SD) rats were provided by Laboratory Animal Center of Dalian Medical University and housed under controlled conditions (temperature, 20-22 ℃; humidity, 55%-65%; lights on between 7 AM and 7 PM). A single rat was confined and fed in a cage and was allowed free access to food and water.

Experimental protocol

The rats were randomly assigned into two weight-matched groups. One group, the GE-treated group, (body weight 121.7 ± 8.1 g, $n=9$) was given drinking water containing GE (provided by Wako Pure Chemical Industries, Osaka, Japan and dissolved in ethanol and sodium hydroxide which the concentrations were 0.5% and 0.01N, respectively.) at a concentration of 800 mg/L. The other group, the control group, (body weight 119.5 ± 6.7 g, $n=8$) was given the drinking water containing the same concentrations of ethanol and sodium hydroxide as control group. Both groups were fed with a high-fat diet (fat content: 20% of total calorie intake). The amount of food intake was recorded daily and the body weight was monitored weekly.

After 24 weeks of treatment, the rats were fasted overnight and then anesthetized by using intraperitoneal pentobarbital (60 mg/kg) for oral glucose tolerance tests. Glucose (2 g/kg) was administered by gavage between 9 and 10 AM, and blood samples were taken by tail vein droplets at time points of 0, 15, 30, 60, and 120 minutes after the oral glucose load. Two days later, the rats were fasted overnight and anesthetized again, and blood samples were collected by heart puncture and then plasma was stored at −20 ℃ before assay.

Biochemical and hormonal assays

Blood samples were collected in EDTA-coated tubes and centrifuged at 1 200 r/min and 4 ℃ for 10 minutes. Blood glucose was measured by glucose oxidase method using a glucose analyzer (Beckman, Palo Alto, CA). Plasma leptin was determined using the rat leptin radioimmunoassay (RIA) kit provided by Linco Research (St. Charles, MO). Plasma insulin was measured by RIA using a reagent kit from Linco Research, with rat insulin as standard. Corticosterone was determined by RIA (RSL ^{125}I corticosterone RIA, ICN Biomedicals, Inc., Costa Mesa, CA). The intra-and inter-assay coefficients of variation were <5%. And the levels of cholesterol and triglyceride were determined with an automatic measuring analyzer (Olympus AU 1000, Japan).

Statistical analysis

All data were expressed as the mean ± SD. Statistical analysis was performed by unpaired Student's t test using SPSS11.0 software. Differences were considered significant at $P<0.05$.

Results

Body weight changes

The food intake amount decreased significantly in the GE-treated group from 6 weeks after GE administration

compared with the control group. The body weight gain was less in the GE-treated group from 6 weeks of GE administration than that in the control group and a significant difference was noted from 8 weeks after GE administration (Table 1 and Figure 1).

Table 1 Body weight changes (g, $\bar{x} \pm s$)

Group	0 w	4 w	8 w	12 w	16 w	20 w	24 w
Control	119.5 ± 6.7	294.3 ± 13.8	381.7 ± 13.4	409.0 ± 24.3	444.2 ± 31.3	471.3 ± 34.2	497.2 ± 35.3
GE-treated	121.7 ± 8.1	277.9 ± 12.5	346.8 ± 21.1[a]	371.4 ± 20.9[b]	391.6 ± 23.9[b]	407.2 ± 25.1[b]	413.7 ± 22.6[b]

Compared with the control group, a $P<0.05$; b $P<0.01$

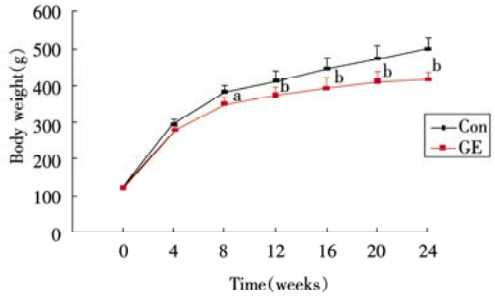

Figure 1 The body weight changes in the GE-treated and the control groups. The body weight gain was significantly reduced in the GE-treated group from 8 weeks of GE administration when compared with the control group (a $P<0.05$; b $P<0.01$).

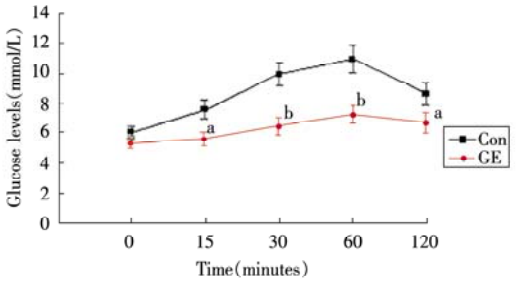

Figure 2 Oral glucose tolerance tests. Blood glucose levels in the GE-treated group were significantly lower 15, 30, 60 and 120 minutes after oral glucose load compared with those in the control group (a $P<0.05$; b $P<0.01$).

Oral glucose tolerance test

After 24 weeks of GE treatment, the fasting blood glucose values were not significantly different between the GE-treated and the control groups (5.3 ± 0.5 mmol/L vs 5.7 ± 0.4 mmol/L). In both groups, blood glucose contents peaked at 60 minutes after oral glucose load. Blood glucose levels in the GE-treated group were significantly lower 15, 30, 60 and 120 minutes after oral glucose load compared with those in the control group (Figure 2).

