Implication of Circulating Irisin Levels with Brown Adipose Tissue and Sarcopenia in Humans

Hae Yoon Choi, Sungeun Kim, Ji Woo Park, Nam Seok Lee, Soon Young Hwang, Joo Young Huh, Ho Cheol Hong, Hye Jin Yoo, Sei Hyun Baik, Byung-Soo Youn, Christos S. Mantzoros,* and Kyung Mook Choi*

Division of Endocrinology and Metabolism, Department of Internal Medicine, College of Medicine (H.Y.C., H.C.H., H.J.Y., S.Y.B., K.M.C.), Korea University, Seoul 136–701, Korea; Department of Nuclear Medicine, College of Medicine (S.K.), Korea University, Seoul 136–701, Korea; AdipoGen, Inc (J.W.P., N.S.L., B.-S.Y.), Incheon 406–840, Korea; Department of Biostatistics, College of Medicine (S.W.H.), Korea University, Seoul 136–701, Korea; and Section of Endocrinology (J.Y.H., C.S.M.), VA Boston Healthcare System, Harvard Medical School, Boston, Massachusetts 02130

Context: Irisin is an exercise-induced novel myokine that drives brown-fat-like conversion of white adipose tissue and has been suggested to be a promising target for the treatment of obesity-related metabolic disorders.

Objective: To assess the association of circulating irisin concentrations with brown adipose tissue (BAT) and/or sarcopenia in humans.

Setting and Design: We examined irisin levels in 40 BAT-positive and 40 BAT-negative women detected by ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}FDG-PET). In a separate study, we also examined 401 subjects with or without sarcopenia defined by skeletal muscle mass index (SMMI) and appendicular skeletal muscle (ASM)/height^2 using dual-energy x-ray absorptiometry.

Results: Among 6877 consecutive ^{18}FDG-PET scans in 4736 subjects, 146 subjects (3.1%) had positive BAT scans. The BAT-detectable group and the matched BAT-undetectable group did not differ in circulating irisin levels measured using two different ELISA kits ($P = .747$ and $P = .160$, respectively). Serum irisin levels were not different between individuals with sarcopenia and those without sarcopenia using either kit ($P = .305$ and $P = .569$, respectively). Also, serum irisin levels were not different between groups defined by ASM/height^2 using either kit ($P = .352$ and $P = .134$, respectively). Although visceral fat area and skeletal muscle mass showed significant difference according to tertiles of SMMI levels, irisin concentrations did not differ.

Conclusions: Circulating irisin levels were not different in individuals with detectable BAT or those with sarcopenia compared with control subjects and were not correlated with SMMI. ***(J Clin Endocrinol Metab* 99: 2778–2785, 2014)**

Regular exercise can lower the risk of chronic metabolic disorders and cardiovascular disease (1, 2). However, the exact molecular mechanisms by which exercise exerts its positive effects have not been fully identified. PPAR-γ coactivator-1 α (PGC-1α) is one of the most important metabolic modulators induced in muscle by ex-

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* C.S.M. and K.M.C. contributed equally to this work.

Abbreviations: ASM, appendicular skeletal muscle; baPWV, brachial-ankle pulse wave velocity; BAT, brown adipose tissue; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; CT, computed tomography; DXA, dual energy x-ray absorptiometry; ^{18}FDG-PET, ^{18}F-fluorodeoxyglucose positron emission tomography; FNDC5, fibronectin type III domain containing protein 5; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; KSOS, Korean Sarcopenic Obesity Study; LDL, low-density lipoprotein; PGC-1α, PPAR-γ coactivator-1 α; PWV, pulse wave velocity; SCFA, sc fat area; SMMI, skeletal muscle mass index; TFA, total fat area; UCP1, uncoupling protein 1; VFA, visceral fat area; WAT, white adipose tissue.

ercise. Increased muscle PGC-1α expression in mice protects against age-related insulin resistance and sarcopenia, a loss of skeletal muscle mass and strength (3). Furthermore, PGC-1α regulates the expression of uncoupling protein 1 (UCP1) and plays a role in thermogenesis in skeletal muscle and brown adipose tissue (BAT) (4).

