Irisin Is Expressed and Produced by Human Muscle and Adipose Tissue in Association With Obesity and Insulin Resistance

José María Moreno-Navarrete, Francisco Ortega, Marta Serrano, Ester Guerra, Gerard Pardo, Francisco Tinahones, Wifredo Ricart, and José Manuel Fernández-Real

Department of Diabetes, Endocrinology, and Nutrition (JM.M.-N., F.O., M.S., E.G., G.P., W.R., J.M.-F.-R.), Hospital Dr. Josep Trueta de Girona, Spain; Institut d’Investigació Biomèdica de Girona, Centro de Investigación Biomédica en Enfermedades del Desarrollo (CIBERObn) (CB06/03/010), and Instituto de Salud Carlos III, 17007 Girona, Spain; and Department of Endocrinology and Nutrition, Hospital Virgen de la Victoria de Málaga, CIBERObn (CB06/03/018), Instituto de Salud Carlos III, Málaga, Spain

Context: Recently irisin (encoded by Fndc5 gene) has been reported to stimulate browning and uncoupling protein 1 expression in sc adipose tissue of mice.

Objective: The objective of the study was to investigate FNDC5 gene expression in human muscle and adipose tissue and circulating irisin according to obesity, insulin sensitivity, and type 2 diabetes.

Design, Patients, and Main Outcome Measure: Adipose tissue FNDC5 gene expression and circulating irisin (ELISA) were analyzed in 2 different cohorts (n = 125 and n = 76); muscle FNDC5 expression was also evaluated in a subcohort of 34 subjects. In vitro studies in human preadipocytes and adipocytes and in induced browning of 3T3-L1 cells (by means of retinoblastoma 1 silencing) were also performed.

Results: In both sc and visceral adipose tissue, FNDC5 gene expression decreased significantly in association with obesity and was positively associated with brown adipose tissue markers, lipogenic, insulin pathway-related, mitochondrial, and alternative macrophage gene markers and negatively associated with LEP, TNFα, and FSP27 (a known repressor of brown genes). Circulating irisin and irisin levels in adipose tissue were significantly associated with FNDC5 gene expression in adipose tissue. In muscle, the FNDC5 gene was 200-fold more expressed than in adipose tissue, and its expression was associated with body mass index, PGC1α, and other mitochondrial genes. In obese participants, FNDC5 gene expression in muscle was significantly decreased in association with type 2 diabetes. Interestingly, muscle FNDC5 gene expression was significantly associated with FNDC5 and UCP1 gene expression in visceral adipose tissue. In men, circulating irisin levels were negatively associated with obesity and insulin resistance. Irisin was secreted from human adipocytes into the media, and the induction of browning in 3T3-L1 cells led to increased secreted irisin levels.

Conclusions: Decreased circulating irisin concentration and FNDC5 gene expression in adipose tissue and muscle from obese and type 2 diabetic subjects suggests a loss of brown-like characteristics and a potential target for therapy. (J Clin Endocrinol Metab 98: E769-E778, 2013)
AMP-activated protein kinase-induced oxidative metabolism and glucose uptake by the muscle (1, 2). Similarly, leptin plays a role as a muscle insulin sensitizer (3, 4). Otherwise, some myokines such as IL-6 and IL-15, mainly produced after physical exercise, display beneficial effects on metabolism interacting with the adipose tissue (5, 6). Exercise-induced muscle IL-6 production increases insulin-stimulated glucose metabolism in muscle and adipose tissue, enhancing insulin-stimulated glucose disposal and fatty acid via AMP-activated protein kinase activation (7). IL-15 leads to reduced adipogenesis and increased fatty acid mobilization from adipose tissue depots (8, 9).

