FNDC5 overexpression and irisin ameliorate glucose/lipid metabolic derangements and enhance lipolysis in obesity

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A B S T R A C T
Irisin is a cleaved and secreted fragment of fibronectin type III domain containing 5 (FNDC5), and contributes to the beneficial effect of exercise on metabolism. Here we report the therapeutic effects of FNDC5/irisin on metabolic derangements and insulin resistance in obesity, and show the lipolysis effect of irisin and its signal molecular mechanism. In obese mice, lentivirus mediated FNDC5 overexpression enhanced energy expenditure, lipolysis and insulin sensitivity, and reduced hyperlipidemia, hyperglycemia, hyperinsulinism, blood pressure and norepinephrine levels; it increased hormone-sensitive lipase (HSL) expression and phosphorylation, and reduced perilipin level and adipocyte diameter in adipose tissues. Subcutaneous perfusion of irisin reduced hyperlipidemia and hyperglycemia, and improved insulin resistance. Either FNDC5 overexpression or irisin perfusion only induced a trend of a slight decrease in body weight in obese mice. In 3T3-L1 adipocytes, irisin enhanced basal lipolysis rather than isoproterenol-induced lipolysis, which were prevented by inhibition of adenylyl cyclase or PKA; irisin increased the HSL and perilipin phosphorylation; it increased PKA activity, and cAMP and HSL mRNA levels, but reduced perilipin expression. These results indicate that FNDC5/irisin ameliorates glucose/lipid metabolic derangements and insulin resistance in obese mice, and enhances lipolysis via cAMP–PKA–HSL/perilipin pathway. FNDC5 or irisin can be taken as an effective therapeutic strategy for metabolic disorders.

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1. Introduction

Irisin was firstly identified in 2012 as a muscle-derived factor that promotes the formation of brown-adipocyte-like cells in mice [1]. Irisin is a cleaved and secreted fragment of fibronectin type III domain containing 5 (FNDC5), a type of transmembrane protein with a signal peptide, two fibronectin domains and one hydrophobic domain inserted in the cell membrane. It is known that exercise stimulates PGC-1α expression, which promotes FNDC5 expression, and thereby increases irisin levels [1]. Cross-sectional studies revealed that circulating irisin levels were positively correlated with biceps circumference, body mass index, glucose and ghrelin, but negatively with age, insulin, cholesterol, and adiponectin levels in human and intrahepatic triglyceride contents in obese adults [2–4]. Levels of irisin were significantly higher in participants with metabolic syndrome than in those without the syndrome [2]. An interesting question is whether long-term administration of irisin could effectively ameliorate glucose/lipid metabolic derangements, insulin resistance and obesity.

Adipose tissues play major roles in the energy homeostasis and in the development of obesity and metabolic syndrome, which may be a new target against obesity and metabolic diseases [5,6]. Adipose tissue insulin resistance and dysfunctional lipid storage in adipocytes are sentinel events in the progression toward metabolic dysregulation with obesity. Both subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) are linked with metabolic risk factors. VAT remains more strongly associated with an adverse metabolic risk profile even after accounting for standard anthropometric indexes [7]. Circulating irisin is mainly attributed to muscle secretion [1]. A recent study showed that FNDC5/irisin was not only a myokine but also an adipokine, and short-term periods of endurance exercise training induced FNDC5 secretion by SAT and VAT [8]. However, the effects of irisin on lipolysis and its downstream signaling components are unknown.
It is known that gene transfer represents a relatively new possibility for treating genetic disorders and common multifactorial diseases by changing the related gene expression. Lentiviral or retroviral vectors allow the permanent integration of a therapeutic transgene in target cells and have provided a delivery platform for several successful clinical approaches in gene therapy. In the present study, we showed that lentiviral vector-mediated FNDC5 overexpression effectively ameliorated metabolic derangements and insulin resistance in high fat diet (HFD)-induced obese mice. Persistent subcutaneous perfusion of irisin caused similar effects to FNDC5 overexpression in obese mice. Irisin enhances lipolysis via cAMP–PKA–hormone-sensitive lipase (HSL)/perilipin pathway. FNDC5/irisin can be taken as an effective therapeutic strategy for metabolic disorders.

