Circulating Irisin in Relation to Insulin Resistance and the Metabolic Syndrome

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Context: Irisin, a recently identified hormone, has been proposed to regulate energy homeostasis and obesity in mice. Whether irisin levels are associated with risk of the metabolic syndrome (MetS), cardiometabolic variables, and cardiovascular disease (CVD) risk in humans remains unknown.

Objective: Our objective was to assess the associations between baseline serum irisin levels and MetS, cardiometabolic variables, and CVD risk.

Design, Setting, and Subjects: We conducted a comparative cross-sectional evaluation of baseline circulating levels of the novel hormone irisin and the established adipokine adiponectin with MetS, cardiometabolic variables, and CVD risk in a sample of 151 subjects.

Results: Baseline irisin levels were significantly higher in subjects with MetS than in subjects without MetS. Irisin was associated negatively with adiponectin ($r = -0.4$, $P < .001$) and positively with body mass index ($r = 0.22$, $P = .008$), systolic ($r = 0.17$, $P = .04$) and diastolic ($r = 0.27$, $P = .001$) blood pressure, fasting glucose ($r = 0.25$, $P = .002$), triglycerides ($r = 0.25$, $P = .003$), and homeostasis model assessment for insulin resistance ($r = 0.33$, $P < .001$). After adjustment for potential confounders, including body mass index, subjects in the highest tertile of irisin levels were more likely to have MetS (odds ratio [OR] = 9.44, 95% confidence interval [CI] = 2.66–33.44), elevated fasting blood glucose (OR = 5.80, 95% CI = 1.72–19.60), high triglycerides (OR = 3.89, 95% CI = 1.16–13.03), and low high-density lipoprotein cholesterol (OR = 3.30, 95% CI = 1.18–9.20). Irisin was independently associated with homeostasis model assessment for insulin resistance and general Framingham risk profile in multiple linear regression analyses after adjustment for confounders. Adiponectin demonstrated the expected associations with outcomes.

Conclusions: Irisin is associated with increased risk of MetS, cardiometabolic variables, and CVD in humans, indicating either increased secretion by adipose/muscle tissue and/or a compensatory increase of irisin to overcome an underlying irisin resistance in these subjects. **(*J Clin Endocrinol Metab* 98: 4899–4907, 2013)**

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received May 29, 2013. Accepted September 13, 2013.
First Published Online September 20, 2013

Abbreviations: BMI, body mass index; BP, blood pressure; CI, confidence interval; CVD, cardiovascular disease; FPG, fasting plasma glucose; FFM, fat-free mass; GFRP, general Framingham risk profile for CVD; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; MetS, metabolic syndrome; OR, odds ratio; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; TG, triglyceride; WC, waist circumference.

The metabolic syndrome (MetS) is a clustering of risk factors for both type 2 diabetes mellitus and cardiovascular disease (CVD). Insulin resistance and obesity have long been considered as the most pivotal components in its pathogenesis (1, 2); atherogenic dyslipidemia, hypertension, and the proinflammatory, prothrombotic environment typically promoted by obesity are closely associated with the MetS (2–4).

Adipose tissue is increasingly recognized as an active endocrine organ, capable of regulating metabolism and insulin resistance via production of adipocytokines that also may modify the chronic inflammation associated with the MetS (5). Adipose tissue is composed of two distinctly different functional compartments. White adipose tissue is the primary site of energy storage but may also modulate whole-body and muscle and/or liver insulin sensitivity via hormonal signals (6). Brown adipose tissue is the second type of adipose tissue, found primarily in mammals, and may also affect insulin sensitivity and whole-body metabolism via its role in thermogenesis (7). Recent studies in mice and humans have demonstrated that enhancing brown fat thermogenesis may lead to improved glucose tolerance, increased insulin sensitivity, lower body weight, and decreased fat mass (8–10).

Muscle tissue has been recently recognized as another endocrine regulator of metabolism. Irisin is a novel hormone whose expression is induced by peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) and exercise. It has been reported to act on white adipose cells in vitro and in vivo to stimulate *UCP1* expression and to alter expression of several molecules leading to brown-fat-like development (11). This conversion of white adipocytes to brown adipocytes and the resultant increase in thermogenesis promotes improved insulin sensitivity, reductions in body weight, and improved glucose tolerance in mice (9, 11). Recent studies in humans have reported on the association between irisin levels and the expression of its precursor *FNDC5* with exercise and PGC1α mRNA levels (12, 13). More recent studies have shown that irisin is also released by adipocytes (14, 15). Previous studies in rodents and humans (12, 14, 16–19) have raised hypotheses on potential associations between circulating irisin levels and energy expenditure, metabolic parameters including diabetes status, and/or other adipokines. Although the effects of exercise training on changes in *FNDC5* mRNA expression or irisin levels remain to be fully elucidated in both animals and/or humans (11, 12, 20, 21), most, but not all, human studies on irisin have reported a positive correlation between circulating irisin levels at baseline or *FNDC5* mRNA expression and body mass index (BMI) (11, 12, 16, 18, 20). In humans, it is well known that obesity is associated with increased risk of insulin resistance, MetS, and CVD. To date, no previous studies have directly examined relationships between irisin and the MetS, insulin resistance, and/or CVD risk after adjusting for potential confounders. In this study, we assessed associations between baseline serum irisin levels and MetS, insulin resistance, and cardiovascular risk in a sample of 151 subjects. We also assessed in similar models associations between the same parameters and adiponectin, an established biomarker of risk for these outcomes.

Subjects and Methods

Study population

A total of 151 Caucasian and African American subjects (71 men and 80 women) were recruited consecutively for this study over an 18-month period through various advertising modalities (radio, newspapers, and flyers) in the greater Boston area. Subjects with a history of myocardial infarction or stroke, an active diagnosis of diabetes mellitus, active iv drug use, hepatitis, cirrhosis, dialysis, long-term steroid use, and/or current treatment for cancer or active infection other than brief antibiotic therapies were excluded on enrollment. Measured biomarkers were not available for all subjects, so 148 samples were analyzed for irisin and 146 samples for adiponectin.

