

Original Article

Decreased irisin secretion contributes to muscle insulin resistance in high-fat diet mice

Zaigang Yang, Xu Chen, Yujuan Chen, Qian Zhao

Geriatric Department of Endocrinology, The First Hospital Affiliated to Zhengzhou University, Zhengzhou 450052, China

Received April 18, 2015; Accepted May 29, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: Aims: Recent studies have revealed the relationship between irisin and insulin signaling, while positive associations of muscle FNDC5 with insulin resistance is observed. However, the functional mechanism of irisin on muscle insulin resistance is still obscure. This study aims to investigate the effect of irisin on muscle insulin action. Methods: Diabetic mouse model was established by high fat diet (HFD) induced obesity in C57BL/6 mice. Body indexes and serum levels of triglyceride (TG), blood glucose and insulin were record. Oral glucose tolerance test (OGTT) was performed before being killed. Circulating irisin level was also detected, while FNDC5/irisin expression was determined by RT-PCR and western blot analysis in both muscle and adipose tissues. Insulin action was further evaluated by the phosphorylation of AKT and Erk, and palmitic acid treated muscle cells were introduced for mimicking diabetic status *in vitro*. Results: Obvious obese features associated with type 2 diabetes were observed in HFD feeding mice, with decreased circulating irisin level and FNDC5/irisin secretion in adipose tissues. Although FNDC5/irisin expression showed little change in skeletal muscle, the insulin action was inhibited significantly. Moreover, palmitic acid treated muscle cells showed similar inhibition of insulin action, and FNDC5/irisin expression change. Besides, insulin action could be reversed by irisin addition in muscle cells. Conclusion: HFD induced obese mice showed decreased irisin secretion from adipose tissues, which might contribute to muscle insulin resistance. Furthermore, irisin addition could recover insulin action in palmitic acid treated muscle cells, indicating the importance of irisin for preserving insulin signaling.

Keywords: High-fat diet, obesity, diabetes, irisin, muscle insulin resistance

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, and has reached epidemic levels in the worldwide with poor prognosis and increased morbidity and mortality [1]. The incidence of diabetes is often associated with the interaction between genetic and environmental factors, with many complications such as cardiovascular disease, kidney disease, neuropathy and retinopathy [2]. Type 2 diabetes (T2D) is the most common form in the human population, more than 90% of cases, and stems from the failure of the body to respond normally to insulin, called insulin resistance, coupled with the inability to produce enough insulin to overcome this resistant state [3]. T2D is often associated with obesity, the most common meta-

bolic disorder in the world, and the current epidemics of these two conditions are seemingly related [4, 5], because of the ability of obesity to engender insulin resistance. Insulin resistance in both obesity and T2D can be manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output [6], however, many details of the mechanisms by which obesity causes systemic insulin resistance remain unknown, except for an increasing understanding of what may now be referred to as the adiponutrient axis [7, 8].

Irisin, identified as a proteolytic cleavage product of the fibronectin type III domain-containing protein 5 (FNDC5), is a novel myokine secreted by contracting skeletal muscle, possibly mediating some exercise health benefits via 'brown-

ing' of white adipose tissue [9]. Irisin can cause a significant increase in total body energy expenditure and resistance to obesity-associated insulin resistance in mice, while controversy still exists concerning irisin origin, regulation and function in humans [10]. Recent clinical studies show that circulating irisin levels are decreased in newly diagnosed Chinese type 2 diabetic patients without clinical angiopathy [11], as well as other T2D patients [12]. The association between irisin and reduced insulin sensitivity has also been investigated with results showing that circulating irisin can predict the insulin resistance onset related to dietary weight regain [13]. Moreover, increasing reports reveal the functional ways of irisin through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling [14, 15], indicating the relevance between irisin and insulin signaling. A few recent studies have observed positive associations of muscle FNDC5 with insulin resistance [16, 17]; speculating on the negative, desensitizing effects of irisin on insulin action.

In the present study, rat model for T2D was established by high fat diet induced obesity in C57BL/6 mice. When impaired glucose tolerance and decreased insulin sensitivity were observed in obese mice, skeletal muscle and subcutaneous abdominal adipose tissues were taken for the detection of FNDC5/irisin secretion and expression. Studies focused on changes in the phosphorylation of key kinases in insulin signaling were also performed to evaluate the insulin resistant in combination with insulin level. Furthermore, mimicking diabetic status *in vitro* by treating muscle cells with palmitic acid was introduced to verify the speculation about the contribution of decreased irisin secretion from adipose tissues to the inhibited insulin signaling in skeletal muscle.