2. Materials and methods

2.1. Mice and obesity model

Male 6-week old C57/BL6j mice were purchased from Comparative Medicine Centre of Yangzhou University (Yangzhou, China). All of our investigations were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011). The mice were housed in a temperature- and humidity-controlled room with food and tap water ad libitum and a 12-h light/12-h dark cycle. Food intake was measured daily. Body weight (BW) was measured at weekly intervals. Mice were fed with high fat diet (HFD, 60% kcal as fat) for 12 weeks to induce obesity. Control mice were fed with standard diet (Ctrl, 12% kcal as fat).

2.2. Cell culture

Adipocytes were differentiated from 3T3-L1 preadipocytes (Keygen Biotech. Co. Ltd., Nanjing, China). Briefly, 3T3-L1 preadipocytes were maintained in high glucose DMEM supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin G sodium, and 100 μg/ml streptomycin and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. Two days after achieving confluence, differentiation to adipocytes was induced by treatment of postconfluent cells with 10% FBS, 10 μg/ml insulin, 1 μM dexamethasone, and 0.5 mM isobutyl-1-methylxanthine. The differentiation medium was withdrawn 2 days later and replaced with medium supplemented with 10% FBS and 10 μg/ml insulin. After 2 days in insulin-containing medium, the cells were then cultured in high glucose DMEM containing 10% FBS. The media were replaced every 2 days thereafter until 85% of the cells contained lipid droplets. Eight days after the induction of differentiation, 3T3L1 adipocytes were ready to be used in experiments [9,10].

2.3. Construction of FNDC5 expression plasmid

Recombinant lentivirus expressing FNDC5 or EGFP vector was purchased from Life Technologies (California, USA). Briefly, the FNDC5 mRNA was amplified by RT-PCR, and the PCR product was inserted into the pLent6.3_MCS-IRES2-EGFP plasmid. The final bicistronic plasmid construct, plenti-FNDC5-IRES-EGFP, was designed to co-express the FNDC5 protein and EGFP. To produce infectious viral particles, the lentiviral plasmid (plenti-FNDC5-IRES-EGFP) using Lipofectamine 2000 (Life Technologies, California, USA) was carried out at the end of the 6th week after the diet application in Ctrl mice or HFD mice. Acute experiments were performed 3 weeks or 6 weeks after the introduction.

2.4. Introduction of FNDC5 plasmid

A single intravenous injection of recombinant lentivirus (1 × 10⁷ TU/ml, 100 μl) expressing FNDC5 or EGFP vector (Life Technologies, California, USA) was carried out at the end of the 6th week after the diet application in Ctrl mice or HFD mice. Acute experiments were performed 3 weeks or 6 weeks after the introduction.

2.5. Subcutaneous perfusion of irisin

Irisin (0.55 nmol/μl, 1.45 nmol/day) or saline was subcutaneously administered with micro-osmotic pump (Model 1004, Alzet) in the HFD mice at the end of the 8th week after diet application. The subcutaneous perfusion site was located at midline of the lower abdomen. Acute experiments were carried out 4 weeks after the pump implantation.

2.6. Insulin and glucose tolerance tests

The mice were fasted for 6 h or overnight before intraperitoneal injection with insulin (0.75 units/kg body weight) or glucose (0.9 g/kg body weight). Blood glucose levels in the tail vein blood samples were measured with a blood glucometer (One Touch, Johnson & Johnson, USA) 15, 30, 60, 90 and 120 min after the injection [11].

2.7. Indirect calorimetry

The mice were individually housed for at least 3 days before the calorimetry measurements. Metabolism parameters were simultaneously measured in individually housed mice with a PhenoMaster system (TSE Systems, Thuringia, Germany). After 2 h of acclimatization, the O₂ consumption, CO₂ production, heat production, and respiratory exchange ratio (RER) for each mouse were continuously measured for a further 24 h. A photobeam-based monitoring system was used to track animal movement including rearing and climbing [12].