Like adipose tissue, skeletal muscle has recently been recognized as an active endocrine organ releasing myokines, which may be responsible for the beneficial effects of exercise (5). Recently, Bostrom et al (6) found that PGC-1α expression in muscle of mice increases expression levels of the fibronectin type III domain containing protein 5 (FNDC5), a membrane protein that is cleaved and secreted as a novel myokine named irisin. Interestingly, irisin is induced by exercise, and stimulates UCP1 expression and browning of adipose tissue, which leads to the development of beige or brite adipocytes in murine adipose tissue (6). Furthermore, this effect was associated with increase in total body energy expenditure, which results in improved glucose tolerance and modest amounts of weight loss (6). Based on these results, Bostrom et al suggested that irisin is responsible for some of the beneficial effects of exercise and might be a promising target for the treatment of metabolic disorders. However, the exercise effects and metabolic influence of irisin in humans have not been fully established yet. Whereas studies on mice shows that exercise-induced irisin may stimulate browning of white adipose tissue (6–8), five well-conducted exercise intervention studies have shown that long-term exercise does not increase circulating irisin levels in humans (9–13).

The existence of BAT in human adults was recently confirmed in several landmark studies using ^{18}F-fluorodeoxyglucose positron emission tomography (PET)/computed tomography (CT) (^{18}FDG-PET/CT) scans and biopsies (14, 15). Human BAT shows gene expression profiles that are more similar to murine beige adipocytes than classical brown adipocytes (16). Although irisin results in brown-fat-like conversion of white adipose tissue (WAT) in mice, to the best of our knowledge, there have been no reports associating irisin with BAT in humans. In addition, whereas physical inactivity and muscle disuse may lead to adverse myokine patterns accompanied by loss of muscle mass, there are only limited data regarding the possible association between irisin and sarcopenia.

In the present study, to identify functional evidence on a potential role for circulating irisin in vivo, we first evaluated irisin levels in individuals with BAT detected using ^{18}FDG-PET/CT scans compared with individuals without BAT matched for age, sex, and season/y of ^{18}FDG-PET/CT scanning. Then, we also examined irisin concentrations in individuals with sarcopenia and explored the relationships between irisin, skeletal muscle mass, and cardiometabolic risk profiles in Asian men and women.

Materials and Methods

Detection of brown adipose tissue by ^{18}F-FDG PET/CT imaging

A total of 6877 consecutive PET/CT scans were evaluated from January 2008 to December 2010 at Korea University Guro Hospital. All PET/CT scans were performed using the Gemini TF 16-Slice PET/CT scanner (Philips Medical Systems), a new high-performance, time-of-flight–capable, fully three-dimensional PET scanner using lutetium-yttrium oxyorthosilicate crystals. After at least 12 h of fasting, ^{18}F-FDG (370–550 MBq) was injected by iv, and then patients rested in a quiet room for 60 min. A whole-body PET image (below cerebellum to inguinal) was acquired for 10 min (1 min per bed), and PET image analysis was performed at a dedicated workstation (Extended Brilliance Workspace 3.5 with PET/CT viewer for automated image registration, Philips). The temperature in the PET scanning room was constantly regulated at 22°C.

Patients were considered to have ^{18}F-FDG BAT when the following criteria were met: 1) ^{18}F-FDG uptake was in the cervical/supraclavicular, mediastinal, paravertebral, and/or perirenal areas; 2) ^{18}F-FDG uptake had a maximum standardized uptake value 2.0 g/mL or greater (an indicator of (^{18}F-FDG uptake intensity); and 3) the tissue corresponded to the density of adipose tissue on CT (−250−−50 Hounsfield units) (17).

A total of 146 subjects out of 4736 subjects (3.1%) tested positive for BAT (Supplemental Figure 1). BAT-positive patients were screened, and potentially eligible individuals were invited to attend a face-to-face assessment to confirm eligibility. This study included only women because of a higher prevalence of BAT among women, the more active participation of women, and concerns regarding potential confounding effects of sex. Participants who had histories of cardiovascular disease (myocardial infarction), unstable angina, stroke, peripheral artery disease, or cardiovascular revascularization), diabetes, stage 2 hypertension (resting blood pressure (BP) ≥160/100 mm Hg), or severe renal or hepatic disease were excluded. Forty out of 146 BAT-positive subjects were enrolled according to the inclusion and exclusion criteria. Forty BAT-negative subjects were then enrolled as controls and were matched with the BAT-positive subjects based on age, sex, and season/y of PET/CT scanning.