This study was approved by the Beth Israel Deaconess Medical Center Committee on Clinical Investigations and Institutional Review Board, in accordance with the Helsinki Declaration of 1975 as revised in 1983. All participants gave written informed consent before participation in the study. As part of the informed consent process, participants' rights as research subjects, exceptions to confidentiality, and the possible risks involved were clearly explained.

Data measurement

Fasting blood samples were collected from each participant at one time point between 2009 and 2011. These blood samples were then processed for serum and plasma within 90 minutes of collection and stored at −80°C. Fasting serum and plasma collected during study visits were used to analyze irisin and adiponectin as previously described (12). Adiponectin was measured by RIA (Millipore). Irisin was measured by colorimetric ELISA (EK-067-52; Phoenix Pharmaceuticals). In this study, interassay and intra-assay coefficients of variation were 6.9% to 9.3% and 1.8% to 3.6% for adiponectin, and $<15\%$ and $<10\%$ for irisin, respectively. Fasting plasma glucose (FPG) was measured with the Roche Cobas c311 clinical chemistry analyzer (Roche Diagnostics), and high-density lipoprotein (HDL) and triglycerides (TGs) were measured by LabCorp through the Clinical Research Center at Beth Israel Deaconess Medical Center.

Anthropometry and body composition were measured by a trained dietician. Height was measured to the nearest 0.1 cm with the use of a wall-mounted stadiometer. Body weight was assessed without shoes to the nearest 0.1 kg, using a calibrated digital electronic scale. Bioelectrical impedance analysis was performed with subjects lying in a supine position using a Quantum II bioelectrical impedance analyzer (22, 23) (RJL Systems). These measurements were performed during the fasting state before blood

sampling with subjects wearing a standard hospital gown and pajama bottoms. BMI was calculated as weight divided by height squared (kilograms per square meter), and waist circumference (WC) was measured along the horizontal plane of the superior border of the iliac crest (24).

Information on physical activity, alcohol consumption, and smoking status was obtained from self-reported questionnaires. Participants were asked questions regarding the type and typical duration of their regular exercise. Energy expenditure by regular exercise was estimated by metabolic equivalent hours per week (25). Regular exercise included both aerobic and anaerobic exercise. Participants' dietary intake was assessed by using the Block Food Frequency Questionnaire (26, 27) (NutritionQuest).

Assessment of MetS, insulin resistance, and CVD risk

Participants were categorized as having MetS according to the National Heart, Lung, and Blood Institute/American Heart Association criteria, which define the presence of MetS as currently having 3 or more of the following 5 cardiovascular risk factors: 1) central obesity (WC ≥102 cm for men and ≥88 cm for women), 2) elevated TG (≥150 mg/dL) or specific medication for this lipid abnormality, 3) low HDL-cholesterol (<40 mg/dL for men and <50 mg/dL for women) or specific medication for this lipid abnormality, 4) systemic hypertension (blood pressure [BP] ≥130/85 mm Hg) or antihypertensive therapy, and 5) elevated FPG (≥100 mg/dL) or history of diabetes mellitus or taking antidiabetic medications (24). Homeostasis model assessment for insulin resistance (HOMA-IR) (28) was calculated as (fasting insulin [units per milliliter] × fasting glucose [milligrams per deciliter]/18)/22.5. To evaluate the 10-year risk of CVDs, the general Framingham risk profile for CVD (GFRP) risk assessment tool was calculated (29).

Evaluation of irisin assay

Validation of commercially available irisin ELISA kits was conducted before analyzing clinical samples, and a detailed report has been published (12). In additional further evaluation, one kit (catalog no. EK-067-52) manufactured by Aviscera, one kit (catalog no. EK-067-29) from Phoenix Pharmaceuticals, and one kit from Adipogen (catalog no. AG-45A-0046EK-KI01) were comparatively evaluated using the same samples and in accordance to methodology as previously described (12).

The results of the additional evaluation showed that the detectable range of each kit was 0.066 to 1024 ng/mL for EK-067-52, 0.1 to 1000 ng/mL for EK-067-29, and 0.001 to 5 μg/mL for the Adipogen kit. In addition, values measured in the same subjects using the Adipogen kit were higher than those that have previously been suggested as a physiological concentrations of irisin (11) and approximately one order of magnitude higher than levels measured in the same subjects by the other two kits above (current study) (12). Although the detectable range in nanograms per milliliter was similar in EK-067-52 and EK-067-29, the EK-067-52 kit showed a wider spectrum of OD values for 10 to 1000 ng/mL standard concentrations (0.11–1.99 for EK-067-52 vs 0.14–1.17 for EK-067-29) and resulted in human irisin levels falling consistently in the linear range of the curve that is required for study validity and provides an advantage in distinguishing small differences among individual samples. Therefore, we have concluded that EK-067-52 was the most appropriate kit available for the analysis of clinical samples in our studies.

Statistical analysis

The Student's *t* test or Mann-Whitney *U* test for continuous variables and χ^2 test for categorical variables were used to compare general clinical characteristics between subjects with MetS and without MetS. Normality of distribution was tested with the Kolmogorov-Smirnov test. Spearman's correlation coefficients were calculated to evaluate the relationship between irisin and anthropometric measurements as well as other biomarkers characteristically associated with MetS. Subjects were divided into tertiles according to their circulating irisin levels. To assess the relationship between irisin and MetS components, we calculated the adjusted odds ratio (OR) and 95% confidence interval (CI) with a multivariable binary logistic regression model after controlling for all of the significant variables identified by univariate analyses. To evaluate the relationship between circulating irisin levels and HOMA-IR as well as GFRP, multiple linear regression analyses were performed. All statistical analyses were performed using SPSS version 18.0 (SPSS, Inc). A two-sided $P < .05$ was considered statistically significant.