Materials and methods

Animal studies

C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 6 weeks of age with body weight in the range of 30-35 g, and randomly divided into two groups: 1) control groups (n=8) fed with standard rodent chow and 2) high-fat diet (HFD) groups (n=8) fed with a high fat (60% kcal) diet (D12492, Research Diets) for 12 weeks. Physiological parameters like body weight, body fat, serum triglyceride

(TG), fasting blood glucose and insulin levels were evaluated. In the end, all mice were sacrificed, and both skeletal muscle tissues and subcutaneous abdominal adipose tissues were taken for immunohistochemistry and total RNA extraction for subsequent studies.

Cell culture

Murine myocytes (C2C12) were purchased from ATCC (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 4500 mg/l glucose, supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/ml penicillin/streptomycin, at 37°C in a humidified atmosphere of 5% CO₂. After incubation, C2C12 myotubes were treated with 1) palmitic acid (Sigma-Aldrich), 2) recombinant irisin (r-irisin, Cayman Chemical) and 3) both of palmitic acid and r-irisin, and then stimulated with human recombinant insulin (human r-insulin, Eli Lilly) for 30min to mimic diabetic status *in vitro*. Before used, human r-insulin and r-irisin were diluted to various concentrations in culture media identified through previous examinations and pilot data [18].

Biochemical assays

Mouse body weights were recorded weekly, with corresponding body fat analyzed using either DXA or quantitative magnetic resonance (QMR; ECHO Medical Systems, Houston, TX) technologies as described before [19]. Blood samples were drawn from the tail vein at the times indicated after 12 h of food deprivation, and fasting glucose was measured with an automatic blood glucose meter (Glutest Pro, Sanwa Chemical, Nagoya, Japan). Whole blood was collected and centrifuged in heparinized tubes, and the plasma was stored at 20°C. Triglyceride (TG) content and insulin levels were measured respectively by triglyceride L-type (Wako, Osaka, Japan) and an insulin radioimmunoassay kit (BIOTRAK, Amersham Biosciences) using rat insulin as the standard [20]. To evaluate features involving type 2 diabetes, these mice were also subjected to oral glucose tolerance test (OGTT) before being killed. In brief, they were orally loaded with glucose at 1.0 mg/g (body weight) after fasted for 12 h. Blood samples were collected at different times, and glucose was immediately measured as described above. After being killed, blood sample, muscle and adipose tissue were col-