Study participants in the sarcopenia cohort

Study subjects were drawn from participants in the Korean Sarcopenic Obesity Study (KSOS), an ongoing prospective, observational, cohort study. The details of the study design and objectives have been published previously (18, 19). Participants in this study include healthy volunteers residing in Seoul, South Korea. None of the participants had histories of cardiovascular disease (myocardial infoarction, unstable angina, stroke, or cardiovascular revascularization), stage 2 hypertension (resting BP, ≥160/100 mm Hg), malignancy, or severe renal or hepatic disease. In the present study, the KSOS cohort of adults greater than 20 years of age for the period of 2009–2011 consisted of 401 subjects (149 men and 252 women). All participants provided written informed consent, and the Korea University Institutional

Review Board, in accordance with the Declaration of Helsinki of the World Medical Association, approved this study protocol.

Anthropometric and laboratory measurements

Body mass index (BMI) was calculated as weight/height^2 (kg/m^2), and waist circumference was measured at the midpoint between the lower border of the rib cage and iliac crest. All blood samples were obtained in the morning after a 12-hour overnight fast and were immediately stored at −80°C for subsequent assays. Serum triglyceride, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol levels were determined enzymatically using a chemistry analyzer (Hitachi 747). A glucose oxidase method was employed to measure plasma glucose and high-sensitivity C-reactive protein (CRP) (hsCRP) levels were determined via a chemiluminescence immunoassay (Beckman Coulter).

Measurement of irisin levels

Circulating irisin levels were measured using both a previously validated Aviscera ELISA kit (now provided as cat#EK-067–52 at Phoenix Pharmaceuticals; intra-CV = 0.6∼4.8%, inter-CV = 8.0∼10.0%; detectable range = 0.066∼1024 ng/mL) and Adipogen ELISA kit (cat# AG-45A-0046EK-KI01; intra-CV = 6.9 ± 1.3%, inter-CV = 9.0 ± 0.8%; detectable range = 0.001∼5 μg/mL). In the sarcopenia study, Aviscera ELISA kit was used in 100 subjects with sarcopenia and 100 age- and sex-matched controls selected randomly to confirm or refute data derived using the Adipogen ELISA kit, whereas Adipogen ELISA kit was used in the whole study group.

Assessment of body composition

Body compositional analysis was performed using dual energy x-ray absorptiometry (DXA) and CT. A whole-body DXA scan was performed for each patient to measure total and regional lean mass (g), total body fat (g), and total body fat percentage (%) using fan-beam technology (Hologic Discovery A, Hologic). Appendicular skeletal muscle mass (ASM (kg)) and skeletal muscle mass index (SMMI (%): total skeletal muscle mass (kg)/weight (kg) × 100) were obtained as previously described (18, 19). The abdominal adipose tissue area was quantified using CT (Brilliance 64, Philips Medical Systems). Visceral fat area (VFA), total abdominal fat area (TFA), and sc fat area (SCFA) were calculated from a CT slice scan image, which was obtained between the fourth and fifth lumbar vertebrae during suspended respiration. Fat attenuation was determined by measuring the mean value of the pixels within a range of −190—−30 Hounsfield units.

Definitions and cut-off points for sarcopenia

Sarcopenia was defined as an SMMI (skeletal muscle mass index) of 2 SD below the sex-specific mean value for a younger reference group (20). For men, the cutoff value for sarcopenia was 37.46%, defined as less than 2 SD below the sex-specific normal mean for the young reference group. For women, the corresponding limits were 32.67% (18). Sarcopenia was also defined as an ASM/height^2 of 2 SD below the sex-specific mean value for a younger reference group (21). The sex-specific cut-off points of ASM/height^2 were 8.16 kg/m^2 in men and 6.21 kg/m^2 in women.

Measurement of brachial-ankle pulse wave velocity for analysis of arterial stiffness

After a subject rested in the supine position for 5 minutes, the brachial-ankle pulse wave velocity (baPWV) was measured using a BP-203RPE II volume-plethymographic apparatus (Colin), which simultaneously records the baPWV and the brachial and ankle blood pressures on the left and right sides. The baPWV was calculated as the mean of the left and right baPWV values.

