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Research on the Expression Level of RIP140 Gene in Adipose Tissue of High Fat Diet-induced C57BL/6J Obesity Mice*

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ABSTRACT Objective: To study the expression level of RIP140mRNA in adipose tissue of high fat diet-induced obese mice and the correlation with insulin resistance. **Methods:** Male C57BL/6J mice were divided into two groups: normal-fat diet group (NFD) and high-fat group (HFD), fed respectively for 14 weeks. Mice in HFD group whose body weight were 20 % higher than average weight of NFD mice were selected as obese mice. The triglycerides (TG), total cholesterol (TC), fast blood glucose (FBG), fast insulin (FIns) levels of the NFD group and obese mice were assayed and the HOMA-IR were calculated. The expression of RIP140 mRNA in epididymal adipose tissue was examined by RT-PCR. **Results:** A total of 12 mice in HDF group were recruited into the obesity group. The level of TG, TC, FBG, FIns (P<0.05),HOMA-IR (P<0.01)in obesity group were higher than those of control group; The expression level of RIP140 mRNA in adipose increased significantly in obesity group (P<0.05), when compared with the controls. The correlation analysis showed that the expression of RIP140 mRNA in mice adipose were positively correlated with HOMA -IR(r=0.465, P<0.05) and TG(r=0.536,P<0.05), but not with TC, FBG and FIns. **Conclusions:** The expression level of RIP140 mRNA in adipose tissue is increased and positively correlated with insulin resistance in mice fed with high fat diet.

Key words: Receptor-interacting protein 140; Obesity; Insulin resistance; Adipose tissue; Mice

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1 Introduction

Obesity has become one of the public health problems of global concern which poses a risk to human health. The studies have shown that energy homeostasis is important for the prevention and control of obesity, diabetes and other metabolic diseases. Energy homeostasis is a process regulated by specific gene networks that requires a strict balance between energy intake, storage, and expenditure. Adipose tissue is an energy storage organ [1] and an endocrine organ that can secrete a variety of adipokines that are involved in metabolic regulation and obesity-related comorbidities [2]. Nuclear receptors (NR) are a super family of transcription factors involved in many physiological processes, such as energy homeostasis, metabolism, fertility, and cell differentiation. The activity of NR is regulated by the binding of ligands[3] and it is modulated by coregulators[4]. Receptor-interacting protein 140 (RIP140) is a 140-kDa ligand-dependent corepressor for NR involved in energy homeostasis by inhibiting energy expenditure. RIP140 is widely expressed in metabolic tissues, mainly in adipose tissue, muscle, and liver [5]. It has been shown that RIP140-deficient mice are lean and resistant to diet-induced obesity. Fritah A has pointed out that, in the absence of RIP140, the expression levels of uncoupling protein 1 (UCP1) gene in brown adipose tissue and carnitine palmitoyl transferase 1β (CPT-1β) genes in white adipose tissue were up-regulated, and thus promote fatty acid oxidation and protein energy consumption [6,7]. It has been described that in RIP140-deficient fat cells the expression of genes associated with fatty acid oxidation, oxidative phosphorylation, glycolysis and Krebs cycle were up-regulated[8]. Recently, Ho, etc. found RIP140 can reduce glucose uptake induced of GLUT4 by inhibiting Akt mediated phosphorylation of AS160 in the cytoplasm of adipocytes[9].

Recent studies show that RIP140, as the regulator of energy balance, plays an important role in the regulation of glucose and lipid metabolism. It may be a new direction for the treatment of obesity, diabetes and other metabolic diseases, but the specific regulatory mechanism is uncertainty. Insulin resistance is an important pathogenesis of obesity and diabetes. In this article, the expression level of RIP140 gene in epididymal adipose tissue of high fat diet-induced obesity mice and its relationship with insulin resistance was studied. It may provide evidence for further research about the role of RIP140 in obesity and related metabolic diseases.