Irisin is a novel myokine, proteolytically processed from the product of the FNDC5 gene prior to being released into the circulation (10). Irisin is regulated by peroxisome proliferator-activated receptor-γ coactivator-1 (PGC1)-α, and it has been proposed to mediate the beneficial effects of exercise on metabolism, inducing the browning of sc adipocytes and thermogenesis by increasing uncoupling protein 1 (UCP1) levels (10). In mice, irisin causes a significant increase in total body energy expenditure and resistance to obesity-associated insulin resistance. In humans, contradictory effects of physical exercise on irisin production have been reported (11–13). Timmons et al (11), using gene expression arrays, detected an exercise-induced increase of FNDC5 mRNA in human muscle biopsies from old but not from young subjects. On the other hand, 2 recent studies found an association of FNDC5 gene expression and irisin levels with physical exercise and PGC1-α mRNA level (12, 13). In human tissues, the distribution of FNDC5 expression was strongly increased in muscle in comparison with adipose tissue, similar to the findings described in mice (12). In fact, age-related muscle loss correlated to decreased circulating irisin concentration, muscle mass being the main predictor of this in humans (12). In this latter study, circulating irisin concentration levels were inversely correlated with adiponectin and positively correlated with body mass index (BMI), fasting glucose, and total cholesterol. Furthermore, after bariatric surgery-induced weight loss, circulating irisin levels as well as muscle FNDC5 gene expression were significantly down-regulated. According to these authors, these correlations may suggest a compensatory role for irisin in response to deterioration of insulin sensitivity and glucose/lipid metabolism (12).

The stimulation of beige adipocytes in human adipose tissue has been postulated as a possible therapeutic way to improve obesity-associated metabolic disturbances (14). Because irisin is a circulating factor that activates beige fat cells in rodents (10), it could represent one way to perform this action in humans. In the current study, we aimed to investigate FNDC5 gene expression in skeletal muscle, human adipose tissue, and circulating irisin in association with the expression of browning and adipogenic genes in both sc and visceral adipose tissue, according to obesity and type 2 diabetes. The possible relationship among obesity-associated metabolic disturbances, insulin sensitivity, and circulating irisin was also investigated. In vitro, we studied the potential irisin secretion from human preadipocytes and after their differentiation into adipocytes. Finally, we explored irisin secretion after inducing browning by retinoblastoma 1 (Rb1) silencing of mouse 3T3-L1 cells.

Subjects and Methods

Subjects’ recruitments for adipose tissue and muscle study

Two hundred forty-three adipose tissue samples (125 visceral and 118 sc abdominal fat depots) from a group of Caucasian participants, with different degree of obesity (BMI between 20 and 58 kg/m²) were analyzed. In a subcohort of the last, consecutive 29 morbidly obese subjects, we also investigated circulating irisin in association with sc and visceral FNDC5 gene expression. Finally, in a subgroup of 34 participants, muscle tissues (rectus abdominis muscle) were also obtained. The cohorts were recruited at the Endocrinology Service of the Hospital Universitari Dr Josep Trueta (Girona, Spain). All subjects reviewed that their body weight had been stable for at least 3 months before the study, and all subjects gave written informed consent after the purpose, nature, and potential risks for the study were explained to them.

Adipose tissue (visceral and sc) and muscle samples were obtained during elective surgical procedures (cholecystectomy, surgery of abdominal hernia, and gastric bypass surgery), washed, fragmented, and immediately flash frozen in liquid nitrogen before be stored at −80°C. The isolation of adipocyte and stroma vascular cell fraction (SVF) is reported as supplementary information.

Subjects’ recruitments for the insulin sensitivity study

Seventy-six Caucasian men were recruited and studied in an ongoing study dealing with nonclassical cardiovascular risk factors in northern Spain. Subjects were randomly localized from a census and they were invited to participate. The participation rate was 71%. A 75-g oral glucose tolerance test according to the American Diabetes Association Criteria was performed in all subjects. Subject characteristics and inclusion criteria were detailed in the Supplemental Data, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org. All subjects gave written informed consent after the purpose of the study was explained to them. The Ethical Committee of the Hospital Universitari Dr Josep Trueta approved the protocol.

Subjects were studied in the postabsorptive state. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Subjects’ waists were measured with a soft tape midway between the lowest rib and the iliac crest; the hip circumference.
was measured at the widest part of the gluteal region; and the waist to hip ratio was accordingly calculated.

Insulin sensitivity was measured using the frequently sampled iv glucose tolerance test as previously reported (Ref 15 and Supplementary Data).