Results

Clinical, laboratory, and demographic characteristics of the study participants are presented in Table 1. The 27.8% of the participants met the criteria for MetS. The mean age of the participants was 45.5 ± 4.3 years, which was not significantly different between those with and without MetS. Compared with subjects without MetS, irisin levels were significantly higher in subjects with MetS (mean [interquartile range], 164.0 [134.0–209.0] vs 211.2 [172.6–255.1] ng/mL, respectively; $P < .001$). Conversely, subjects with MetS had lower adiponectin levels than subjects without MetS ($P = .001$). Additionally, participants with MetS showed lower regular exercise-related physical activities than participants without MetS ($P = .008$). With respect to anthropometric and body composition measures, subjects with MetS had significantly higher BMI, percent body fat, percent fat-free mass (FFM), and WC relative to subjects without MetS ($P < .01$ for all associations; Table 1). Thirty-eight subjects were taking medication for diabetes (n = 4), hypertension (n = 24), or dyslipidemia (n = 18). Eleven subjects were taking a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor (n = 9 in the MetS group), 2 were taking fibrates (n = 2 in MetS group), 1 was taking an ω3 agent (n = 1 in the MetS group), 1 was taking an HMG-CoA reductase inhibitor plus fibrate (n = 1 in the MetS group), and 3 were taking unknown medications (n = 3 in the MetS group), respectively.

Spearman's correlation analyses demonstrated that circulating irisin levels were negatively correlated with adiponectin levels ($r = -0.4$, $P < .001$) (Table 2). With respect to anthropometric measurements, irisin levels were

Table 1. General Characteristics of Study Subjects[a]

	No MetS (n = 107)	MetS (n = 44)	All (n = 151)	*P* Value[b]
Age, y	46.0 (43.0–48.0)	46.0 (44.3–47.0)	46.0 (43.0–47.0)	.64
Male gender, n (%)	48 (44.9)	23 (52.3)	71 (47.0)	.41
Race, white, n (%)	50 (46.7)	22 (50.0)	72 (47.7)	.72
Current smokers, n (%)	34 (31.8)	14 (31.8)	48 (31.8)	.99
Irisin, ng/mL[c]	162.2 (133.5–206.9)	214.4 (174.4–254.2)	176.7 (141.9–220.0)	<.001
Adiponectin, μg/mL[d]	9.69 (5.86–13.07)	4.65 (3.24–7.48)	8.1 (4.64–12.58)	<.001
BMI, kg/m^2	27.0 (23.5–31.6)	32.9 (29.1–38.2)	28.6 (25.1–33.9)	<.001
Body fat, %	28.8 ± 11.1	33.5 ± 10.5	30.1 ± 11.1	.007
Fat mass, kg	22.2 (14.9–32.6)	31.8 (26.1–44.4)	25.4 (16.5–36.0)	<.001
Fat-free mass, %	71.4 ± 11.4	66.5 ± 10.5	70.0 ± 11.3	.002
Fat-free mass, kg	56.7 (47.6–67.4)	63.8 (55.6–76.0)	58.2 (49.0–70.6)	.001
WC, cm	92.1 (85.4–103.7)	111.2 (101.5–118.3)	97.1 (87.6–111.6)	<.001
BP, mm Hg				
Systolic	121.2 ± 15.2	129.6 ± 13.6	123.7 ± 15.2	.002
Diastolic	75.4 ± 11.5	83.3 ± 10.0	77.7 ± 11.6	<.001
TG, mg/dL	75.0 (56.0–94.0)	141.5 (103.0–182.8)	82 (60.0–117.0)	<.001
HDL-cholesterol, mg/dL	59.0 (50.0–69.0)	43.5 (35.3–48.0)	53.0 (45.0–65.0)	<.001
Glucose, mg/dL	89.0 (83.2–93.0)	100.1 (93.3–109.0)	91.0 (84.4–98.0)	<.001
HOMA-IR[e]	0.89 (0.42–1.64)	3.16 (1.79–6.70)	1.33 (0.62–2.57)	<.001
GFRP, %	3.71 (2.29–6.24)	7.56 (5.48–13.33)	4.83 (2.88–7.29)	<.001
Physical activity, METs-h/wk	12.4 (2.15–31.5)	9.3 (0.0–14.6)	11.0 (1.70–27.9)	.02
Alcohol consumption, g/d	2.08 (0.0–11.05)	2.28 (0.0–7.57)	2.08 (0.0–8.23)	.21
Total energy intake, kcal/d	1868.9 (1337.5–2647.8)	1734.2 (1270.8–2260.8)	1843.3 (1313.2–2564.0)	.16
Vegetable or fruit consumption, servings/d	5.22 (3.29–7.81)	4.49 (2.44–6.32)	4.85 (2.93–7.36)	.03
Medication history	12 (11.2)	26 (59.1)	38 (25.2)	<.001
Lipid-modifying agent	2 (1.9)	16 (36.4)	18 (11.9)	<.001
Antihypertensive	10 (9.3)	14 (31.8)	24 (15.9)	.001
Antidiabetic	0 (0)	4 (9.1)	4 (2.6)	.007

Abbreviation: MET, metabolic equivalent.

[a] Data are means ± SD for continuous parametric variables, median (interquartile range) for continuous nonparametric variables, or number (column percentage) for categorical variables.

[b] *P* values are from Student's *t* test (for continuous parametric variables) or nonparametric Mann-Whitney *U* test (for continuous nonparametric variables) and χ^2 tests for categorical variables.

[c] n = 148.

[d] n = 146.