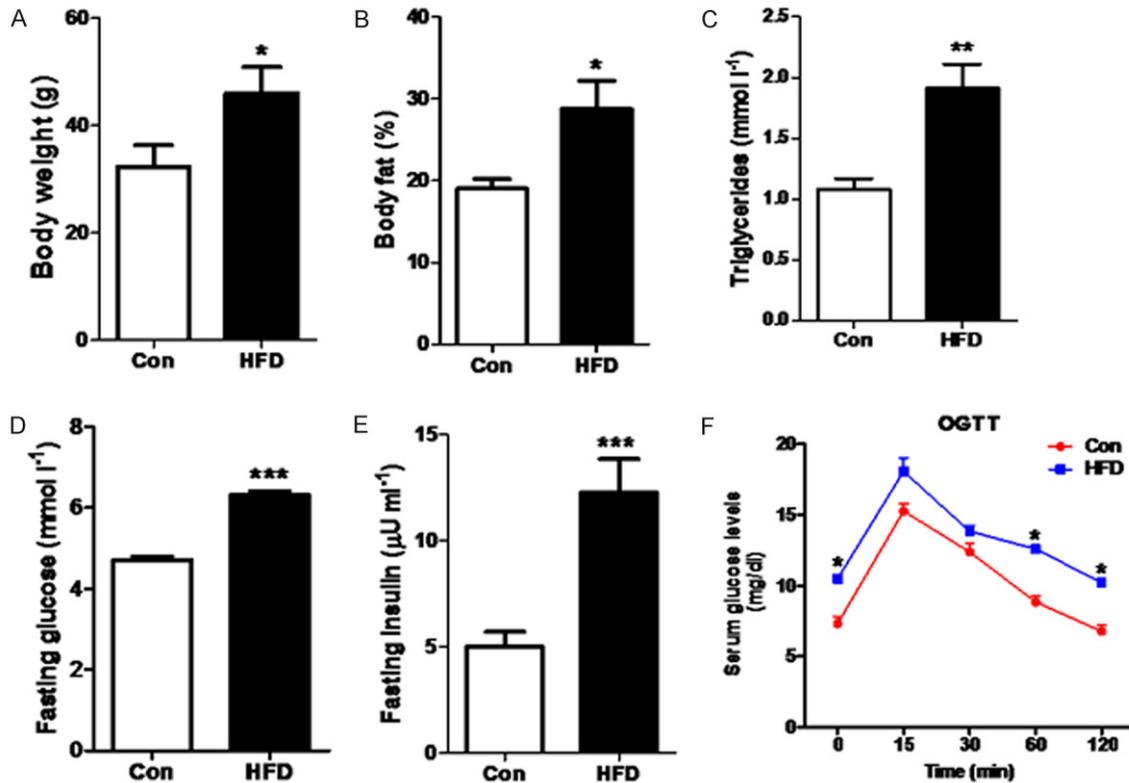


Figure 1. Obesity associated characters began to increase gradually from 4 weeks of high-fat diet feeding in C57BL/6J mice, indicating omens of type II diabetes. Characters like body weight (A), body fat (B), TG (C), glucose (D) and insulin (E) were detected, and the data results from HFD group all showed significant higher than control. Moreover, results from OGTT test indicated impaired glucose tolerance in HFD group (F). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; comparisons between the mice fed with high-fat diet (HFD) and standard diet (Con), $n = 8$ per group.

lected and stored respectively for further studies.

RNA isolation and real-time PCR

Total RNA from skeletal muscle, adipose tissue, and muscle cells were isolated using TriReagent (Molecular Research Center, Inc., USA). After purified and DNase-treated using RNeasy mini Kit (Qiagen, USA), RNA was quantified spectrophotometrically in a NanoPhotometer (NanoDrop 2000, Implen, Germany), and cDNA was produced with a High Capacity RNA to cDNA kit (Applied Biosystems, USA). Gene expression was measured by qRT-PCR (ABI7900HT, Applied Biosystems, USA). Primers used here were: FNDC5-forward: 5'-TGAGGTTGTCATCGG-ATT TGC-3', reverse: 5'-GCGGGTGGTGGTGTTCAC-3'; β -actin-forward: 5'-AAAGACCTGTACGGAAACAC-3', reverse: 5'-GTCATACTCCTGCTTGCTGAT-3'. The results were analyzed using the

2^{- $\Delta\Delta$ CT} method [21], with β -actin used as the internal reference gene.

Western blot analysis

Tissues and cells were homogenized and lysed with ice-cold buffer (25 mM Tris-HCl, pH 7.4, 10 mM sodium orthovanadate, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10mM EDTA, 10 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride). After centrifugation, immunoprecipitation of muscle and adipose proteins was performed as described previously [20]. Briefly, samples were separated on polyacrylamide gels and transferred to Hybond-P PVDF transfer membrane (Amersham Biosciences), blocked for 1 h with 5% fat-free milk at room temperature. After incubating the membrane with antibodies for FNDC5/irisin, β -actin, AKT, phospho-AKT, ERK1/2 and phospho-ERK1/2, bands were detected by ECL detection reagents (Amersham Biosciences)