2 Materials and Methods

2.1 Experimental Animals

30 health 4-5 week-old SPF male C57BL/6J mice, 16-20 g were used in this study, and they were purchased from the Experimental Animal Hayes Lake LLC, license number: SCXK (Shang-

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hai) 2012-0002. These mice were maintained under temperature 20 ℃, relative humidity of 60 %, 12 h/d light cycle and free access to water during feeding. Experimental methods for the animals conformed the relevant requirements of <<Guidance Suggestions for the Care of Laboratory Animals>>, which is issued by the Ministry of Science and Technology People's Republic of China.

2.2 Experimental methods

2.2.1 Established the model of obesity mice, specimen collection and blood glucose testing These mice were randomly divided into 2 groups: normal diet (NFD) group 10, high-fat diet (HFD) group 20 after one week adaptive feeding. Normal diet group was given standard rodent diet (12 % fat of total calories provided by the Affiliated Hospital of Qingdao University Experimental Animal Center) feeding, while HFD group was given high-fat diet (60 % fat of total calories from Beijing Nuokang source biotechnology Limited) feeding. After 14 weeks, mice in HFD group whose body weight were 20 % higher than average weight of NFD mice were selected as obese mice [10]. After 10 h of overnight fasting, the fasting blood glucose of after peak was measured in the control group and the obese group mice with Roche blood glucose meter. Then mice were anesthetized by intraperitoneal injection with chloral hydrate, cardiac blood, centrifugation supernatant, and store at -20 ℃ refrigerator for later use. Take epididymal adipose tissue, placed in liquid nitrogen cryopreservation to be seized.

2.2.2 Biochemical parameters The levels of triglycerides and total cholesterol were analyzed by automatic biochemical analyzer.

2.2.3 Detection of blood insulin and calculation of insulin resistance index The levels of fasting insulin (FIns) were determined by ELISA. And the insulin resistance index was calculated using the homeostasis model, which can evaluate insulin resistance. HOMA-IR = FPG (mmol / L) × FINS (mU/mL) / 22.5.

2.2.4 Determining of RIP140 mRNA Extract the total RNA with Trizol (Omega Biotechnology) and synthesize cDNA according to the instructions of reverse transcription reagents (Thermo Fisher Company). Design of primers for each gene with the software of PCR Designer, and the primers were synthesized in Shanghai HanYu Biological Technology corporation. Primer sequences of RIP140: the forward primer 5'-GAACATGACTCATGGAGAAGAG-3', the reverse primer 5'-GAATCAGACAGCCTCTTCCG -3', product length of 315 bp, GAPDH: the forward primer 5'-TGAGCATCTCCCTCACAATTTC-3', the reverse primer 5'-GTGCAGCGAACTTTATTGATGG- 3', product length of 104 bp, adopt the method of SYBR-Green obtaining amplification of PCR.

RT-PCR reaction system 20 μL, reaction conditions were as follows: 95 ℃ denaturation for 10 min, 95 ℃ denaturation 15 sec, 56 ℃ annealing l min, 72 ℃ extension 1 min, 40 cycles, after the reaction the amplification curve and melting curve was analyzed and CT values were recorded too.

2.2.5 Statistical analysis Measurement data were showed by ($\bar{x}\pm s$). SPSS17.0 was utilized for statistical analysis. Data were compared between the two groups with two independent samples t test, and $P<0.05$ was considered statistically significant; the correlation between the variables were evaluated with Pearson correlation coefficient.

3 Results

3.1 Quantitative analysis of experimental animals

12 mice were counted as obese mice in HFD group, there was no mouse shedding in NFD group. Finally 12 mice in the obese group and 10 mice in the control group were included in the statistical analysis.

3.2 Change in body weight of mice

Before the experiment the difference of mice weight in NFD group and HFD group did not show statistical significance ($P>0.05$); the weight in HFD group was significantly higher than NFD group after 10 weeks ($P<0.05$); at the end of the experiment, the difference was highly statistically significant ($P<0.01$), as shown in Table 1.

Table 1 Change in body weight of mice in two groups($\bar{x}\pm s$)

Group	Quantity	5w(g)	15w(g)	19w(g)
NFD	10	18.48± 1.092	26.84± 1.135	28.86± 1.136
HFD	20	18.36± 1.084	30.16± 1.142[a]	36.43± 2.606[b]

Note: Compared with normal control group, [a] $P<0.05$, [b] $P<0.01$.