[e] n = 138.

positively correlated with BMI ($r = 0.22$, $P = .008$), body fat mass ($r = 0.21$, $P = .01$), WC ($r = 0.24$, $P = .003$). With respect to components of MetS and insulin resistance, irisin was positively correlated with systolic BP ($r = 0.17$, $P = .04$), diastolic BP ($r = 0.27$, $P = .001$), FPG ($r = 0.25$, $P = .002$), total circulating TG ($r = 0.25$, $P = .003$), and HOMA-IR ($r = 0.33$, $P < .001$). Irisin was also negatively correlated with regular exercise-related physical activities ($r = -0.16$, $P = .05$).

Additionally, in similar models, adiponectin was negatively correlated with BMI, fat mass, FFM, WC, GFRP, FPG, TG, and HOMA-IR.

In unadjusted logistic regression models, higher circulating irisin levels were significantly associated with higher odds of having MetS (Table 3). On the other hand, serum levels of adiponectin were negatively associated with the odds of having MetS (Table 3). In comparison with subjects in the lowest tertile of irisin levels, subjects in the highest tertile of irisin levels were more likely to have central obesity (OR = 2.33, 95% CI = 1.03–5.29), elevated BP or hypertension (OR = 2.50, 95% CI = 1.11–5.63), dyslipidemia (OR = 3.25, 95% CI = 1.25–8.4 for elevated TG; OR = 3.25, 95% CI = 1.25–8.40 for low HDL-cholesterol), and elevated FPG (OR = 5.11, 95% CI = 1.71–15.23). After adjustment for age, gender, race, and lifestyle-related factors such as smoking status, physical activity, vegetable or fruit consumption, and alcohol consumption, the adjusted ORs (95% CIs) for MetS or components of MetS in relation to tertile of irisin levels were 7.84 (2.52–24.37) for MetS, 2.78 (1.11–6.99) for central obesity, 3.15 (1.21–8.22) for elevated BP, 6.04 (1.87–19.48) for elevated FPG, 4.23 (1.31–13.69) for high TG, and 3.49 (1.27–9.61) for low HDL-cholesterol, respectively. After adding BMI to the multivariate regression model with age, gender, race, BMI, and lifestyle-related factors such as smoking status, physical activity, vegetable

Table 2. Spearman's Correlation Coefficients Between MetS Components and Anthropometric Measurements

	Irisin	Adiponectin	Age	BMI	% Fat	Fat Mass	% FFM	FFM	WC	GFRP	PA	HDL	SBP	DBP	FPG	TG	HOMA-IR
Irisin	1	−0.40[a]	−0.01	0.22[b]	0.14	0.21[c]	−0.14	0.12	0.24[b]	0.25[b]	−0.16[d]	−0.15	0.17[c]	0.27[b]	0.25[b]	0.25[b]	0.33[a]
Adiponectin		1	−0.07	−0.31[a]	−0.04	−0.21[c]	0.05	−0.40[a]	−0.40[a]	−0.40[a]	0.15	0.41[a]	−0.13	−0.16[d]	−0.30[a]	−0.38[a]	−0.41[a]
Age			1	−0.07	−0.08	−0.09	0.07	−0.03	−0.01	0.42[a]	0.05	0.01	0.12	0.19[c]	0.12	0.10	0.05
BMI				1	0.63[a]	0.86[a]	−0.63[a]	0.48[a]	0.89[a]	0.21[b]	−0.22[b]	−0.23[b]	0.39[a]	0.32[a]	0.32[a]	0.20[c]	0.49[a]
% Fat					1	0.90[a]	−1.00[a]	−0.27[b]	0.58[a]	−0.18[c]	−0.28[b]	−0.01	0.20[c]	0.12	0.12	0.02	0.33[a]
Fat mass						1	−0.89[a]	0.15	0.84[a]	0.04	−0.28[a]	−0.14	0.33[a]	0.26[b]	0.23[b]	0.11	0.48[a]
% FFM							1	0.28[b]	−0.58[a]	0.17[c]	0.27[b]	0.01	−0.20[c]	−0.12	−0.12	−0.02	−0.331
FFM								1	0.52[a]	0.48[a]	0.01	−0.30[a]	0.27[b]	0.28[a]	0.27[b]	0.21[b]	0.27[b]
WC									1	0.32[a]	−0.23[b]	−0.34[a]	0.40[a]	0.38[a]	0.37[a]	0.29[a]	0.53[a]
GFRP										1	−0.09	−0.40[a]	0.53[a]	0.55[a]	0.48[a]	0.50[a]	0.37[a]
PA											1	0.06	−0.13	−0.19[c]	−0.14	−0.18[c]	−0.23[b]
HDL												1	−0.06	−0.12	−0.29[a]	−0.51[a]	−0.42[a]
SBP													1	0.78[a]	0.27[b]	0.14	0.33[a]
DBP														1	0.31[a]	0.27[b]	0.36[a]
FPG															1	0.43[a]	0.52[a]
TG																1	0.33[a]
HOMA-IR																	1

Abbreviations: DBP, diastolic BP; PA, physical activity assessed by metabolic equivalent; SBP, systolic BP.

[a] $P < .001$.

[b] $P < .01$.

[c] $P < .05$.

[d] $P = .05$.

or fruit consumption, and alcohol consumption, the participants in the highest tertile of irisin levels were significantly more likely to exhibit MetS (OR = 9.44, 95% CI = 2.66–33.44), elevated FPG (OR = 5.80, 95% CI = 1.72–19.60), high TG (OR = 3.89, 95% CI = 1.16–13.03), and low HDL-cholesterol (OR = 3.30, 95% CI = 1.18–9.20), respectively (Table 3). There were also significant linear increases of ORs for MetS, elevated FPG, elevated TG, and lower HDL-cholesterol with increasing tertiles of irisin and linear decreases of ORs with increasing tertiles of adiponectin levels (all P for trends $<.05$). After adjustment for body fat mass instead of BMI in multivariate logistic regression, the odds of subjects in the highest tertile of circulating irisin levels having MetS (OR = 7.25, 95% CI = 2.20–23.93), elevated FPG (OR = 5.13, 95% CI = 1.55–16.99), elevated TG (OR = 3.73, 95% CI = 1.13–12.30), and lower HDL-cholesterol (OR = 3.10, 95% CI = 1.11–8.68) were slightly attenuated, but the significance of each of these associations remained (data not shown).