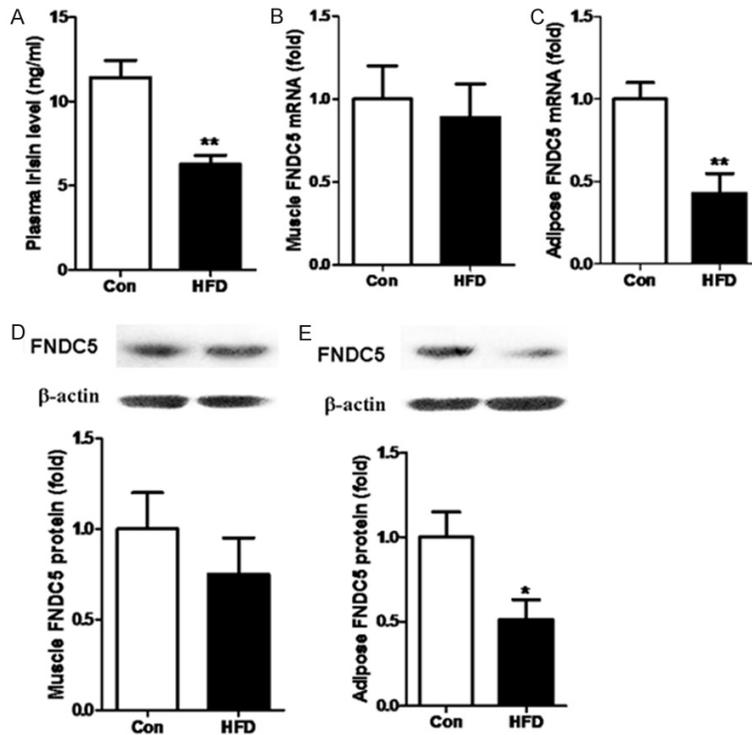


Figure 2. Effect of high-fat diet on irisin secretion in C57BL/6J mice. Plasma irisin level showed significantly decreased in HFD group compared to control (A). FNDC5/irisin expression at both mRNA and protein levels were detected in the muscle and adipose tissues, with the results indicating that FNDC5/irisin expression was significantly inhibited in adipose tissues (C, E) but not in muscle tissues (B, D). ** $P < 0.01$; comparisons between the mice fed with high-fat diet (HFD) and standard diet (Con), $n = 8$ per group.

and quantified by densitometric analysis using Alpha Imager 2200.

Statistical analysis

Results were expressed as the means \pm S.E. Differences between groups were examined for statistical significance using Student's *t* test, analysis of variance (ANOVA) with Fisher's protected least significant difference test, or ANOVA with the Games-Howell test. $P < 0.05$ was considered to be statistically significant.

Results

HFD induced obesity with type 2 diabetes-associated features

Diet induced obese rat model, commonly used for diabetes related studies [1], was established here in C57BL/6 mice by fed with a high fat (60% kcal) diet for few weeks. Mouse body weights were recorded at 1, 2, 4, 8 or 12 week, and significant increased body weight as well

as body fat was observed in the HFD group from the fourth week (Figure 1A and 1B). Moreover, the content of TG, glucose and insulin from blood samples from obese mice showed significantly higher than control (Figure 1C-E). Additionally, results from OGTT test also indicated the impaired glucose tolerance in HFD feeding mice compared to control (Figure 1F). These results demonstrated diabetes complication with HFD induced obesity, involving the mechanism of insulin resistance related pathways.

Decreased FNDC/irisin levels with down-regulated expression in adipose tissues of obese mice

Since mildly increased amount of circulating irisin has been reported to contribute to the improvements in obesity and glucose homeostasis [9], circulating irisin levels were also recorded to investigate the relevance between irisin secretion and insulin action. Here, significantly decreased circulating irisin level was observed in HFD feeding mice at the sixth week (Figure 2A) meanwhile body weight increased all the way, as well as blood glucose. Previous studies have revealed that most of circulating irisin was attributed to muscle secretion, involving a muscle-adipose tissue crosstalk through a regulatory feedback mechanism [22]. Thus, the expression of FNDC5/irisin was detected in both skeletal muscle and subcutaneous adipose tissues at both mRNA and protein level. Results indicated that FNDC5/irisin protein was significantly down-regulated in the adipose tissues of HFD feeding mice compared to control (Figure 2C and 2E), whereas this change was not obvious in the muscle tissues (Figure 2B and 2D). Therefore, HFD induced decrease of circulating irisin level mainly due to the down-regulated FNDC5/irisin expression in adipose tissues.