Analytical methods
Serum glucose, glycosylated hemoglobin (HbA1c), serum insulin, total serum triglycerides, and high-density lipoprotein (HDL) cholesterol measurements are detailed in Supplemental Data. Plasma irisin concentrations were measured using an ELISA (SK00170-01; Aviscera Bioscience Inc, Santa Clara, California). The intra- and interassay coefficients of variation for these determinations were between 4% and 6% and between 8% and 10%, respectively.

Human adipocyte differentiation
Subcutaneous human preadipocytes (Zen-Bio Inc, Research Triangle Park, North Carolina) were cultured and differentiated as previously described (16). The release of irisin by adipocytes was corrected by the number of cells, the time, and the volume of the medium and expressed as femtomoles per (thousand cells × hours).

Induction of browning of 3T3-L1 adipocytes
The embryonic fibroblast mouse cell line 3T3-L1 (American Type Culture Collection, Manassas, Virginia) was maintained in DMEM containing 20 mM glucose, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Two days after confluence, an insulin (5 μg/mL), dexamethasone (0.5 μM), and isobutylmethylxanthine (0.5 mM) mixture was added for 2 days, followed by 5 days with insulin (5 μg/mL) alone. Differentiation was verified by morphological assessment and adipogenic gene expression.

Permanent silencing was performed using Rb1-targeted and control short hairpin RNA lentiviral particles (sc-29468-V and sc-108080l; Santa Cruz Biotechnology, Santa Cruz, California) according to the manufacturer instructions. Positive 3T3-L1 adipocytes harboring short hairpin RNA cassette for Rb1 were selected by puromycin (3 μg/mL) selection 60 hours after infection. In 3T3-L1 Rb1 knockdown cells, white adipogenesis was significantly decreased in parallel with the increase of brown adipocyte differentiation (data not shown, J. M. Moreno-Navarrete, unpublished data). The release of irisin was corrected by the number of cells, the time, and the volume of the medium and expressed as femtomoles per (thousand cells × hours).

Gene expression
RNA was prepared from all these samples using RNeasy lipid tissue mini kit (QIAGEN, Izasa SA, Barcelona, Spain). The integrity of each RNA sample was checked by an Agilent bioanalyzer (Agilent Technologies, Palo Alto, California). Total RNA was quantified by means of a spectrophotometer (GeneQuant; GE Health Care, Piscataway, New Jersey) and reverse transcribed to cDNA using a high-capacity cDNA archive kit (Applied Biosystems Inc, Madrid, Spain) according to the manufacturer’s protocol.

Real-time quantitative PCR was conducted using a Light Cycler 480 Probes Master (Roche Diagnostics, Barcelona, Spain) using Taqman or Sybr Green technology suitable for relative genetic expression quantification and running the same amount of tissue total mRNA (10 ng) to compare gene expression between tissues.

The commercially available and prevalidated TaqMan primer/probes sets used were as follows: endogenous control cyclophilin A (PPIA; 4333763) and target genes such as fibronectin type III domain containing 5 (FNDC5; Hs00401006_m1); peroxisome proliferator-activated receptor-γ (PPAR-γ; Hs00234592_m1); solute carrier family member 4 (SLC2A4 or GLUT4; Hs00168966_m1); insulin receptor substrate 1 (IRS-1; Hs00178563_m1); fatty acid synthase (FASN; Hs00188012_m1); acetyl-CoA carboxylase (ACACA; Hs00167385_m1); and PR domain containing 16 (PRDM16; Hs00922674_m1); leptin (LEP; Hs00174877_m1); uncoupling protein 1 (mitochondrial, proton carrier) (UCP1, Hs00222453_m1); interleukin 10 (IL10, Hs0016622_m1); tumor necrosis factor (TNF, Hs01113624_g1); CD68 (CD68, Hs00154355_m1); mannose receptor, C type 1 (MRC1 or CD206, Hs00267207_m1).