2.8. In vitro and in vivo lipolysis

Lipolysis was performed in vitro on differentiated 3T3-L1 adipocytes. Briefly, cells were pre-treated without or with 1 ng/μl of irisin for 30 min in the presence or absence of adenylyl cyclase inhibitor SQ22536 or PKA inhibitor H-89. Media were then collected for glycerol and FFA level determinations. Lipolysis was also performed in vivo. Briefly, mice were fasted for 4 h or 24 h. Blood was collected for the determination of plasma FFA level.

2.9. Quantitative RT-PCR analysis

Total RNAs from tissues or differentiated 3T3L1 adipocytes were isolated using trizol and subjected to reverse transcription with PrimeScript RT reagent (Takara Biotechnology Co., Ltd., Tokyo, Japan) according to the manufacturer’s instructions. For quantitative PCR, cDNA fragments were subjected to SYBR Green RT-PCR (Takara Biotechnology Co., Ltd., Tokyo, Japan) using StepOne Plus system. The quantitative measures were obtained using the ΔΔCT method. Primers for all qPCR experiments were included as follows: FNDC5: ATGAAAGGAGATGGGAGGA (F), GCGCGAGGAAGAGCTAATACA (R); UCP1: ACTGCCACACCTCCAGCTCATT (F), CTITGCTCCTACCTCAAGTGG (R); HSL: CCGTGAGCCAGACTCTTC (F), CACGCCAATCTGGGTCTAT (R); β-actin: CTGCGTGTTCACACCCTTCITTG (F), GGCATGGCAATGGTCTCCTAT (R); PPARα: ACACACTCAGCTGCTGCTG (F), TTCTGAGGCTCTGAGTGG (R); and PPARγ: CAAAGACACAGTGTAATT (F), ACCATGGAATTTCTGTA (R).

2.10. Statistical analysis

All data were presented as mean ± SEM and analyzed by Tukey’s honestly significant difference (HSD) test. Differences were considered significant at *P* < 0.05.
The frozen tissues or differentiated 3T3-L1 adipocytes were homogenized using a sonicator in ice-cold RIPA buffer containing 1% Nonidet P-40, 0.1% SDS, and protease inhibitor. Homogenates were centrifuged for 30 min at 12,000 rpm at 4 °C, and supernatants were collected. Protein amounts from all samples were assessed using a BCA-kit followed by protein concentration normalization before all Western blot experiments. The lysates (20–50 μg) were resolved by SDS-PAGE. Proteins were transferred to a PVDF membrane (Bio-Rad), which was incubated with the specific antibody (FNDC5, UCP1, HSL, P-HSL, perilipin or P-perilipin). Blots were re-probed with the respective antibodies against β-actin for normalization. Immunoreactive proteins were visualized with a gel imaging system (Tanon Science and Technology, Shanghai, China), and densitometric analysis was performed with Quantity One software (Bio-Rad).

2.11. ELISA analysis

Commercial ELISA or EIA kits were used for irisin, insulin, leptin, cAMP, angiotensin II, norepinephrine, FFA, cholesterol, triglyceride and glycerol. PKA activity was measured with PKA kinase activity kit (ADI-EKS-390A, Enzo Life Sciences Inc., Farmingdale, NY, USA). Irisin kits were bought from Phoenix Pharmaceuticals (EK-067-16, Burlingame, USA). Minimum detectable concentration of irisin is 6.8 ng/ml. The cross-reactivity is 100% with irisin or irisin (42–112), and 8% with FNDC. No cross-reactivity is found with FNDC5 (165–212), FNDC5 (162–205) or irisin (42–95). The recovery is 104%, 82% and 88.7% for spiked irisin at 5, 10 and 20 ng/ml, respectively. Intra- and inter-assay variations are <10% and <15%, respectively. Recently, it was reported that the interobserver and intraobserver variabilities of measurements with the kits were 6.2% and 5.9%, respectively [13]. The serum and muscle samples for measuring irisin were diluted 2 and 10 times, respectively.