Adding adiponectin in a regression model assessing jointly both adiponectin and irisin as predictors of study outcomes revealed that higher circulating irisin levels were still significantly associated with MetS (OR = 7.22, 95% CI = 1.88–27.68) and elevated FPG (OR = 4.24, 95% CI = 1.23–14.61) (data not shown).

Table 4 presents associations between insulin resistance assessed by HOMA-IR and 10-year general CVD risk assessed by GFRP. Multiple linear regression analyses showed that irisin was independently and positively associated with HOMA-IR ($\beta = 0.003 \pm 0.001$, $P = .002$) and GFRP ($\beta = 0.002 \pm 0.000$, $P < .001$) after adjustment for age, gender, race, physical activity, vegetable and fruit consumption, smoking status, alcohol consumption, and BMI. Adiponectin was negatively and significantly associated with HOMA-IR and GFRP. These associations remained significant even after adjustment for BMI and mutually for hormones with other confounders in model 5. After adjustment for body fat mass or WC instead of BMI in multivariate linear regression models, the significant associations remained unchanged (data not shown).

After adding medication history of lipid-altering agents to multiple logistic and linear regression models to consider the possible impact of lipid-altering agents on irisin levels, the significance of these associations also remained unchanged (data not shown). These significant associations between irisin and MetS, HOMA-IR, and GFRP remained unchanged even after excluding subjects who were taking medications for diabetes, hypertension, and dyslipidemia.

Discussion

This study demonstrated that circulating irisin levels were higher in subjects with MetS and were independently associated with the odds of having MetS even after controlling for confounders including BMI. An additional novel finding is that irisin is also positively associated with insulin resistance and CVD risk. This study also replicated well-known negative associations between adiponectin and study outcomes.

Irisin is a recently identified novel hormone that has been proposed to play a significant role in energy homeo-

Table 3. OR (95% CI) by Multiple Logistic Regression Models for Metabolic Syndrome Components in Relation to Biomarkers[a]

	Irisin (n = 148)				Adiponectin (n = 146)			
	T1, n = 49	T2, n = 50	T3, n = 49	*P* for Trend	T1, n = 48	T2, n = 49	T3, n = 49	*P* for Trend
MetS, n	5/49	13/50	25/49		25/48	13/49	5/49	
Model 1	1	3.09 (1.01–9.48)	9.17 (3.11–27.0)	<.001	1	0.33 (0.14–0.78)	0.11 (0.04–0.31)	<.001
Model 2	1	2.69 (0.84–8.59)	9.80 (3.12–30.8)	<.001	1	0.26 (0.10–0.68)	0.07 (0.02–0.24)	<.001
Model 3	1	2.46 (0.67–9.02)	12.43 (3.42–45.2)	<.001	1	0.28 (0.10–0.78)	0.07 (0.02–0.28)	<.001
WC,[b] n	23/49	30/50	33/49		33/48	28/49	24/49	
Model 1	1	1.70 (0.76–3.76)	2.33 (1.03–5.29)	.04	1	0.61 (0.26–1.39)	0.44 (0.19–0.999)	.05
Model 2	1	1.16 (0.68–4.06)	2.78 (1.11–6.99)	.03	1	0.42 (0.16–1.11)	0.21 (0.07–0.59)	.003
Model 3	1	2.64 (0.28–24.5)	1.99 (0.22–17.7)	.58	1	0.54 (0.06–4.57)	0.27 (0.03–2.53)	.25
BP,[c] n	18/49	25/50	29/49		23/48	31/49	18/49	
Model 1	1	1.77 (0.77–3.84)	2.50 (1.11–5.63)	.03	1	1.87 (0.83–4.21)	0.63 (0.28–1.42)	.80
Model 2	1	1.49 (0.59–3.72)	3.15 (1.21–8.22)	.02	1	2.16 (0.84–5.58)	0.44 (0.16–1.21)	.12
Model 3	1	1.31 (0.47–3.63)	2.51 (0.91–6.88)	.07	1	3.12 (1.10–8.85)	0.70 (0.22–2.21)	.59
Glucose,[d] n	5/49	9/50	18/49		16/48	11/49	5/49	
Model 1	1	1.93 (0.60–6.24)	5.11 (1.71–15.2)	.002	1	0.58 (0.24–1.42)	0.23 (0.08–0.69)	.007
Model 2	1	2.12 (0.62–7.28)	6.04 (1.87–19.5)	.002	1	0.58 (0.22–1.52)	0.22 (0.07–0.71)	.01
Model 3	1	1.80 (0.50–6.46)	5.80 (1.72–19.6)	.003	1	0.65 (0.24–1.77)	0.28 (0.08–0.95)	.04
TG,[e] n	8/49	10/50	19/49		17/48	14/49	6/49	
Model 1	1	1.28 (0.46–3.58)	3.25 (1.25–8.4)	.001	1	0.73 (0.31–1.72)	0.25 (0.09–0.72)	.01
Model 2	1	1.19 (0.35–4.04)	4.23 (1.31–13.7)	.01	1	0.52 (0.18–1.48)	0.14 (0.04–0.55)	.005
Model 3	1	0.97 (0.27–3.24)	3.89 (1.16–13.0)	.02	1	0.55 (0.19–1.63)	0.18 (0.04–0.73)	.02
HDL,[f] n	9/49	18/50	23/49		26/48	14/49	8/49	
Model 1	1	2.5 (0.99–6.31)	3.93 (1.57–9.82)	.003	1	0.34 (0.15–0.78)	0.17 (0.06–0.43)	<.001
Model 2	1	2.96 (1.09–8.0)	4.82 (1.79–13.0)	.002	1	0.19 (0.07–0.50)	0.07 (0.02–0.24)	<.001
Model 3	1	2.75 (1.002–7.54)	4.56 (1.68–12.4)	.003	1	0.19 (0.07–0.53)	0.08 (0.02–0.27)	<.001