Statistical analyses

Each variable was assessed for a normal distribution. Data are expressed as mean ± SD or median (interquartile range [25–75%]). Differences between groups were tested using an independent two-sample t test or Mann-Whitney U test for continuous variables, and the χ^2 test was used to test for differences in the distribution of categorical variables. Multiple stepwise regression analyses with circulating irisin levels as the dependent variable was performed to identify independently associated factors in the study subjects. Age, sex, BMI, waist circumference, systolic and diastolic BP, VFA, SCFA, fasting plasma glucose, total cholesterol, triglycerides, HDL cholesterol, hsCRP, ASM, and SMMI were analyzed as covariates. All statistical results were based on two-sided tests. Differences between tertile groups were tested using one-way ANOVA, Kruskal-Wallis H-test for continuous variables, and Fisher's exact test or Pearson's χ^2 test for categorical variables. Data were analyzed using SAS 9.2 (SAS Institute). P values $< .05$ were considered statistically significant.

Results

Characteristics of the study subjects with or without brown adipose tissue detected by ^{18}FDG-PET/CT

The BAT-positive group and the matched BAT-negative group did not differ significantly regarding anthropometric and metabolic profiles (Table 1, Supplemental Figure 1). In particular, there were no significant differences in VFA or SCFA between BAT-positive and BAT-negative groups, nor was there a significant difference in hsCRP and pulse wave velocity (PWV) levels between the two groups. Importantly, circulating irisin levels measured using two kinds of irisin ELISA kit showed no differences in subjects with BAT detected using ^{18}FDG-PET/CT compared with those without BAT (969.0 [861.1–1078.2] vs. 979.0 [808.0–1128.7] ng/mL, P = .747 (Adipogen); 56.7 [50.5, 63.9] vs. 60.2 [55.1, 63.6] ng/mL, P = .160 (Aviscera); Figure 1A).

Clinical and laboratory characteristics of the study subjects with or without sarcopenia

The clinical and biochemical characteristics of the study subjects in the sarcopenia cohort are presented in Table 2. The prevalence of sarcopenia in the entire sample was 31.4% and was lower in men than in women (13.4% and 42.1%, respectively). Subjects with sarcopenia were

Table 1. Clinical and Laboratory Characteristics of the Study Subjects with or without BAT Detected by ^{18}FDG-PET/CT

Characteristic	BAT Negative	BAT Positive	*P* Value
N	40	40	
Age, y	42 (35, 44)	40 (36, 42)	.307
Body weight, kg	55.2 (50.0, 61.2)	55.7 (49.3, 61.2)	.966
BMI, kg/m^2	21.9 (19.8, 24.5)	21.91 (20.42, 23.8)	.799
Waist circumference, cm	70.0 (67.0, 76.5)	74.0 (68.0, 77.0)	.188
Systolic BP, mm Hg	111.0 ± 12.0	114.5 ± 13.3	.221
Diastolic BP, mm Hg	71.5 ± 9.4	74.1 ± 13.3	.320
Glucose, mmol/L	4.6 (4.2, 5.2)	4.7 (4.4, 5.1)	.368
Insulin, μU/L	8.4 (5.7, 12.2)	9.1 (7.4, 11.8)	.273
Total cholesterol, mmol/L	4.1 ± 0.9	3.9 ± 0.9	.369
Triglycerides, mmol/L	0.8 (0.6, 1.1)	0.9 (0.6, 1.4)	.242
HDL cholesterol, mmol/L	1.3 ± 0.3	1.2 ± 0.3	.235
LDL cholesterol, mmol/L	2.4 ± 0.7	2.2 ± 0.7	.207
hsCRP, mg/L	0.5 (0.3, 0.7)	0.4 (0.3, 1.0)	.697
Mean PWV, cm/sec	1132 (1052, 1228)	1214 (1122, 1326)	.018
VFA, cm^2	72.5 (53.8, 90.3)	71.1 (47.3, 89.0)	.855
SCFA, cm^2	169.1 (134.0, 219.7)	197.5 (162.7, 227.8)	.107

older and more likely to be female, had higher body weight, BMI, waist circumference, BP, fasting plasma glucose, total cholesterol, triglycerides, LDL cholesterol, and hsCRP compared with subjects without sarcopenia. Furthermore, VFA, SCFA, and PWV were higher, whereas SMMI and ASM/height^2 values were significantly lower in subjects with sarcopenia compared with those without sarcopenia. However, circulating irisin levels were not different between groups by both ELISA kits (951.1 [770.6–1141.8] vs. 1003.0 [761.3–1211.7] ng/mL, $P = .305$ (Adipogen); 117.4 [96.9, 138.6] vs. 120.0 [102.3, 142.1] ng/mL, $P = .569$ (Aviscera); Figure 1B). Also, serum irisin levels were not different between individuals with sarcopenia and those without sarcopenia defined by ASM/height^2 by both kits (1056.6 [676.0–1236.1] vs. 959.5 [770.1–1160.0] ng/mL, $P = .352$ (Adipogen); 116.4 [88.2–137.2] vs. 122.7 [103.3–142.1] ng/mL, $P = .134$ (Aviscera)). Although irisin levels measured using Adipogen ELISA kit were significantly correlated with those measured using Aviscera ELISA kit ($r = 0.219$, $P = .002$, Supplemental Figure 2), the magnitude of correlation is relatively low and absolute values were quite different.