2.12. Tissue embedding and H&E staining

The mice were deeply anesthetized with isoflurane. Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) were quickly removed, rinsed in ice-cold PBS, and incubated overnight in formalin. SAT and VAT were then washed with PBS for 15 min and dehydrated by successive incubation in 70% to 100% ethanol solutions. Adipose tissues and VAT were then washed with PBS for 15 min and dehydrated by successive incubation in 70% to 100% ethanol solutions. Adipose tissues were embedded in paraffin, and slices were cut every 7 mm. H&E staining was performed on adipose tissues after rehydration, and pictures were taken with an Olympus camera mounted on an optical microscope.

2.13. Chemicals

Antibodies against UCP1 (ab155117), FNDC5 (ab174833) and β-actin (ab6276) were obtained from Abcam (Cambridge, England). Antibodies against HSL (sc-25843) were purchased from Santa Cruz (California, USA); antibodies against perilipin (#9349) and phosphorylated HSL at Ser563 (#4139), Ser565 (#4137) and Ser660 (#4126) were purchased from Cell Signaling (Massachusetts, USA). Irisin (ChinaPeptides Co., Ltd, Shanghai, China) was derived from Escherichia coli. The purified and identified irisin was subjected to the removal of the endotoxin with Pierce high-capacity endotoxin removal resin (Thermo Scientific, MA, USA). Adenylate cyclase inhibitor SQ22536 and PKA inhibitor H-89 were from Sigma (St Louis, MO). ELISA kits for insulin were obtained from ALPCO (New Hampshire, USA); ELISA kits for leptin, cAMP, angiotensin II and norepinephrine were bought from R&D systems (Minneapolis, USA); ELISA kits for FFA was from Wako Chemicals (Osaka, Japan); ELISA kits for cholesterol, triglyceride and glycerol were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Primers for all qPCR experiments were from Life Technologies (California, USA).

2.14. Statistics

Data are presented as mean ± SEM, and a value of P < 0.05 was considered statistically significant. Two-tailed, unpaired Student’s t-tests were used to compare two treatment groups. One-way ANOVA and two-way ANOVA were used for data analysis of more than two groups followed by Bonferroni’s post-hoc analysis.

3. Results

3.1. Effectiveness of FNDC5 overexpression

FNDC5 mRNA, FNDC5 protein and irisin levels in skeletal muscle were higher in obese mice than those in control mice. Although there was a tendency for the serum FNDC5 and irisin levels to increase in obese mice, this did not reach statistical significance. Administration of recombinant lentivirus expressing FNDC5 via tail vein increased the FNDC5 mRNA, FNDC5 protein and irisin levels in skeletal muscle and serum in both control and HFD mice 3 and 6 weeks after gene transfer (Fig. 1A–C). FNDC5 gene transfer failed to cause significant increase in mRNA levels in the liver, SAT and VAT except in muscles (Fig. S1). The significant increases in the serum irisin levels and GFP fluorescence in skeletal muscle were found 1, 2, 4, and 6 weeks after FNDC5 gene transfer in HFD mice (Fig. S2). These results confirmed the effectiveness of the FNDC5 introduction in increasing circulating FNDC5 and irisin levels.

3.2. FNDC5 overexpression increases energy expenditure

FNDC5 overexpression increased the O2 consumption, CO2 and heat production in HFD mice without significant effect on the total activity (Fig. 2A), indicating that FNDC5/irisin increased energy expenditure. FNDC5 overexpression had no significant effects on body weight and food intake in obese mice, but induced a tendency toward a slight decrease in body weight (−4.3% vs. vehicle at the end of the 6th weeks after the gene transfer, P = 0.288) in obese mice (Fig. 2B.C). Intraperitoneal injection of irisin had no significant effect on core temperature in obese mice (Fig. S3), suggesting that circulating irisin does not alter the central setpoint of body temperature. The increased heat production effect on body temperature may be offset by increased heat dissipation due to the mechanisms of body temperature regulation.

3.3. FNDC5 overexpression improves lipid metabolism

Serum cholesterol, triglyceride and free fatty acid (FFA) levels were increased in HFD mice, which were reduced by FNDC5 overexpression, indicating that FNDC5/irisin effectively attenuated the disturbance of lipid metabolism in obesity (Fig. 3A). FNDC5 overexpression had no significant effect on the increased serum leptin level in HFD mice (Fig. 3B).