3.3 Comparison of FBG, TG, TC, FIns and HOMA-IR

The obese group compared with the control group, FBG, TG, TC, FIns were increased ($P<0.05$), HOMA-IR were increased ($P<0.01$), as shown in Table 2.

Table 2 Comparison of FBG, TG, TC, FIns and HOMA-IR($\bar{x}\pm s$)

Group	Quantity	FBG (mmol/L)	TG (mmol/L)	TC (mmol/L)	FIns (mU/L)	HOMA-IR.
Control group	10	9.38± 1.69	0.98± 0.32	2.48± 0.21	9.88± 2.72	2.76± 0.72
Obese group	12	11.1± 3.64[a]	1.20± 0.36[a]	4.52± 0.40[a]	13.1± 2.39[a]	5.64± 0.69[b]

Note: Compared with normal control group, [a] $P<0.05$, [b] $P<0.01$.

3.4 Comparison of RIP140mRNA in two groups

Compared with the control group, the expression level of RIP140mRNA in the experimental group was increased (P <0.05), as shown in Table 3.

Table 3 Comparison of RIP140mRNA in two groups($\bar{x}\pm s$)

Group	Quantity	RIP140mRNA
Control group	10	0.42± 0.16
Obese group	12	2.34± 0.72[a]

Note: Compared with normal control group, [a] P<0.05.

3.5 Correlation analysis

The expression of RIP140 mRNA in obese group were positively correlated with HOMA -IR (r=0.465, P<0.05) and TG(r=0.536, P<0.05), but not with TC, FBG and FIns.

4 Discussion

The increasing prevalence of obesity is related to both increased energy intake and reduced energy expenditure. Obesity has been shown to increase the risk of developing type 2 diabetes mellitus, cardiovascular diseases, osteoarthritis, sleepapnea, and several types of cancer [11-14]. But the molecular mechanisms of obesity are not fully understood, and thus the analysis of specific genes that regulate energy homeostasis can be crucial.

RIP140, as a corepressor for NR, is highly expressed in adipose tissue, regulating metabolism and maintaining energy balance. It has been described that in RIP140-deficient fat cells the expression levels of genes associated with fatty acid oxidation, oxidative phosphorylation, glycolysis and Krebs cycle were up-regulated[8]. It has been shown that in the absence of RIP140, the genes expression level of UCP1 and CPT-1β were increased, as a result, more chemical energy was transformed into heat, and therefore, enhancing metabolic rate [6,15,16]. But currently the research on the expression level of RIP140 gene in obesity mice is uncommon. In this study we found the expression level of RIP140 gene in adipose tissue of high fat diet-induced obesity mice was up-regulated as well as the levels of FBG, TG, TC, and FIns (P <0.05). The increased TG and TC in obesity mice was mainly due to the long-term intaking of high fat diet. Furthermore, we surmise RIP140 may reduce oxidative decomposition of fatty acids by inhibiting the expression UCP1 gene and CPT-1β gene, leading to elevated levels of TG. Meanwhile, the experiment also showed a positive correlation between RIP140 and TG, this founding emphasized the role of RIP140 in inhibiting energy expenditure, and suggested that RIP140 may be an important factor in inducing obesity. Analysis of the correlation between RIP140 and TC was not statistically significant, it may be due to the oxidation of fatty acids were inhibited and thus the metabolite of CoA decreased, affecting the synthesis of cholesterol. Meanwhile, RIP140 may cause elevated FBG by inhibiting the process of glycolysis, Krebs cycle and other genes associated catabolism, thus insulin levels were elevated. But there is no statistically significant correlation analysis RIP140 with glucose and insulin, indicating that RIP140 may be not a major factor can affect FIns and FBG in obesity mice.