Levels of corticosterone, leptin, insulin, cholesterol and triglyceride

There were no significant differences in plasma corticosterone levels between the GE-treated and the control groups 24 weeks after GE treatment. The plasma levels of leptin and insulin in the GE-treated group decreased significantly than those in the control group ($P<0.05$). The serum levels of cholesterol and triglyceride were also markedly reduced in the GE-treated group when compared with the control group (Table 2).

Table 2 Levels of corticosterone, leptin, insulin, cholesterol and triglyceride ($\bar{x} \pm s$)

Group	Corticosterone(nmol/L)	Leptin(ng/mL)	Insulin(mIU/L)	Cholesterol(mmol/L)	Triglyceride(mmol/L)
Control	368.7 ± 47.2	15.48 ± 3.58	38.29 ± 9.85	2.49 ± 0.36	1.31 ± 0.48
GE-treated	341.5 ± 51.3	11.04 ± 3.46[a]	22.48 ± 9.42[a]	1.62 ± 0.42[b]	0.82 ± 0.16[a]

Compared with the control group, a $P<0.05$; b $P<0.01$

Discussion

Accumulating evidence suggests that obesity has been associated with alterations in glucocorticoid metabolism in both humans and rodents. In fact, the tissue glucocorticoid action has been implicated as a pathophysiological factor of obesity and type 2 diabetes through dysregulation of 11β-HSD [14]. Two isoforms of 11β-HSD have been cloned and characterized. 11β-HSD1 is abundant in glucocorticoid target tissues including liver, adipose tissue and the central nervous system, and it is thought to serve as a tissue-specific amplifier of glucocorticoid action. It has reductase activity *in vivo* and works to regenerate active glucocorticoids (cortisol in humans and corticosterone in rodents, respectively) from inactive 11-keto metabolites cortisone (in humans) or 11-dehydrocorticosterone (in rodents), thus increasing local glucocorticoid levels [15]. 11β-HSD2 is expressed widely in mineralocorticoid target tissues, such as the kidney, gut and placenta, and acts as a potent dehydrogenase that oxidizes active glucocorticoids to their inactive 11-keto forms, thereby preventing glucocorticoids from binding to mineralocor-

ticoid receptors. The dysregulation of 11β-HSD1 is related to the development of obesity and obesity-related metabolic disorders including insulin resistance [16], and the pharmacologic inhibition of 11β-HSD can alter intracellular glucocorticoid levels and may be therapeutic for metabolic syndrome [17,18]. Previous work has shown that administration of high-fat diet to rats induced the development of obesity and obesity-related metabolic disorders [19]. This study observed the effect of GE on preventing diet-induced obesity and insulin resistance in rats fed with a high fat diet and found that long-term GE treatment significantly suppressed food intake and slowed body weight gain compared with control rats, which is consistent with Li's study in Zucker rats [20]. Glucocorticoids have multiple effects on lipid metabolism. These hormones increase lipolysis in adipocytes and regulate hepatic lipoprotein production such that the patients with Cushing's syndrome have elevated serum triglycerides and cholesterol concentrations. Long-term inhibition of 11β-HSD caused the reduction of serum cholesterol and triglycerides contents in rats fed with a high fat diet. These results are also consistent with the research on 11β-HSD1 inhibition ameliorates metabolic syndrome and prevents progression of atherosclerosis in mice [21].

Leptin, which is secreted by the adipocytes in proportion to the size of adipose tissue, participates in body weight regulation by inhibiting food intake and enhancing energy expenditure [22]. In this study, the decreased leptin levels in GE-treated rats suggest that leptin is not the causal factor of the anorexic and weight-reducing effects of GE. The decreased leptin levels may be explained by the reduced adipose tissue mass caused by GE inhibition of 11β-HSD activity in adipose tissue.

It is well known that glucocorticoids have many actions on carbohydrate metabolism, usually antagonizing insulin effects. They stimulate hepatic gluconeogenesis by increasing activities of the crucial enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase. In addition, glucocorticoids provide gluconeogenic substrates by increasing the release of amino acids from muscle and glycerol from fat and they also have a permissive action on glucagon- and epinephrine-stimulated gluconeogenesis. Glucocorticoids directly inhibit insulin secretion from pancreatic β-cells and decrease glucose uptake in peripheral tissues through glucose transporter translocation from the plasma membrane to intracellular sites. Therefore excessive tissue glucocorticoids may contribute to hyperglycemia and insulin resistance associated with type 2 diabetes and pharmacologic inhibition of intracellular glucocorticoid activation can effectively decrease fasting glucose and insulin levels in diet-induced obese mice [21]. In this study, although the fasted glucose levels were not lower significantly in GE-treated rats, insulin levels decreased significantly, and oral glucose tolerance tests showed that there was a significant decrease in blood glucose levels at 15, 30, 60 and 120 minutes after oral glucose load, suggesting that the inhibition of glucocorticoid activation has an effective improvement for insulin sensitivity.

The long-term inhibition of 11β-HSD by GE did not affect the terminal circulating corticosterone levels in this experiment, suggesting that partial pharmacologic inhibition of 11β-HSD may not be enough to measurably alter HPA function and that the local dysregulation of glucocorticoids may be more important in the pathogenesis of obesity and insulin resistance [23,24].

In summary, long-term GE treatment slowed body weight gain, lowered the levels of plasma leptin and insulin, reduced the levels of serum cholesterol and triglyceride, and improved glucose tolerance in rats fed with a high-fat diet. These findings suggest that chronic inhibition of glucocorticoid activity may contribute to resisting diet-induced obesity and insulin resistance.

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