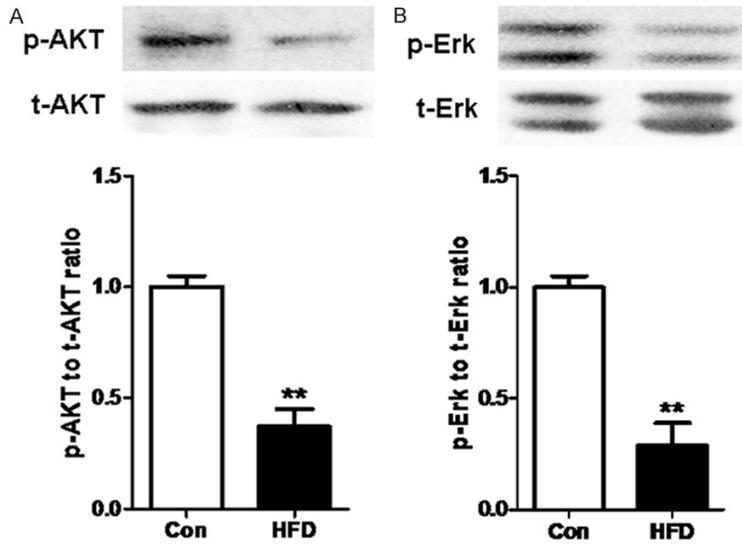


Figure 3. High-fat diet induced insulin resistance in the muscle tissues of C57BL/6J mice. Phosphorylation of AKT and Erk were introduced here to indicate insulin resistance by western blotting analysis. And, the results showed that phosphorylation of AKT (A) and Erk (B) both decreased in the muscle tissues indicating insulin resistance. **P<0.01; comparisons between the mice fed with high-fat diet (HFD) and standard diet (Con), n=8 per group.

impaired glucose tolerance, was further evaluated by the activation of signaling molecules in the insulin signaling pathway. Previous study has showed that irisin potentially prevent obesity and associated type 2 diabetes through the p38 MAPK and ERK pathways [15]. So, the activation of AKT, commonly used insulin signal molecules, and Erk, another potential signal molecules from branched downstream pathways of insulin signaling [23], was investigated. As shown in **Figure 3**, the p-AKT to t-AKT ratio and p-Erk to t-Erk ratio both showed significantly decrease in the skeletal muscle of HFD induced obese mice. This result revealed relevance between inhibited muscle insulin action and decreased circulating irisin levels.

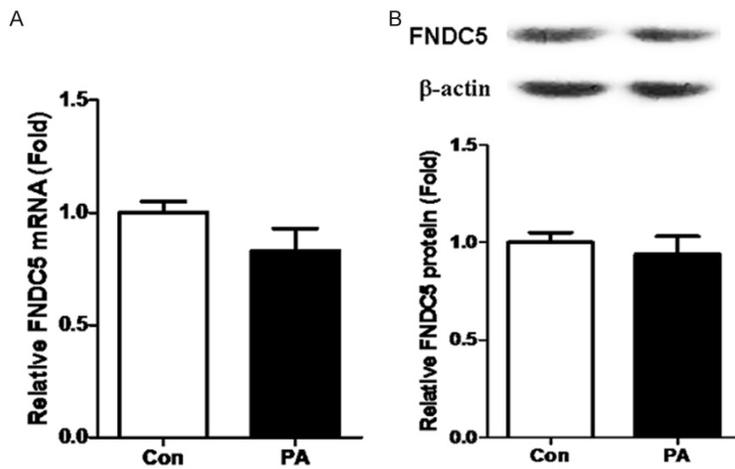


Figure 4. Effect of palmitate addition on the expression of FNDC5/irisin in C2C12 cells. After incubation with 20 mM palmitic acid for 20 min, C2C12 cells were collected for the detection of FNDC5/irisin expression. Results showed that palmitic acid cannot regulate FNDC5/irisin expression at both mRNA (A) and protein (B) levels.

Irisin could recover the palmitic acid-induced insulin resistance of C2C12 cells

In order to verify speculation about the contribution of decreased irisin levels to the insulin resistance of muscle, C2C12 cells, commonly used murine myocytes, were chosen and treated with 20 mM palmitic acid, one representatives of free fatty acids [24], for diabetic status mimicking in vitro as described before [10]. As shown in **Figure 4**, FNDC5/irisin expression was also not influenced under palmitic acid treatment in C2C12 cells. However, similar inhibition of insulin signaling was detected after insulin stimulation in palmitic acid-treated C2C12 cells compared to control (**Figure 5**). Furthermore, this inhibition can be recovered if moderate amount of irisin (100 nM) was added at the same time with palmitic acid, while individual irisin addition could also promote the insulin action.