[a] Model 1 is unadjusted. Model 2 is adjusted for age, gender, race, smoking status, physical activity, vegetable or fruit consumption, and alcohol consumption. Model 3 is adjusted as for model 2 plus BMI. Tertile values of biomarkers are expressed as T1, T2, and T3. (For irisin [nanograms per milliliter]: T1, 72.19–157.09; T2, 157.1–209.00; T3, 209.01–749.34; and for adiponectin [micrograms per milliliter]: T1, 1.12–5.60; T2, 5.61–10.6; T3, 10.7–28.8).

[b] WC ≥102 cm for men and ≥88 cm for women.

[c] Systemic hypertension (BP ≥130/85 mm Hg) or antihypertensive therapy.

[d] Elevated FPG (≥100 mg/dL) or history of diabetes mellitus or taking antidiabetic medications.

[e] Elevated TG (≥150 mg/dL) or specific medication for elevated TG.

[f] Low HDL-cholesterol (men, <40 mg/dL; women, <50 mg/dL) or specific medication for low HDL.

stasis and obesity (11, 20, 21, 30–32). To date, no study has investigated associations between irisin and the risk of either MetS or CVD in humans. Irisin stimulates *UCP1* expression and activity in mice and induces browning of adipocytes in white adipose tissue depots, thereby promoting thermogenesis, increased total energy expenditure, and decreased obesity (11). Irisin overexpression in mice fed a high-fat diet resulted in improvement of hyperinsulinemia and glucose tolerance. In both animal and human studies, expression of PGC1α, a molecule upstream of irisin that is important in the conversion of white adipose tissue to brown adipose tissue (7), is increased after exercise (33, 34) and is also associated with aerobic performance (13). The regulation of irisin in humans as well as the role of irisin in glucose metabolism remains to be clarified, because studies published to date (12, 15–18) have reported conflicting results on the association between irisin levels and glucose levels, insulin levels, and insulin resistance. Its role in CVD risk remains totally unknown. Our results clearly indicate that baseline circulating irisin levels are significantly positively correlated with BMI as well as with both MetS components and insulin resistance as assessed by HOMA-IR (Table 2). Our findings are consistent with those of previous studies that have also shown a positive correlation between irisin and BMI (12, 16, 18), fasting blood glucose (12, 16, 18), TG (18), and diastolic BP (18) and a negative correlation between irisin and circulating HDL-cholesterol (12). Given the correlation of *FNDC5* mRNA expression or irisin levels with BMI, we also ran appropriate models to demonstrate that irisin is related to insulin resistance and the MetS even after adjusting for BMI or body fat mass. These associations were also independent from adiponectin. This indicates that irisin is associated with MetS independently from obesity and that this association can be mediated not only by increased BMI or adiposity (thus the attenuation of

Table 4. β-Regression Coefficients for Log-Transformed HOMA-IR and GFRP Relative to Irisin and Adiponectin[a]

	Irisin						Adiponectin					
	n	R^2	β	SE	Standardized β	P	n	R^2	β	SE	Standardized β	P
Log HOMA	136						134					
Model 1		0.10	0.005	0.001	0.33	<.001		0.15	−0.09	0.018	−0.40	<.001
Model 2		0.09	0.005	0.001	0.34	<.001		0.16	−0.10	0.019	−0.45	<.001
Model 3		0.25	0.004	0.001	0.26	.001		0.28	−0.07	0.019	−0.33	<.001
Model 4		0.29	0.003	0.001	0.24	.002		0.31	−0.07	0.019	−0.31	<.001
Model 5		0.33[b]	0.003[b]	0.001[b]	0.18[b]	.03[b]		0.33[c]	−0.06[c]	0.019[c]	−0.26[c]	.004[c]
Log GFRP	148						146					
Model 1		0.03	0.002	0.001	0.20	.02		0.10	−0.05	0.01	−0.33	<.001
Model 2		0.51	0.002	0.001	0.23	<.001		0.52	−0.04	0.009	−0.26	<.001
Model 3		0.54	0.002	0.001	0.20	.001		0.55	−0.03	0.009	−0.22	.001
Model 4		0.71	0.002	0.000	0.18	<.001		0.70	−0.02	0.007	−0.16	.003
Model 5		0.71[b]	0.001[b]	0.000[b]	0.14[b]	.004[b]		0.71[c]	−0.02[c]	0.007[c]	−0.12[c]	.03[c]

[a] Model 1 is unadjusted. Model 2 is adjusted for age, gender, and race. Model 3 is adjusted as for model 2 plus BMI. Model 4 is adjusted for model 3 plus physical activity, vegetable or fruit consumption, smoking status, and alcohol consumption. Model 5 is adjusted as for model 4 plus mutual adipomyokines as indicated below. R^2 represents the adjusted R^2 values of each model. The β-values are unstandardized regression coefficients, and standardized β-values are standardized regression coefficients.

[b] Adjusted for adiponectin.