3.4. FNDC5 overexpression improves glucose metabolism and insulin resistance

The fasting blood glucose and serum insulin levels were increased in HFD mice, which were reduced by FNDC5 overexpression (Fig. 3C,F). Glucose tolerance test (ITT) and insulin tolerance test (ITT) are often used to evaluate insulin resistance. HFD mice manifested significantly elevated glucose excursions following glucose challenge compared with control mice in the GTT, and the glucose excursion was reduced by FNDC5 overexpression (Fig. 3D). The efficiency of insulin was quantified by its ability in reducing blood glucose level in the ITT. Insulin was less effective in HFD mice than that in control mice, which were prevented by FNDC5 overexpression (Fig. 3E). Akt is known to mediate the effects of insulin on glucose metabolism. FNDC5 overexpression promotes the Akt phosphorylation in both control and HFD mice.
These results indicate that FNDC5/irisin improves glucose metabolism and insulin resistance in obesity.

3.5. FNDC5 overexpression reduces blood pressure

Obesity is known to be a marker of cardiovascular risk, and is an important and primary contributor to the pathophysiology of hypertension [14]. Increased afferent activity from the white adipose tissue (WAT) and enhanced adipose afferent reflex are involved in sympathetic activation and hypertension in HFD-induced obesity rats [15,16]. HFD mice showed an increased serum norepinephrine level (an index of sympathetic activity) and a tendency of increased blood pressure. FNDC5 overexpression significantly reduced blood pressure (Fig. S5) and serum norepinephrine level in obese mice, but had no significant effect on serum angiotensin II level (Fig. 3G). These results indicate that FNDC5/irisin plays a beneficial role in attenuating hypertension and sympathetic activation in obesity.

3.6. FNDC5 overexpression increases UCP1 expression

UCP1 is the key protein to the thermogenic capacity of adipose tissues, which enables the separation of lipid oxidation from ATP production, allowing a higher metabolic rate and the conversion of nutritional energy to heat [17,18]. Irisin acts on white adipose cells in vitro and in vivo to stimulate UCP1 expression and causes a broad program of brown-fat-like development [1,19]. The present study showed that FNDC5 overexpression significantly increased UCP1 mRNA and protein expression in the SAT but not in the VAT in both control and HFD mice (Fig. 4A,F). The UCP1 protein level in brown adipose tissues (BAT) was much higher than that in SAT and VAT, but FNDC5 overexpression failed to cause a significant increase in UCP1 protein in the BAT (Fig. S6). Moreover, the mRNA levels of other brown fat genes were measured. It was found that FNDC5 overexpression caused a mild increase in PGC1α mRNA in SAT, but not in Cidea, Prdm16 and PPARγ mRNA (Fig. S7).
Fig. 3. Effects of FNDC5 overexpression on lipid/glucose metabolism, insulin sensitivity and lipolysis in Ctrl mice and HFD mice 6 weeks after gene transfer. A, serum cholesterol, triglyceride and free fatty acid (FFA) levels; B, serum leptin level; C, fasting blood glucose; D, glucose tolerance test (GTT); E, insulin tolerance test (ITT); F, serum insulin level; G, serum angiotensin II and norepinephrine levels; H, lipolysis effects represented with serum FFA levels after 4 h and 24 h fasting. Values are mean ± SEM. *P < 0.05 vs. Vector with same diet. †P < 0.05 vs. Ctrl with same treatment. n = 6 for each group.