The prevalidated SybrGreen primers were purchased from Sigma S.A. (Barcelona, Spain), and the primers sequences used were as follows: endogenous control human cyclophilin A (PPIA) forward, 5′-CAATGGCTGACCACCACAA-3′; and reverse 5′-CCTCCCAATATATTGCGTCTT-3′; peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) forward, 5′-GATAATTGAAGAGINCAGAT-5′; and reverse 5′-CCTCCATCATCCCGAGAT-5′; and peroxisome proliferator-activated receptor-γ coactivator 1β (PGC1β) forward, 5′-GCTGACAGATAGGAGAGC-3′; and reverse 5′-GAAATGGGATCTGAGTAGT-3′; transcription factor A, mitochondrial (TFAM) forward, 5′-AAGATCTCGAGAAGTACTAGTG-3′; and reverse 5′-CAGATGTCAGACAGTTTTCCAGTTT-3′; cytochrome c oxidase III (MTCO3) forward, 5′-GCCCCTAAGAGATCAAT-3′; and reverse 5′-GAGAATGTTTCTGAGAGGACTC-3′; fat-specific protein 27 (FSP27) forward 5′-gaggtccaaacagctcag-3′; and reverse: 5′-ctgccgtgctagcctgag-3′.

The reaction was performed in a final volume of 7 μL. The cycle program consisted of an initial denaturing of 10 minutes at 95°C and then 40 cycles of a 15-second denaturizing phase at 95°C and a 1-minute annealing and extension phase at 60°C. A threshold cycle (Ct) value was obtained for each amplification curve, and a ΔCt value was first calculated by subtracting the Ct value for human PPIA RNA from the Ct value for each sample. Fold changes compared with the endogenous control were then determined by calculating 2^-ΔΔCt, so gene expression results are expressed as an expression ratio relative to PPIA gene expression according to the manufacturers’ guidelines.

Protein preparation
Proteins were extracted from adipose tissue by using a Polytron PT-1200C homogenizer (Kinematica AG, Lucerne, Switzerland) directly in radioimmunoprecipitation assay buffer (0.1% sodium dodecyl sulfate; 0.5% sodium deoxycholate; 1% Nonident P-40; 150 mM NaCl; and 50 mM Tris-HCl, pH 8.0), supplemented with protease inhibitors (1 mM phenylmethysulfonyl fluoride). Cellular debris and lipids were eliminated by centrifugation of the solubilized samples at 13 000 rpm for 60 minutes at 4°C, recovering the soluble fraction below the fat supernatant and avoiding the nonhomogenized material at the bottom of the centrifuge tube. Protein concentration was determined using the RC/DC protein assay (Bio-Rad Laboratories, Hercules, California).
**Statistics**

Statistical analyses were performed using SPSS version 12.0 software for Windows (SPSS, Chicago, Illinois). All assays were performed at least in duplicate and reported as mean ± SD. The comparison between groups was performed using a 2-way ANOVA followed by post hoc analysis (using DMS and Bonferroni post hoc tests). Parameters that did not fulfill normal distribution were mathematically transformed (log10) to improve symmetry for subsequent analyses. The relation between variables was analyzed by bivariate correlation (Pearson’s or Spearman’s test) and multiple linear regression models (using a stepwise method). To analyze in vitro experiments, a Mann Whitney U test (nonparametric test) was used.

**Results**

**Human adipose tissue**

Anthropometric and clinical characteristics of all participants are detailed in Table 1. In this cohort of subjects, LEP and CD68 gene expression in visceral adipose tissue (VAT) were significantly associated with BMI (r = 0.47, P < .0001, and r = 0.30, P < .0001) and fasting glucose (r = 0.38, P < .0001 and r = 0.20, P = .01). TNFα gene expression was significantly associated with BMI (r = 0.27, P = .004). In sc adipose tissue (SAT), LEP gene expression was associated with BMI (r = 0.25, P = .008).