[c] Adjusted for irisin.

effect estimates) but also by other mechanisms yet to be fully elucidated, including less efficient metabolism and/or direct effects on risk factors for MetS. Given the role of irisin to promote metabolism (11), we propose that our findings in humans reflect either an increased baseline secretion of irisin by the increased adipose and muscle tissue in obesity and/or a compensatory increase of irisin levels to combat obesity and the MetS and/or irisin resistance, similar in a way to the well-documented leptin resistance in obesity and the MetS (35). It is possible that the factors regulating irisin after exercise are different from those that regulate baseline irisin levels. Moreover, Boström et al (11) have suggested that moderate increase in circulating irisin after treatment may be causally related to improved energy homeostasis and glucose tolerance in mice. Thus, the results from our study indicate that, similar to the elevated insulin and leptin levels in insulin and leptin resistance associated with human obesity, irisin levels are also elevated and, similar to insulin or leptin, may play a compensatory role with respect to regulating energy expenditure and glucose metabolism in obese humans and/or that human obesity and the MetS may be irisin-resistant states. Alternatively, an elevated secretion by the increased muscle and fat mass as previously described in mice (36) and humans (12, 16) cannot be excluded, but this would not fully explain the association of irisin with many cardiometabolic variables, independent from BMI.

Importantly, we also found that irisin was associated with an increased 10-year risk of general CVD, as assessed by GFRP, after adjusting for other confounders. Considering the positive associations observed in this study between irisin and MetS components and between irisin and 10-year general CVD risk, these findings suggest that irisin levels may be used as an independent predictor of future risk of general CVD, apparently reflecting an underlying association with obesity and the MetS. The role of the magnitude of irisin changes after exercise in regulating these variables remains to be shown.

In the present study, we also replicate the finding that adiponectin is negatively correlated with obesity-related anthropometric measurements, MetS components such as glucose and TG, and HOMA-IR and was positively correlated with HDL as expected. These results are consistent with those from several previous studies (37, 38) regarding circulating adipokine levels.

The strength of our study is that this is the first study that evaluated the relationship of irisin to MetS and insulin resistance in humans and provided a more detailed analysis including MetS components and regression models adjusted for various anthropometric measurements such as BMI, WC, or body fat mass and mutually for adiponectin. Data presented herein demonstrate consistency in terms of the association between irisin and metabolic parameters as well as anthropometric measurements providing internal validity to the study. Another strength of the study is the fact that we did take into account numerous potential confounders such as lifestyle, racial-ethnic, and socioeconomic factors when performing our analyses. We also took into account various potential confounders such as total energy intake and medication history of lipid-altering agents that have not been reported here, and we restricted analyses only in the treatment-naive (diabetes,

hypertension, and dyslipidemia) group. We found that the significance of the results remained unaltered. Replication of expected associations between adiponectin and the study outcomes also provides validity in terms of the methodology used here.

Limitations of this study include the relatively small sample size, which, however, provided more than 80% power to detect significant associations at the $r > 0.20$ and the conventional $P = .05$ level. Because this study is not confined to specific age groups, gender, or race, the diversity of our population sample increases the generalizability of our results and increases the applicability of the associations observed here between irisin and metabolic pathways across populations. Future studies with larger sample sizes and examining irisin in various conditions and populations will be needed.

To minimize the limitations in terms of the measurement of dietary intake, physical activities, body composition, and insulin resistance, validated questionnaires (25–27) and assessment tools (22, 23, 28) that have been widely used in other large-scale epidemiologic studies were used in our study. To eliminate bias from laboratory sources, previously validated and specific assays were run blindly and under code by laboratory personnel who were not aware of the hypotheses underlying our studies. Moreover, any random laboratory error in hormonal and other assessments is a possibility, but random misclassification would have been expected to lead to depressed effect estimates, suppressing statistical significance toward the null and, thus, could not have materially altered the statistically significant results of this study. Although no previous study has evaluated CVD risk and MetS per se, two previous studies in humans have observed a negative association between circulating irisin levels and type 2 diabetes mellitus (15, 17) and a positive association with insulin sensitivity (15). These studies have used different assay kits, and it remains possible that differences in kits used and/or in the ethnic background of study subjects might have been responsible for differences in results. Given the data in our pilot assay evaluations, we used kits deemed most reliable as per our previously validated assay (12) used here, which is specific for the measurement of circulating irisin. Cross-validation of commercially available assays is needed before this field can move forward, but we are confident in our data on the basis of the assay kit validations we performed in our laboratory.

In summary, the results of this study demonstrate that irisin is associated with increased odds of having MetS and with increased risk of insulin resistance and 10-year risk of CVD. Furthermore, these results suggest that irisin may play a possible role in glucose metabolism. Whether the associations between high circulating irisin levels, MetS prevalence, and insulin resistance could be explained by a physiological compensatory mechanism that would result in increased irisin levels due to an underlying decreased sensitivity to irisin's effects (ie, obesity and MetS being irisin-resistant states, similar to insulin resistance) or another possible hypothesis such as increased secretion by the increased muscle and fat tissue in obesity, the potential mechanisms supporting these associations should be clarified by further mechanistic studies.

Acknowledgments

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This study was supported by National Institute on Aging Grant R01 AG032030 and National Institute of Diabetes and Digestive and Kidney Diseases Grant 81913. The project described was supported by Grant UL1 RR025758 to the Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health.

K.H.P. and C.S.M. designed the study, analyzed the data, interpreted the data, and wrote the first draft of the paper; K.H.P., L.Z., M.A.T., and C.S.M. contributed to the writing of the manuscript, the interpretation of data, and critical revision of the manuscript; L.Z., M.B., B.T., A.S., K.E.J., J.Y.H., F.D., E.V.G., C.R.D., and J.A.C. were involved in study recruitment and acquisition of the data. All authors were involved in the writing of the manuscript, reviewed the manuscript, and gave approval of the final manuscript.

Disclosure Summary: No potential conflicts of interest relevant to this article were reported. The authors have nothing to disclose.