Correlation analysis between SMMI and irisin levels

The SMMI was negatively correlated with BMI ($P < .001$), systolic BP ($P = .032$), VFA ($P < .001$), SCFA ($P < .001$), TFA ($P < .001$), total cholesterol ($P = .017$), and LDL cholesterol ($P = .002$). Importantly, correlation analysis adjusted for age and sex showed that SMMI was not correlated with circulating irisin level ($r = 0.05$, $P = .358$). Moreover, in multiple stepwise regression analyses, irisin concentrations measured using Adipogen ELISA kit were associated with fasting plasma glucose ($\beta = -1.566$) and total cholesterol levels ($\beta = -1.678$), whereas irisin levels measured using Aviscera ELISA kit were associated with age ($\beta = -1.044$), diastolic BP ($\beta = 0.460$), and VFA ($\beta = 0.153$), but not with SMMI.

Anthropometric and clinical characteristics according to SMMI

Table 3 presents clinical and laboratory variables stratified by SMMI level tertiles. Body weight, BMI, waist circumference, BP, total cholesterol, LDL cholesterol, and PWV levels were significantly different with increasing SMMI levels. Furthermore, VFA and SCFA levels were decreased, whereas ASM and ASM/height^2 were increased according to increment of SMMI tertiles. However, irisin levels measured using two kinds of ELISA kit did not differ ($P = .303$ and $P = .357$, respectively).

Discussion

Skeletal muscle has recently been identified as an active endocrine organ, secreting myokines, which may communicate with other organs to mediate the beneficial effects of exercise on systemic metabolism. Exogenous administration of irisin in the mouse induces the expressions of UCP1 and other BAT-associated genes through increased PPAR-α expression (6). Moreover, irisin directly communicates with WAT and induces browning, which results in the increase of systemic energy expenditure and improvement of metabolic profiles (6). These results support that irisin might be a novel, promising target for the treatment of chronic metabolic disorders. Interestingly, Roca-Rivada et al (22) recently reported that visceral adipose tissue and especially sc adipose tissue also express and secrete