Fig. 4. Effects of FNDC5 overexpression on UCP1, perillipin and HSL expressions, HSL phosphorylation and adipocyte diameter in SAT and VAT of Ctrl mice and HFD mice 6 weeks after gene transfer; A, relative values of UCP1 mRNA and protein; B, relative values of perillipin; C, relative values of HSL mRNA; D, phosphorylated HSL; E, adipocyte diameter; F, representative photos of Western blot show the perillipin, UCP1, P-HSL (Ser563, Ser565, Ser660) and HSL proteins. Values are mean ± SEM. *P < 0.05 vs. Vector with same diet. †P < 0.05 vs. Ctrl with same treatment. n = 3 for each group in A–D; n = 6 for each group in E.
3.7. FNDC5 overexpression promotes lipolysis and reduces the size of adipocytes in SAT

FNDC5 overexpression increased the plasma FFA levels after 4-h or 24-h fasting in obese mice (Fig. 3H), suggesting that FNDC5/irisin enhances the fasting-induced lipolysis. The diameter of adipocytes was increased in both SAT and VAT of HFD mice. FNDC5 overexpression reduced the adipocyte size in SAT of HFD mice (Figs. 4E, S8) and attenuated the fat accumulation in the liver of HFD mice (Fig. S9).

3.8. FNDC5 overexpression enhanced lipolysis via cAMP–PKA–perilipin/HSL pathway

There is no significant difference of perilipin levels in both SAT and VAT between control and HFD mice, while FNDC5 overexpression reduced the perilipin levels in both SAT and VAT (Fig. 4B,F). The HSL mRNA and phosphorylated HSL at Ser660 in both SAT and VAT were increased in HFD mice. FNDC5 overexpression increased HSL mRNA and the phosphorylated HSL at Ser563 and Ser660 but not at Ser565 in the SAT and VAT in both control and HFD mice (Fig. 4C,D,F).

3.9. Persistent perfusion of irisin improves lipid metabolism and insulin resistance

Persistent subcutaneous perfusion of irisin with micro-osmotic pump for 4 weeks had no significant effects on body weight and food intake in obese mice, but a tendency toward a slight decrease in body weight (−4.0% vs. saline at the end of the 4th weeks, \( P = 0.486 \)) was induced by irisin perfusion in obese mice (Fig. 5A,B). Similar to FNDC5 overexpression, irisin perfusion reduced serum cholesterol, triglyceride and FFA levels, as well as fasting blood glucose level in HFD mice (Fig. 5C,D). GTT and ITT showed that irisin perfusion improved insulin sensitivity in HFD mice (Fig. 5E). The serum irisin level in the mice perfused with irisin was about three times higher than that with saline (Fig. 5F). On the other hand, irisin infusion increased UCP1 mRNA and protein expression in SAT (Fig. S10).

3.10. Irisin enhances lipolysis via cAMP–PKA–HSL/perilipin pathway in vitro

In 3T3-L1 adipocytes, irisin concentration-dependently enhanced basal lipolysis, reaching its maximal effect at about 1 h after the irisin treatment. However, irisin had no significant effect on isoproterenol (ISO)-induced lipolysis (Fig. 6A,B). The lipolysis effect of irisin was prevented by adenylyl cyclase inhibitor SQ22536 or PKA inhibitor H-89 (Fig. 6C). Irisin caused similar increase in cAMP levels and PKA activity to ISO or ISO plus irisin (Fig. 6D). On the other hand, irisin increased the phosphorylation of HSL at Ser563 or Ser660 but not at Ser565 as well as the phosphorylation of perilipin at Ser522 (Fig. 7A,C); it increased HSL mRNA level but reduced perilipin level (Fig. 7B,D).

3.11. Irisin enhances UCP1 mRNA and Akt phosphorylation in vitro

To define the timeframe of irisin in inducing the browning of white adipocytes, we examined the effects of irisin on the UCP1 mRNA in 3T3L1 adipocytes administered during the differentiation and after the differentiation period, respectively. Irisin increased the UCP1 mRNA in differentiated 3T3L1 adipocytes. Irisin given during the differentiation period caused a greater effect on the UCP1 mRNA than that given after the differentiation (Fig. S11). Irisin caused a similar level of Akt phosphorylation with insulin in 3T3-L1 adipocytes (Fig. S12).