---

**Table 1. Anthropometric and Clinical Parameters of Study Subjects From Cohort 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonobese</th>
<th>Obese</th>
<th>Nonobese T2D</th>
<th>Obese T2D</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>35</td>
<td>56</td>
<td>6</td>
<td>28</td>
<td>.5</td>
</tr>
<tr>
<td>Gender (men/women)</td>
<td>11/24</td>
<td>9/47</td>
<td>2/4</td>
<td>6/22</td>
<td>.02</td>
</tr>
<tr>
<td>Age, y</td>
<td>51.1 ± 13.4</td>
<td>45.9 ± 10.9</td>
<td>55.5 ± 13.2</td>
<td>43.9 ± 10.4</td>
<td>.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.8 ± 2.7</td>
<td>45.4 ± 8.2</td>
<td>28.19 ± 1.38</td>
<td>44.5 ± 5.3</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>5.03 ± 0.63</td>
<td>5.2 ± 0.72</td>
<td>8.91 ± 3.59</td>
<td>8.22 ± 3.64</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>67.32 ± 54.82</td>
<td>73.22 ± 28.45</td>
<td>56.56 ± 11.10</td>
<td>124.78 ± 62.59</td>
<td>.02</td>
</tr>
<tr>
<td>VAT</td>
<td>2.36 ± 2.2</td>
<td>2.53 ± 1.36</td>
<td>3.04 ± 0.28</td>
<td>5.1 ± 3.2</td>
<td>.04</td>
</tr>
</tbody>
</table>

**Abbreviations:** HOMA-IR, homeostasis model assessment insulin resistance index; R.U., relative units; T2D, type 2 diabetes. Bold values mean that the value is statistically significant, P < .05.

a P < 0.05 compared with nonobese and nondiabetic participants performing DMS and Bonferroni post hoc tests.
b P < 0.05 compared with non-obese and T2D participants performing DMS and Bonferroni post hoc tests.
c P < 0.05 compared with obese and non-diabetic participants performing DMS and Bonferroni post hoc tests.
d P < 0.05 compared with nonobese and non-diabetic participants performing DMS and Bonferroni post hoc tests.
These data were in agreement with the known inflammatory state of VAT vs SAT (17, 18).

In both SAT and VAT, FNDC5 gene expression was significantly decreased in association with obesity (Table 1 and Figure 1A). In SAT, FNDC5 gene expression was decreased significantly in type 2 diabetic participants (0.0029 ± 0.002 vs 0.0042 ± 0.003 R.U., \( P = 0.01 \)), but this association was lost after controlling for BMI. No differences were found in SAT (0.0040 ± 0.002 vs 0.0038 ± 0.003 R.U., \( P = 0.7 \)) and VAT (0.0051 ± 0.0028 vs 0.0046 ± 0.003 R.U., \( P = 0.5 \)) FNDC5 gene expression according to gender. In both SAT and VAT, FNDC5 gene expression was negatively associated with BMI and positively associated with GLUT4, IRS1, FASN, ACC, PRDM16, UCP1, and MTCO3 gene expression (Table 2 and Figure 2 and Supplemental Figure 1). In addition, in SAT, FNDC5 gene expression was negatively associated with LEP and FSP27 and positively associated with PGC1α, IL10, CD206, and CD68 gene expressions. In VAT, FNDC5 was also negatively associated with TNFα gene expression (Table 2).

In multiple linear regression models, BMI (\( \beta = -0.31, P = .005 \)) contributed independently to SAT FNDC5 gene expression variance after controlling for age and gender. Furthermore, UCP1 (\( \beta = .24, P = .01 \)), PRDM16 (\( \beta = .22, P = .04 \)), IRS1 (\( \beta = .32, P = .003 \)), FASN (\( \beta = .36, P = .001 \)), MTCO3 (\( \beta = .32, P = .003 \)), LEP (\( \beta = -0.47, P < .0001 \)), FSP27 (\( \beta = -0.32, P = .003 \)), and CD206 (\( \beta = .38, P = .001 \)) contributed independently to FNDC5 gene expression variance after controlling for age, gender, and BMI. Otherwise, BMI (\( \beta = -0.33, P = .001 \)) contributed independently to VAT FNDC5 gene expression variance after controlling for age and gender, and only UCP1 (\( \beta = .24, P = .01 \)) contributed independently to the FNDC5 gene expression variance after controlling for age, gender, and BMI. The associations with IRS1, FASN, PRDM16, and MTCO3 gene expressions were lost after controlling for BMI.