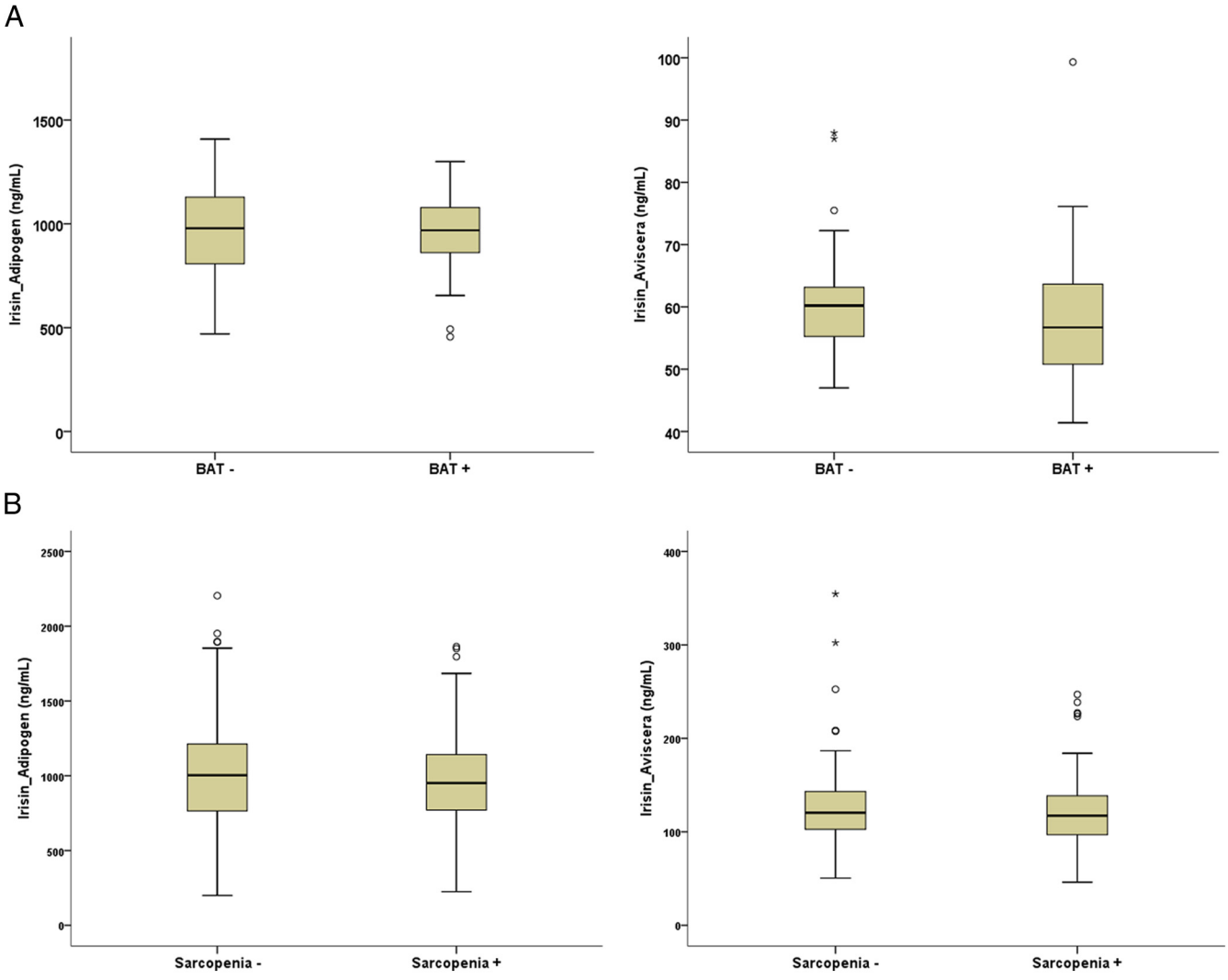

Figure 1. Circulating irisin levels measured using both Aviscera and Adipogen ELISA kit according to the presence of brown adipose tissue (BAT) (A) and sarcopenia (B) in humans.

FNDC5/irisin in rat adipocytes and human adipose tissue. Therefore, they suggested that FNDC5/irisin is not only a myokine but is also an adipokine (22). Considering that 72% of circulating FNDC5/irisin is attributed to muscle secretion, the regulatory feedback mechanism between muscle and adipose tissue may be critical. However, the impacts of irisin on anthropometric and metabolic phenotypes in humans are controversial. Although several studies have reported that circulating irisin levels are positively correlated with BMI (9, 23), some studies have reported null (24) or even negative correlations (25). The present study, including a relatively large sample of Asian men and women, found that circulating irisin levels were not associated with BMI or waist circumference in multiple regression analyses. Lately, Huh et al (9) reported very low mRNA expression of FNDC5 in human fat compared with muscle using a TissueScan qPCR array.

In the initial report, irisin was shown to stimulate the formation of brown-like adipocytes, the beige adipocytes, which have a unique gene expression signature in a murine model (6). Zhang et al (8) also demonstrated that recombinant irisin stimulates browning of white adipocytes through MAPK p38 MAP kinase and ERK MAP kinase signaling in mice. Although classic brown adipocytes have pronounced basal expression of UCP1, beige adipocytes show basal expression of UCP1 similar to that of WAT. However, after stimulation, the expression of UCP1 in beige adipocytes reaches the same high level as that seen in BAT (26). Previously, BAT was thought to be lost after infancy in humans. However, recent studies found persistence of functional BAT in human adults using ^{18}FDG-PET/CT and biopsy (14, 15). Cypess et al (27) reported that human BAT share many similarities in gene expression profiling with classical rodent BAT. Jespersen et al (28) demonstrated a coexistence of classical BAT and brite/beige adipocytes in human BAT. Biopsies of human BAT contain mRNA of genes characteristic of beige cells, such as CD137 and T-box1, suggest that human BAT re-

Table 2. Clinical and Laboratory Characteristics of the Study Subjects with or without Sarcopenia