Insulin resistance (IR), also known as decreased insulin sensitivity, usually refers to insulin-mediated glucose utilization reduced, IR is one of the main pathogenesis of obesity and type 2 diabetes. IR occurs mainly in peripheral tissues (muscle and fat) and liver, the former showed that decreased ability of glucose uptake, usage and storage by skeletal muscle and adipose tissue, while the latter showed the weakened ability to inhibit hepatic glucose outputting.

Present study has confirmed the peroxisome proliferator-activated receptor PPAR-γ and adiponectin play an important role in the mechanism of insulin resistance in obesity [17, 18]. Under conditions of high fat diet, the binding of PPAR-γ gene and the natural ligand can make the excess energy storing in adipose tissue, and induce obesity and insulin resistance. Adiponectin can promote fatty acid oxidation in skeletal muscle and glucose intake and reduce hepatic glucose output, and the lack of adiponectin will lead to insulin resistance [19]. Our study found that a positive correlation between RIP140 and HOMA-IR in high fat diet-induced obesity mice. In adipocytes, RIP140 can be phosphorylated by protein kinase C epsilon (PKCε), followed by arginine methylation, and exported to the cytoplasm[20]. The protein kinase C (PKC) and protein arginine methyl transferase enzyme (PRMT) in adipose tissue can stimulate the phosphorylation and methylation of RIP140, and facilitate its transport to the cytoplasm. Ho, etc. found RIP140 can reduce glucose uptake induced of GLUT4 by inhibiting Akt mediated phosphorylation of AS160 in the cytoplasm of adipocytes[9], and then reduce insulin sensitivity, suggesting that RIP140 may participate in the mechanisms of insulin resistance in obesity.

It may promote its cytoplasmic translocation, thereby reduce glucose uptake induced by the GLUT4, and then increase insulin resistance by the above way that the expression level of RIP140mRNA in adipose tissue of high fat diet-induced obesity mice was increased. In this study a positive correlation between RIP140 and HOMA-IR in obesity mice was found, suggesting that RIP140 may be involved in the pathophysiology of diabetes development, but it needs further research on the mechanism of influence FINs, FBG and HOMA-IR. Also, when changing the expression level of RIP140, whether the oxidation and heat-related genes will be affected? And what about the insulin signaling system? These questions need to be more in-depth and meticulous research.

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高脂饮食诱导的 C57BL/6J 肥胖小鼠脂肪组织 RIP140 基因表达水平的研究 *

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摘要 目的：探讨高脂饮食致肥胖小鼠脂肪组织 RIP140mRNA 表达水平的变化及其与胰岛素抵抗的关系。**方法：**将 C57BL/ 6J 雄性小鼠随机分为正常饮食（NFD）组、高脂饮食（HFD）组分别喂养 14 周后，测量两组小鼠体重，以 NFD 组小鼠体重作为对照，选取 HFD 组中体重大于对照组小鼠平均体重 20 % 的小鼠作为肥胖组小鼠。对照组和肥胖组小鼠取血测甘油三酯（TG）、总胆固醇（TC）、空腹血糖（FBG）、空腹胰岛素水平（FIns），计算稳态模型胰岛素抵抗指数（HOMA-IR）；采用 RT-PCR 技术检测两组小鼠附睾脂肪组织 RIP140 mRNA 的表达水平，并进行统计学分析。**结果：**HDF 组小鼠中有 12 只符合标准计入肥胖组。肥胖组小鼠 TG、TC、FBG、FIns（P<0.05），HOMA-IR（P<0.01）均明显高于对照组；肥胖组小鼠脂肪组织 RIP140 mRNA 的表达高于对照组，差异具有统计学意义（P<0.05）；相关分析显示小鼠脂肪组织 RIP140 mRNA 表达水平与 TG 水平呈正相关（r=0.536，P<0.05），与胰岛素抵抗指数呈正相关（r=0.465，P<0.05），而与 TC、FBG、FIns 水平相关分析无统计学意义（P>0.05）。**结论：**高脂饮食诱导的肥胖小鼠脂肪组织 RIP140 mRNA 表达增加，并与胰岛素抵抗程度呈正相关。

关键词：受体相互作用蛋白 140；肥胖；胰岛素抵抗；脂肪组织；小鼠

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