Only a few numbers of participants with type 2 diabetes were under treatment [metformin (n = 4), glitazones (n = 3), and insulin (n = 4)]. No drug effects were found to influence FNDC5 gene expression in VAT and SAT.

Irisin levels in adipose tissue (AT) lysates were measured by an ELISA in 12 SAT and 16 VAT samples. AT irisin levels strongly correlated with FNDC5 gene expression in both SAT (\( r = 0.65, P = .02 \)) and visceral (\( r = 0.54, P = .02 \)) adipose tissues. Interestingly, VAT irisin protein levels were positively associated with insulin pathway-related gene expression [such as IRS1 (\( r = 0.63, P = .015 \)) and GLUT4 (\( r = 0.70, P = .01 \))] and negatively with BMI (\( r = -0.55, P = .02 \)).

Figure 1. A, FNDC5 gene expression in SAT and VAT according to obesity and type 2 diabetes. B, FNDC5 gene expression in adipose tissue fractions [adipocytes (MA) and stromovascular cells (SVCs)]. C, FNDC5 gene expression in CD14+ and CD14− cells from SVCs from SAT (n = 5) and VAT (n = 4).
Circulating irisin and adipose tissue FNDC5 expression

In a subcohort of consecutive 29 morbidly obese subjects, circulating irisin concentration was significantly associated with both FNDC5 gene expression in sc (r = 0.44, P = .01) and visceral (r = 0.38, P = .04) adipose tissues. These associations were maintained after controlling for age and BMI. No significant differences in circulating irisin according to gender were found (424.4 ± 72.7 ng/mL in men vs 509.1 ± 122.2 ng/mL in women, P = .2).

Circulating irisin, BMI, and insulin sensitivity

Anthropometrical and analytical characteristics of this cohort are shown in Tables 3 and 4. Excluding diabetic subjects, circulating irisin decreased in association with obesity (Table 3). In fact, circulating irisin was negatively associated with BMI (r = −0.37, P = .001), percent fat mass (r = −0.26, P = .03), waist to hip ratio (r = −0.31, P = .008) and positively associated with insulin sensitivity (r = 0.24, P = .04). No correlation was found with fasting glucose, HbA1c, fasting triglycerides, HDL cholesterol, or C-reactive protein. In a multiple linear regression analysis, BMI (β = −.33, P = .004) contributed independently to circulating irisin variance after controlling for age, waist to hip ratio and insulin sensitivity. Circulating irisin concentration was decreased significantly in participants with type 2 diabetes (Table 4).

In vitro studies

Human adipocytes

Irisin secretion into the media was measured before and after the differentiation process (at day 14) of human sc adipocytes. Secreted irisin concentration was 0.071 ± 0.010 fmol/(thousand cells × hour) in media from the preadipocytes and decreased to 0.035 ± 0.003 fmol/(thousand cells × hour) in the adipocyte media after differentiation (Figure 3A).

In 3T3-L1 cells after inducing browning features

The strong association between FNDC5 and brown adipocyte genes is supported by the browning effects of irisin on white adipose tissue (10). Because we found that
Irisin was produced at substantial levels in human preadipocytes and adipocytes, we aimed to investigate the effects of induced browning in 3T3-L1 Rb1 knockdown (KD) cells on irisin production. These cells expressed high levels of brown adipocyte genes, such as 

**UCP1**

and 

**PRDM16**

(data not shown, J. M. Moreno-Navarrete, unpublished data). Interestingly, irisin secretion was significantly higher in 3T3-L1 Rb1 KD than 3T3-L1 C cells (Figure 3B).