Characteristic	Sarcopenia	No Sarcopenia	*P* Value
N	126	275	
Age, y	61 (54, 67)	52 (40, 63)	<.001
Sex, M/F	20/106	129/146	<.001
Body weight, kg	64.3 (58.0, 72.7)	61.0 (54.0, 70.0)	<.001
BMI, kg/m^2	26.4 (24.3, 28.9)	23.2 (21.4, 24.9)	<.001
Waist circumference, cm	85 (80, 93)	80 (74, 87)	<.001
Systolic BP, mm Hg	127.0 ± 16.0	121.7 ± 14.5	.001
Diastolic BP, mm Hg	80.4 ± 10.7	78.1 ± 10.4	.047
Glucose, mmol/L	4.4 (3.8, 5.1)	4.1 (3.6, 4.7)	.004
Total cholesterol, mmol/L	4.1 ± 0.9	3.8 ± 1.0	.013
Triglycerides, mmol/L	1.0 (0.7, 1.4)	0.8 (0.6, 1.3)	.044
HDL cholesterol, mmol/L	1.1 (0.9, 1.3)	1.0 (0.9, 1.3)	.480
LDL cholesterol, mmol/L	2.5 ± 0.8	2.2 ± 0.7	.007
hsCRP, mg/L	0.6 (0.3, 1.1)	0.4 (0.2, 0.8)	<.001
Mean PWV, cm/sec	1422 (1208, 1577)	1314 (1170, 1481)	.010
VFA, cm^2	145.0 (106.0, 190.9)	100.3 (66.8, 141.3)	<.001
SCFA, cm^2	244.3 (204.9, 297.7)	151.0 (115.1, 191.0)	<.001
TFA, cm^2	402.5 (337.5, 450.8)	265.6 (208.6, 312.8)	<.001
ASM, kg	17.4 (15.7,19.8)	20.8 (17.5, 25.3)	<.001
ASM/height^2, kg/m^2	7.2 (6.2, 8.0)	8.0 (6.9, 9.1)	<.001
SMMI, %	30.9 (29.7, 32.2)	38.9 (35.6, 42.2)	<.001

sembles murine brite/beige adipocytes more closely than classical brown adipocytes (16). In the present study, we first showed that circulating irisin levels did not differ between individuals with BAT and without BAT using ^{18}FDG-PET/CT. These results may suggest that there is an essential difference between rodent and human thermogenesis. Most energy expenditure is funneled to BAT in mice housed under nonthermal neutral condition, which does not usually occur under physiologic conditions in humans (29). On the other hand, in a study using metabolic chamber, plasma irisin levels were not correlated with 24-hour energy expenditure in 17 postmenopausal women (30). Furthermore, Norheim et al (12) reported no correlation between circulating irisin and UCP1 mRNA expression in sc adipose tissue in humans. Raschke et al (31) found no effect of recombinant irisin or FNDC5 on browning of primary human adipocytes. Based on these results, they concluded that the beneficial effect of irisin observed in mice is unlikely to be translated to humans (31). Further