References

1. **Reaven GM.** Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes*. 1988;37:1595–1607.
2. **Reaven GM.** Insulin resistance: the link between obesity and cardiovascular disease. *Med Clin North Am*. 2011;95:875–892.
3. **Lakka HM, Laaksonen DE, Lakka TA, et al.** The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709–2716.
4. **Carr DB, Utzschneider KM, Hull RL, et al.** Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes*. 2004;53:2087–2094.
5. **Mantzoros CS, Li T, Manson JE, Meigs JB, Hu FB.** Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes. *J Clin Endocrinol Metab*. 2005;90:4542–4548.
6. **Cinti S.** Between brown and white: novel aspects of adipocyte differentiation. *Ann Med*. 2011;43:104–115.
7. **Gesta S, Tseng YH, Kahn CR.** Developmental origin of fat: tracking obesity to its source. *Cell*. 2007;131:242–256.
8. **Stanford KI, Middelbeek RJ, Townsend KL, et al.** Brown adipose

tissue regulates glucose homeostasis and insulin sensitivity. *J Clin Invest*. 2013;123:215–223.
9. **Yang X, Enerbäck S, Smith U.** Reduced expression of FOXC2 and brown adipogenic genes in human subjects with insulin resistance. *Obes Res*. 2003;11:1182–1191.
10. **Lowell BB, S-Susulic V, Hamann A, et al.** Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature*. 1993;366:740–742.
11. **Boström P, Wu J, Jedrychowski MP, et al.** A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481:463–468.
12. **Huh JY, Panagiotou G, Mougios V, et al.** 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.
13. **Lecker SH, Zavin A, Cao P, et al.** Expression of the irisin precursor FNDC5 in skeletal muscle correlates with aerobic exercise performance in patients with heart failure. *Circ Heart Fail*. 2012;5:812–818.
14. **Roca-Rivada A, Castelao C, Senin LL, et al.** FNDC5/irisin is not only a myokine but also an adipokine. *PLoS One*. 2013;8:e60563.
15. **Moreno-Navarrete JM, Ortega F, Serrano M, et al.** 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–E778.
16. **Stengel A, Hofmann T, Goebel-Stengel M, Elbelt U, Kobelt P, 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.
17. **Choi YK, Kim MK, Bae KH, et al.** Serum irisin levels in new-onset type 2 diabetes. *Diabetes Res Clin Pract*. 2013;100:96–101.
18. **Liu JJ, Wong MD, Toy WC, et al.** Lower circulating irisin is associated with type 2 diabetes mellitus. *J Diabetes Complications*. 2013;27:365–369.
19. **Swick AG, Orena S, O'Connor A.** Irisin levels correlate with energy expenditure in a subgroup of humans with energy expenditure greater than predicted by fat free mass. *Metabolism*. 2013;62:1070–1073.
20. **Timmons JA, Baar K, Davidsen PK, Atherton PJ.** Is irisin a human exercise gene? *Nature*. 2012;488:E9–E10; discussion E10–E11.
21. **Fain JN, Company JM, Booth FW, et al.** Exercise training does not increase muscle FNDC5 protein or mRNA expression in pigs. *Metabolism*. 2013;62(10):1503–1511.
22. **Fakhrawi DH, Beeson L, Libanati C, et al.** Comparison of body composition by bioelectrical impedance and dual-energy x-ray absorptiometry in overweight/obese postmenopausal women. *J Clin Densitom*. 2009;12:238–244.
23. **Nichols J, Going S, Loftin M, Stewart D, Nowicki E, Pickrel J.** Comparison of two bioelectrical impedance analysis instruments for determining body composition in adolescent girls. *Int J Body Compos Res*. 2006;4:153–160.
24. **Grundy SM, Cleeman JI, Daniels SR, et al.** Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112:2735–2752.
25. **Ainsworth BE, Haskell WL, Herrmann SD, et al.** 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43:1575–1581.
26. **Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L.** A data-based approach to diet questionnaire design and testing. *Am J Epidemiol*. 1986;124:453–469.
27. **Block G, Woods M, Potosky A, Clifford C.** Validation of a self-administered diet history questionnaire using multiple diet records. *J Clin Epidemiol*. 1990;43:1327–1335.
28. **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.** Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412–419.
29. **D'Agostino RB Sr, Vasan RS, Pencina MJ, et al.** General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation*. 2008;117:743–753.
30. **Villarroya F.** Irisin, turning up the heat. *Cell Metab*. 2012;15:277–278.
31. **Wu J, Boström P, Sparks LM, et al.** Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell*. 2012;150:366–376.
32. **Polyzos SA, Kountouras J, Shields K, Mantzoros CS.** Irisin: A renaissance in metabolism? *Metabolism*. 2013;62(8):1037–1044.
33. **Baar K, Wende AR, Jones TE, et al.** Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J*. 2002;16:1879–1886.
34. **Goto M, Terada S, Kato M, et al.** cDNA Cloning and mRNA analysis of PGC-1 in epitrochlearis muscle in swimming-exercised rats. *Biochem Biophys Res Commun*. 2000;274:350–354.
35. **Moon HS, Dalamaga M, Kim SY, et al.** Leptin's role in lipodystrophic and nonlipodystrophic insulin-resistant and diabetic individuals. *Endocr Rev*. 2013;34:377–412.
36. **Roberts MD, Bayless DS, Company JM, et al.** Elevated skeletal muscle irisin precursor FNDC5 mRNA in obese OLETF rats. *Metabolism*. 2013;62:1052–1056.
37. **Izadi V, Farabad E, Azadbakht L.** Epidemiologic evidence on serum adiponectin level and lipid profile. *Int J Prev Med*. 2013;4:133–140.
38. **Yamamoto Y, Hirose H, Saito I, et al.** Correlation of the adipocyte-derived protein adiponectin with insulin resistance index and serum high-density lipoprotein-cholesterol, independent of body mass index, in the Japanese population. *Clin Sci (Lond)*. 2002;103:137–142.