### Discussion

**FNDC5** gene expression has been described in skeletal muscle of mice, and PGC1α is one of its main regulators (10). After the initial submission of this article, 3 independent papers have confirmed **FNDC5** gene expression in human skeletal muscle (11–13), being the main source of circulating irisin in both animal models and humans. In agreement with these results, we found a 200-fold in-

---

**Table 3.** Anthropometric and Clinical Parameters of Study Subjects (Cohort 2) According to Obesity Status (Excluding Subjects With Type 2 Diabetes)

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Overweight</th>
<th>Obese</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>34</td>
<td>17</td>
<td>.17</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>47.28 ± 10.15</td>
<td>51.62 ± 10.64</td>
<td>54 ± 11.83</td>
<td>.17</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>23.33 ± 1.2</td>
<td>27.23 ± 1.58</td>
<td>32.47 ± 2.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Percent fat mass, %</td>
<td>0.89 ± 0.034</td>
<td>0.93 ± 0.065</td>
<td>1.01 ± 0.065</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>4.9 ± 3.6</td>
<td>9.95 ± 9.01</td>
<td>20.53 ± 12.6</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.27 ± 0.66</td>
<td>5.25 ± 0.47</td>
<td>5.49 ± 0.49</td>
<td>.3</td>
</tr>
<tr>
<td>Insulin sensitivity, 10^{-4}·min^{-1}·pM^{-1}</td>
<td>4.75 ± 0.44</td>
<td>4.78 ± 0.27</td>
<td>4.94 ± 0.37</td>
<td>.2</td>
</tr>
<tr>
<td>Fasting triglycerides, mg/dL</td>
<td>1.44 ± 0.21</td>
<td>1.23 ± 0.22</td>
<td>0.96 ± 0.31</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>91.66 ± 62.5</td>
<td>87.64 ± 49.1</td>
<td>107.5 ± 43.4</td>
<td>.43</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>54.01 ± 15.51</td>
<td>54.45 ± 13.28</td>
<td>52.43 ± 11.1</td>
<td>.87</td>
</tr>
<tr>
<td>Circulating irisin, ng/mL</td>
<td>2157.9 ± 600.7</td>
<td>1783.7 ± 426.9</td>
<td>1652.2 ± 421.8</td>
<td>.006</td>
</tr>
</tbody>
</table>

Bold values mean that the value is statistically significant, P < .05.

*P < .05 vs lean participants performing DMS and Bonferroni post hoc tests.

*P < .05 vs overweight participants performing DMS and Bonferroni post hoc tests.
creased expression of FNDC5 in muscle relative to adipose tissue and a strong relationship among FNDC5, PGC1α, and the expression of other mitochondrial genes (PGC1β, TFAM, and MTCO3) but not with IL15 (a specific myokine).

In line with the browning effects of muscle-produced irisin, observed only in SAT (10), we have found that FNDC5 in muscle was linked to the expression of FNDC5 and UCP1 in SAT but not in VAT.

In humans, muscle FNDC5 gene expression increased with obesity (11, 12). Huh et al (12) speculated that this association might be a compensatory mechanism. Reinforcing this hypothesis, we found that muscle FNDC5 gene expression was positively associated with BMI (r = 0.42, P = .02) but negatively associated with age (r = −.44, P = .01). In fact, age (β = −.43, P = .02) contributed independently to the FNDC5 gene expression variance in muscle after controlling for gender and BMI. Timmons et al (11) detected an exercise-induced increase of FNDC5 mRNA in human muscle biopsies from old (precisely the subjects with the lowest FNDC5 levels in our study) but not from young subjects. These data are not comparable with our data because FNDC5 gene expression in our study was measured only in the basal state. In addition, we also found that muscle FNDC5 mRNA was significantly decreased in subjects with type 2 diabetes. Whether this is pathophysiologically linked to type 2 diabetes should be explored more in depth in future studies.

In the current study, we found an inverse association of FNDC5 gene expression in human SAT and VAT with obesity. In addition, we found a positive association of FNDC5 gene expression with brown adipose tissue markers (PRDM16 and UCP1), lipogenic (FASN and ACC), and the expression of insulin-pathway related genes (GLUT4 and IRS1), mitochondrial (MTCO3 and PGC1α), and alternative macrophage markers (IL-10 and CD206).