Inhibited insulin signaling was observed in the muscle tissues of obese mice

Though FNDC5/irisin expression was not down-regulated in muscle tissues, muscle insulin resistance, indicated by increased insulin but

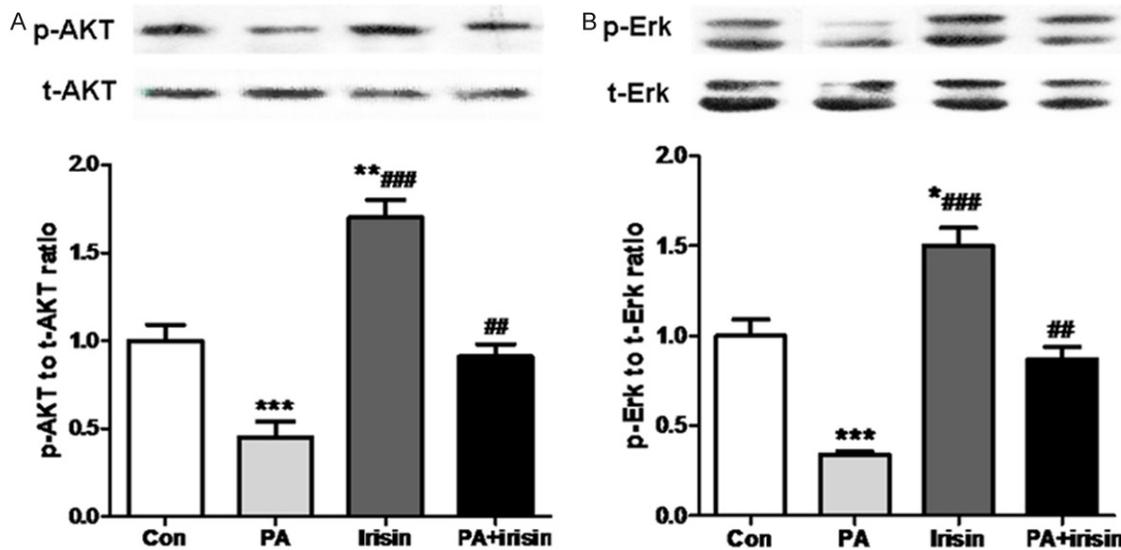


Figure 5. Effect of palmitate or irisin addition on the phosphorylation of AKT and Erk in C2C12 cells. After treated either with 100 nM irisin, 20 mM palmitate or with both of them for 2 h, and then stimulated with 200 nM insulin for 30min, C2C12 cells were collected to detect the phosphorylation of AKT and Erk at protein level. Results indicated that the phosphorylation of AKT (A) and Erk (B) could be inhibited or promote by palmitate or irisin treatment respectively. Moreover, irisin could recover the decreased phosphorylation of AKT and Erk under palmitate treatment in C2C12 cells. PA: palmitate treatment. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control (Con); ## $P < 0.01$, ### $P < 0.001$ vs. PA.

Taken together, irisin could promote insulin signaling in myocytes, and improve insulin resistance induced by free fatty acids like palmitic acid.

Discussion

There is much evidence showing an association between obesity, hypertension and diabetes. In animal studies this is produced by consumption of a high fat diet (called the Western diet) which causes hypertension, lipid abnormalities and arterial hypertrophy [25]. C57BL/6 mice fed with a high fat/high carbohydrate diet can develop a form of non-insulin dependent diabetes as seen by the high glucose and insulin [26]. Here, high fat diet induced obese model, with early stage of type 2 diabetes associated features, was introduced to investigate the correlation of insulin resistance and irisin, a novel cytokines with widespread controversy.

Since the discovery of the PGC1 α -dependent myokine irisin [9], which is able to trigger white fat 'browning' development and thereby promote thermogenesis, great interest has been created concerning its origin, regulation and function. It has been shown that irisin can also be expressed and secreted by adipose tissue

[22]. Moreover, adipose tissue FNDC5/irisin expression seems to represent only a fraction of that expressed in muscle, but it is adipose tissue not skeletal muscle expression that correlates with levels of circulating irisin [27]. In line with these reports, it was demonstrated that FNDC5 protein expression in adipose tissues not skeletal muscle contributed to the level change of circulating irisin in HFD-induced obese mice.