4. Discussion

This study shows that systemic delivery of lentivirus expressing FNDC5 reduces blood glucose level, improves insulin resistance, and increases UCP1 expression and energy expenditure in obese mice. Persistent subcutaneous infusion of irisin causes similar effects to FNDC5 overexpression in obese mice. More important novel findings in this study are that FNDC5/irisin in obese mice attenuates hyperlipidemia and enhances lipolysis via cAMP–PKA–HSL/perilipin pathway. It increases HSL expression and phosphorylation, and reduces perilipin level and adipocyte diameter in adipose tissues in obese mice. The

Fig. 5. Effects of subcutaneous perfusion of irisin with micro-osmotic pump for 4 weeks on body weight, food intake, lipid/glucose metabolism, insulin sensitivity and serum irisin levels in HFD mice. A, body weight; B, food intake; C, serum cholesterol, triglyceride and free fatty acid (FFA) levels; D, fasting blood glucose; E, glucose tolerance test (GTT) and insulin tolerance test (ITT); F, serum irisin levels. Values are mean ± SEM. * \( P < 0.05 \) vs. Saline. \( n = 6 \) for each group.
The present study provides substantial evidence that persistent increased FNDC5/irisin ameliorates metabolic derangements and insulin resistance in obesity. FNDC5/irisin can be taken as an effectively therapeutic strategy for glucose/lipid metabolic disorders. In addition, FNDC5 overexpression decreased serum norepinephrine level and blood pressure in obese mice, suggesting an important role in preventing obesity-related sympathetic activation and hypertension.

FNDC5 overexpression enhanced the rising plasma FFA response to fasting in obese mice, and irisin enhanced glycerol and FFA levels in cultured 3T3-L1 adipocytes. The results indicate that irisin promotes lipolysis in vitro and in vivo, which may be an important mechanism for the therapeutic roles of irisin in attenuating the disturbance of glucose/lipid metabolism and insulin resistance. Therefore, the downstream signal pathway of irisin in promoting lipolysis was investigated in obese mice and in 3T3-L1 adipocytes.

Perilipin is a protein that coats the lipid droplets in adipocytes and acts as a protective coating from HSL. Perilipin phosphorylation exposes the stored lipids to HSL and then promotes lipolysis. PKA-mediated phosphorylation of perilipin is required for inducing the translocation of HSL from the cytosol to the lipid droplets to initiate lipolysis. It is known that activation of β-adrenergic receptors stimulates lipolysis via cAMP-dependent PKA activation in fat cells. HSL is important for the degradation of triacylglycerol in adipose tissues. The phosphorylation at HSL Ser563 or Ser660 activates HSL and lipolysis, while phosphorylation at HSL Ser565 prevents HSL activation. The findings in the present study indicate that FNDC5/irisin promotes lipolysis, which is mediated by cAMP-dependent PKA activation and the phosphorylation of HSL and perilipin. Irisin-induced perilipin down-regulation and HSL up-regulation partially contribute to its lipolysis-promoting effect. It is noted that irisin enhanced basal lipolysis.
but not ISO-induced lipolysis, and caused similar increase in cAMP levels and PKA activity to ISO or ISO plus irisin, possibly because irisin shares its downstream cAMP–PKA pathway with ISO.

Recent evidence has revealed several biological and genetic differences between VAT and SAT [26]. Such differences are reflected in their contrasting roles in the pathogenesis of obesity-related cardiovascular and metabolic problems [27]. It has been found that either SAT or VAT was strongly correlated with insulin resistance in African Americans [28]. Although the VAT is more strongly associated with an adverse metabolic risk, both SAT and VAT are correlated with metabolic risk factors [7]. UCP1 is a transmembrane protein that decreases the proton gradient generated in oxidative phosphorylation. UCP1-mediated heat generation in adipocytes uncouples the respiratory chain, allowing for fast substrate oxidation with a low rate of ATP production [29,30]. It is known that irisin stimulates UCP1 expression and brown-fat-like development [1,31]. We found that FNDC5 overexpression significantly increased UCP1 mRNA and protein expressions, and reduced the adipocyte diameter in the SAT but not in the VAT in both control and HFD mice. Irisin increased UCP1 mRNA in 3T3L1 adipocytes. These results suggest that SAT is more sensitive to FNDC5/irisin than VAT in the effects on brown-fat-like development and heat production.