FNDC5 gene expression was also negatively associated with LEP, TNFα, and FSP27, which is a negative regulator of white transdifferentiation to brown adipose tissue (19, 20). Interestingly, FSP27-deficient mice showed improved insulin sensitivity and were resistant to diet-induced obesity, and FSP27-deficient white adipocytes had reduced lipid storage, smaller lipid droplets, increased mitochondrial activity, and a higher expression of several brown adipose tissue-selective genes. Furthermore, in adipose tissue, in FSP27-deficient mice (with browning phenotype), the expression levels of genes involved in oxidative phosphorylation, the TCA cycle, fatty acid synthesis,
and fatty acid oxidation were increased (20). Otherwise, no correlation was found in association with the master adipogenic transcription factor PPARγ (21). Interestingly, we confirmed the presence of irisin in adipose tissue lysates, being significantly correlated with AT and capacity of adipose tissue (ie, increasing the expression of adipogenic genes) and enhancing adipose tissue (by increasing the adipose tissue capacity to burn fuel (glucose and fatty acids)). The cross-sectional associations suggest a scenario in which irisin enhances white adipose tissue, leading to improved function and capacity of adipose tissue (ie, increasing the expression of adipogenic genes) and enhancing adipose tissue FNDC5 gene expression, irisin being produced in a positive feedback by the adipose tissue itself.

All these associations led us to speculate para- or autocrine production of irisin from adipose tissue, which may act by increasing the adipose tissue capacity to burn fuel (glucose and fatty acids). The cross-sectional associations suggest a scenario in which irisin enhances white adipocyte transdifferentiation (by increasing UCP1 and PRDM16) into beige adipocytes and as a consequence, the capacity of adipose tissue (mainly SAT) to uptake glucose (by increasing IRS1 and GLUT4) or to synthesize fatty acids (by increasing FASN and ACC), to promote lipid mobilization (by decreasing FSP27) or to burn these fuels (by increasing MTCO3 and PGC1α). In parallel to these effects, irisin could contribute to the modulation of obesity-induced inflammatory/antiinflammatory balance (by increasing CD206 and IL10 and decreasing TNFa and LEP). The strong association between FNDC5 and CD206 in SAT also suggests that alternative macrophages might contribute to irisin production at the same level of other cells contained within the stroma vascular cell fraction (such as endothelial cells). In fact, FNDC5 gene expression was similar in CD14<sup>+</sup> (containing alternative and classical macrophages) and CD14<sup>-</sup> (containing endothelial cells and preadipocytes) in SAT.

Circulating irisin has been recently found to be directly associated with muscle mass and estradiol levels and inversely associated with age in a cohort of 117 middle-aged women (12). Circulating irisin also tended to be associated with BMI, glucose, adiponectin, and cholesterol levels, but these correlations were lost after controlling for estradiol levels and muscle mass. In the current study, BMI was the main contributor to circulating irisin after controlling for age, waist to hip ratio, and insulin sensitivity. These different associations could be interpreted in the context of the well-known inverse associations between muscle mass and fat mass or BMI in men (22).

van Marken Lichtenbelt et al. (23) showed that the amount of brown adipose tissue was significantly decreased in association with obesity, with a negative linear relationship between brown adipose tissue, BMI, and percent body fat. Possibly the decreased para- or autocrine production of irisin in muscle/adipose tissue in obese subjects could be behind the obesity-associated lower amounts of brown or beige adipocytes in human adipose tissue. In agreement with this hypothesis, circulating irisin levels were significantly decreased with obesity and type 2 diabetes.

Acknowledgments

We acknowledge the clinical help of Oscar Rovira.

Address all correspondence and requests for reprints to: J. M. Fernandez-Real, MD, PhD, Section of Diabetes, Endocrinology, and Nutrition, Hospital of Girona “Dr Josep Trueta,” Carretera de Franca s/n, 17007, Girona, Spain. E-mail: jmfreal@idibgi.org.

This work was supported by research grants from the Ministerio de Educación y Ciencia (Grant FISPI1100214). The Centro de Investigación Biomédica en Red de Fisiopatología de la Obesidad y Nutrición Fisiopatología de la Obesidad y Nutrición is an initiative from the Instituto de Salud Carlos III (Spain).

Disclosure Summary: The authors declared no conflict of interest.

References

5. Pedersen BK, Akerström TC, Nielsen AR, Fischer CP. Role of myo-