For the putative role of irisin in the protection against obesity-related metabolic disease, positive associations of muscle FNDC5/irisin with insulin resistance have been reported [16, 17, 22], speculating on the negative, desensitizing effects of irisin on insulin action. In this study, impaired glucose tolerance was observed in obese mice, indicating insulin resistance [28], which is caused by the decreased ability of peripheral target tissues (especially muscle) to respond properly to normal circulating concentrations of insulin [29]. Inhibited insulin action in muscle tissues was also demonstrated here with decreased phosphorylation of AKT and Erk. Here, not muscle FNDC5/irisin but adipose tissue FNDC5/irisin was associated with insulin resistance. Moreover, it was speculated that decreased circulating irisin level, due to down-

regulated FNDC5/irisin expression in adipose tissues, might contribute to muscle insulin resistance, speculating on the positive, preserving effects of circulating irisin on muscle insulin action.

During the development of insulin resistance in skeletal muscle, an impairment of glucose utilization and insulin sensitivity has been related to the presence of elevated plasma free fatty acids (FFA) [24]. Palmitic acid, one representative of FFA, has been used for diabetic status mimicking in muscle cells [10]. Thus, palmitic acid treated muscle cells were also introduced to verify our speculation about the function of irisin on muscle insulin action. As expected, muscle cells showed similar inhibited insulin action under palmitic acid treatment, and FNDC5/irisin expression was also detected unchanged. Furthermore, inhibited insulin action could be recovered by irisin addition in palmitic acid treated muscle cells. Therefore, it was concluded that irisin level could promote muscle insulin action, indicating the functional role of irisin in the improvement of muscle insulin resistance.

In conclusion, it was demonstrated that HFD-induced obesity associated with type 2 diabetic features was accompanied by decreased circulating irisin level in the mouse model. Further studies revealed the downregulated FNDC5/irisin expression in adipose tissues but not muscle tissues, though muscle insulin resistance was also observed in HFD-induced obese mice. In vitro diabetic status mimicking showed that irisin might play important role in promoting muscle insulin action. More experiments could be established to verify the functional role of irisin in the improvement of muscle insulin resistance in vivo.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zhao Qian, Geriatric Department of Endocrinology, The First Hospital Affiliated to Zhengzhou University, 1 Jianshe Road, Zhengzhou 450052, Henan Province, China. Tel: +86 1352677003; E-mail: zhaoqian880914@163.com

References

[1] De Angelis K, Irigoyen MC and Morris M. Diabetics and cardiovascular autonomic dysfunction:

- application of animal models. *Auton Neurosci* 2009; 145: 3-10.
- [2] Sander HW and Chokroverty S. Diabetic amyotrophy: current concepts. *Semin Neurol* 1996; 16: 173-178.
- [3] Lazar MA. How obesity causes diabetes: not a tall tale. *Science* 2005; 307: 373-375.
- [4] Mokdad AH, Ford ES, Bowman BA, Dietz WH, Vinicor F, Bales VS and Marks JS. Prevalence of obesity, diabetes, and obesity-related health risk factors, 2001. *JAMA* 2003; 289: 76-79.
- [5] Gesta S, Tseng YH and Kahn CR. Developmental origin of fat: tracking obesity to its source. *Cell* 2007; 131: 242-256.
- [6] Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev* 1995; 75: 473-486.
- [7] Amitani M, Asakawa A, Amitani H and Inui A. The role of leptin in the control of insulin-glucose axis. *Front Neurosci* 2013; 7: 51.
- [8] Kahn BB and Flier JS. Obesity and insulin resistance. *J Clin Invest* 2000; 106: 473-481.
- [9] Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, Rasbach KA, Bostrom EA, Choi JH, Long JZ, Kajimura S, Zingaretti MC, Vind BF, Tu H, Cinti S, Hojlund K, Gygi SP and Spiegelman BM. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; 481: 463-468.
- [10] Kurdiova T, Balaz M, Vician M, Maderova D, Vitek M, Valkovic L, Srbecky M, Imrich R, Kyselovicova O, Belan V, Jelok I, Wolfrum C, Klimes I, Krssak M, Zemkova E, Gasperikova D, Ukropec J and Ukropcova B. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. *J Physiol* 2014; 592: 1091-1107.
- [11] Xiang L, Xiang G, Yue L, Zhang J and Zhao L. Circulating irisin levels are positively associated with endothelium-dependent vasodilation in newly diagnosed type 2 diabetic patients without clinical angiopathy. *Atherosclerosis* 2014; 235: 328-333.
- [12] Alis R, Sanchis-Gomar F, Pareja-Galeano H, Hernandez-Mijares A, Romagnoli M, Victor VM and Rocha M. Association between irisin and homocysteine in euglycemic and diabetic subjects. *Clin Biochem* 2014; 47: 333-335.
- [13] Crujeiras AB, Zulet MA, Lopez-Legarrea P, de la Iglesia R, Pardo M, Carreira MC, Martinez JA and Casanueva FF. Association between circulating irisin levels and the promotion of insulin resistance during the weight maintenance period after a dietary weight-lowering program in obese patients. *Metabolism* 2014; 63: 520-531.
- [14] Song H, Wu F, Zhang Y, Zhang Y, Wang F, Jiang M, Wang Z, Zhang M, Li S, Yang L, Wang XL, Cui