FNDC5 overexpression increased oxygen consumption, carbon dioxide and heat production in obese mice but did not significantly affect the total activity and food intake. There seems to be two crucial reasons for the increased energy expenditure. One is that FNDC5/irisin induced enhancement in lipolysis via cAMP–PKA–HSL/perilipin pathway. Another reason is that FNDC5/irisin induced more heat generation due to the increased UCP1 expression which causes a fast substrate oxidation with a low rate of ATP production (Fig. 8). The increased lipolysis, UCP1 expression and energy expenditure contribute to the beneficial effects of FNDC5/irisin on attenuating glucose/lipid metabolic derangements and insulin resistance in obesity. It is known that the PI3K–Akt pathway primarily mediates the effects of insulin on glucose metabolism [32]. We found that FNDC5 overexpression promotes the Akt phosphorylation in both control and HFD mice. Irisin caused a similar level of Akt phosphorylation with insulin in 3T3-L1 adipocytes. These results suggest that irisin-induced Akt activation may improve glucose metabolism, which may partially contribute to the improved insulin sensitivity in mice with FNDC5 overexpression or irisin infusion. Bariatric surgery has been shown to be an effective and durable therapy for the treatment of morbid obese patients [33,34]. In this study, FNDC5/irisin promoted lipolysis and reduced lipid in adipocytes and hepatocytes. The lipolysis effect of irisin may play beneficial metabolic roles in reducing the lipid accumulation in tissues, decreasing serum lipid levels and preventing obesity.

It is unexpected that irisin had no significant role in reducing body weight in obese mice regardless of striking effect on lipolysis. We noted that irisin induced a tendency in increasing food intake and in reducing body weight in obese mice. It is possible that the role of irisin

![Fig. 8. Schematic diagram showing FNDC5 overexpression or irisin prevents glucose/lipid metabolic derangements, improves insulin resistance and increases energy expenditure via the enhanced lipolysis and the uncoupling of oxidative phosphorylation. Irisin induces the phosphorylation of both hormone-sensitive lipase (HSL) and perilipin via cAMP/PKA pathway. Perilipin is a protein localized on the surface of lipid droplets that serves as a gatekeeper and inhibits lipolysis. The phosphorylation of HSL causes the activation of HSL, and the phosphorylation of perilipin induces the translocation of HSL from the cytosol to the surface of lipid droplets for converting stored triglycerides to glycerol and free fatty acids (FFAs). Irisin-induced down-regulation of perilipin and up-regulation of HSL promote the lipolysis. On the other hand, irisin increases the uncoupling protein 1 (UCP1) expression, which causes the uncoupling of oxidative phosphorylation in mitochondria, and increases the heat production and energy expenditure. P, phosphorylation; +, promoting effect.](image-url)
in reducing body weight is offset by a compensatory slight increase in food intake. The irisin-induced tendency of slight increase in food intake in obesity may be attributed to the up-regulated setpoint in the brain for energy balance and body weight control [35–37]. Interestingly, the effects of irisin in improving the glucose/lipid metabolic derangements are beneficial to the whole body in obesity or metabolic syndrome. A main limitation in the present study is that we have not explored what happens to all the lipolyzed fat, which is worthy of further studies.

5. Conclusion

FNDC5 overexpression or persistent subcutaneous perfusion of irisin increased energy expenditure, ameliorated glucose/lipid metabolic derangements, and attenuated insulin resistance in HFD-induced obese mice. FNDC5/irisin enhanced lipolysis via cAMP→PKA→HSL/perilipin pathway. Irisin increased HSL and UCP1 expressions as well as HSL phosphorylation. It increased perilipin phosphorylation but reduced perilipin expression. These effects contribute to the increased energy expenditure in obesity. FNDC5/irisin can be used as an effective strategy in attenuating metabolic derangements and insulin resistance in obesity and diabetes.

Transparency document

The Transparency document associated with this article can be found online.

Conflict of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jbbadis.2015.06.017.

References


Acknowledgments

None declared.

Appendix A. Supplementary data

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None declared.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jbbadis.2015.06.017.

References


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