Irisin and muscle insulin resistance

- T and Tang D. Irisin promotes human umbilical vein endothelial cell proliferation through the ERK signaling pathway and partly suppresses high glucose-induced apoptosis. *PLoS One* 2014; 9: e110273.
- [15] Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui T, Yang LJ and Tang D. Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes* 2014; 63: 514-525.
- [16] Huh JY, Panagiotou G, Mougios V, Brinkoetter M, Vamvini MT, Schneider BE and Mantzoros CS. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. *Metabolism* 2012; 61: 1725-1738.
- [17] Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P and Klapp BF. Circulating levels of irisin in patients with anorexia nervosa and different stages of obesity—correlation with body mass index. *Peptides* 2013; 39: 125-130.
- [18] Vaughan RA, Gannon NP, Barberena MA, Garcia-Smith R, Bisoffi M, Mermier CM, Conn CA and Trujillo KA. Characterization of the metabolic effects of irisin on skeletal muscle in vitro. *Diabetes Obes Metab* 2014; 16: 711-718.
- [19] Romanatto T, Cesquini M, Amaral ME, Roman EA, Moraes JC, Torsoni MA, Cruz-Neto AP and Velloso LA. TNF-alpha acts in the hypothalamus inhibiting food intake and increasing the respiratory quotient—effects on leptin and insulin signaling pathways. *Peptides* 2007; 28: 1050-1058.
- [20] Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, Ohtsuka-Kowatari N, Kumagai K, Sakamoto K, Kobayashi M, Yamauchi T, Ueki K, Oishi Y, Nishimura S, Manabe I, Hashimoto H, Ohnishi Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Nagai R and Kadowaki T. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem* 2006; 281: 26602-26614.
- [21] Schmittgen TD and Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc* 2008; 3: 1101-1108.
- [22] Roca-Rivada A, Castelao C, Senin LL, Landrove MO, Baltar J, Belen Crujeiras A, Seoane LM, Casanueva FF and Pardo M. FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One* 2013; 8: e60563.
- [23] Carvalheira JB, Torsoni MA, Ueno M, Amaral ME, Araujo EP, Velloso LA, Gontijo JA and Saad MJ. Cross-talk between the insulin and leptin signaling systems in rat hypothalamus. *Obes Res* 2005; 13: 48-57.
- [24] Hirabara SM, Curi R and Maechler P. Saturated fatty acid-induced insulin resistance is associated with mitochondrial dysfunction in skeletal muscle cells. *J Cell Physiol* 2010; 222: 187-194.
- [25] Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC and Cassis LA. Activation of the systemic and adipose renin-angiotensin system in rats with diet-induced obesity and hypertension. *Am J Physiol Regul Integr Comp Physiol* 2004; 287: R943-949.
- [26] Surwit RS, Kuhn CM, Cochrane C, McCubbin JA and Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* 1988; 37: 1163-1167.
- [27] Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, Ricart W and Fernandez-Real JM. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab* 2013; 98: E769-778.
- [28] Kraegen EW, Clark PW, Jenkins AB, Daley EA, Chisholm DJ and Storlien LH. Development of muscle insulin resistance after liver insulin resistance in high-fat-fed rats. *Diabetes* 1991; 40: 1397-1403.
- [29] Petersen KF and Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. *Am J Cardiol* 2002; 90: 11G-18G.