Table 3. Anthropometric and Clinical Characteristics According to SMMI Tertiles

Characteristic	1st Tertile	2nd Tertile	3rd Tertile	*P* Value
N	133	134	134	
SMMI, %	31.1 (29.8, 32.3)	36.3 (35.2, 37.6)	42.3 (40.6, 43.9)	<.001
Age, y	61.0 (54.0, 66.0)	49.5 (38.0, 64.0)	50.5 (40.0, 62.0)	<.001
Weight, kg	61.0 (56.4, 68.0)	58.1 (52.0, 67.0)	67.5 (60.0, 73.6)	<.001
BMI, kg/m^2	25.7 (23.6, 27.9)	23.1 (21.2, 25.3)	23.3 (21.7, 25.2)	<.001
Waist circumference, cm	84 (78, 88)	77 (73, 87)	84 (78, 89)	<.001
SBP, mm Hg	126 (116, 135)	119.5 (109, 132)	124 (114, 133)	.005
DBP, mm Hg	79.3 ± 10.1	75.75 ± 10.9	81.47 ± 9.9	<.001
Glucose, mmol/L	4.3 (3.8, 4.9)	4.1 (3.5, 4.7)	4.1 (3.7, 4.7)	.154
Total cholesterol, mmol/L	4.1 ± 0.9	3.8 ± 0.9	3.8 ± 1.0	.005
Triglyceride, mmol/L	1.0 (0.7, 1.2)	0.8 (0.6, 1.2)	0.8 (0.5, 1.3)	.395
HDL cholesterol, mmol/L	1.1 (0.9, 1.3)	1.0 (0.9, 1.3)	1.0 (0.9, 1.3)	.372
LDL cholesterol, mmol/L	2.5 ± 0.8	2.3 ± 0.7	2.2 ± 0.8	<.001
hsCRP, mg/L	0.5 (0.3, 1.0)	0.4 (0.2, 0.9)	0.4 (0.2, 0.9)	.119
Mean PWV, cm/sec	1424 (1209, 1580)	1258 (1116, 1449)	1337 (1201, 1506)	.001
VFA, cm^2	126.9 (99.5, 168.8)	95.2 (63.8, 149.2)	113.0 (70.6, 147.8)	<.001
SCFA, cm^2	242.8 (205.8, 297.0)	176.7 (141.6, 202.4)	125.0 (98.3, 154.0)	<.001
TFA, cm^2	383.0 (320.5, 437.7)	277.5 (229.0, 344.2)	238.6 (190.1, 300.7)	<.001
ASM, kg	16.6 (15.6, 18.7)	18.5 (16.8, 21.4)	25.1 (22.2, 27.3)	<.001
ASM/height^2, kg/m^2	6.8 (6.1, 7.6)	7.3 (6.6, 8.2)	8.9 (8.1, 9.6)	<0.001
Irisin, Adipogen, ng/mL	939.7 (761.3, 1128.6)	1007.6 (770.1, 1216.9)	1029.2 (770.1, 1211.3)	.303
Irisin, Aviscera, ng/mL	116.2 (94.0, 137.3)	119.1 (99.2, 144.8)	124.2 (110.4, 144.4)	.357

studies to evaluate the role of irisin on browning of WAT or BAT in humans are warranted.

Physical inactivity and muscle disuse leads to loss of muscle mass (sarcopenia) and the accumulation of visceral adipose tissue, which in turn promotes the development of insulin resistance, type 2 diabetes, and atherosclerosis (32, 33). Bostrom et al (6) showed that endurance training for 10 weeks caused increases in mRNA levels of FNDC5 in skeletal muscle and circulating irisin levels in obese individuals. However, five exercise intervention studies did not confirm long-term exercise-induced increase in circulating irisin levels (9–13). In healthy women, circulating irisin levels had positive associations with biceps circumference (used as a surrogate marker of muscle mass) (9). Stengel et al (34) showed that circulating irisin concentrations were positively correlated with fat-free mass using a bioelectrical impedance analyzer. However, these studies included relatively small numbers of Caucasian subjects and did not use clinical criteria to identify sarcopenia. Moreover, although bioelectrical impedance analyzer is a quick, noninvasive method for measuring body composition, its reliability has been called into question according to individual's hydration status, ethnicity, physical fitness, and age (35). In the present study, which included relatively large number of Asian subjects and the standard definition of sarcopenia measured by DXA, which is the current gold standard method for the measurement of muscle mass, there was no significant difference in serum irisin levels between subjects with and without sarcopenia. Moreover, SMMI was not significantly associated with circulating irisin levels measured using two kinds of ELISA kits.

Irisin concentrations have been reported to be lower in patients with type 2 diabetes compared with normal controls (23, 25). Moreno-Navarrete et al (23) reported decreased FNDC5 expression in adipose tissue and muscle from obese and type 2 diabetic subjects. Furthermore, Kurdiova et al (11) found that FNDC5 in adipose tissue and plasma irisin levels were reduced in patients with type 2 diabetes. In agreement with those results, a multiple regression analysis showed that fasting plasma glucose levels were associated with irisin levels in the present study, although we excluded subjects with type 2 diabetes. In addition, circulating irisin concentrations were negatively correlated with total cholesterol levels in this study. Huh et al (9) also found that circulating irisin levels were inversely correlated with total and HDL cholesterol. On the other hand, Choi et al (25) showed that serum irisin was significantly negatively correlated with 2-hour plasma glucose, HbA1c, and triglycerides. However, Park et al (36) reported that irisin concentrations were positively associated with components of metabolic syndrome including fasting plasma glucose and triglycerides. Discrepancies in relationship between circulating irisin levels and metabolic parameters may need additional research using fully validated assays.

There are some limitations to this study. First, because it was a cross-sectional study, no causality could be identified. Second, we enrolled only relatively healthy Asian men and women, so the results of the current study should be further evaluated in other ethnic populations. Last, ELISA kits used in this study showed only a weak correlation and considerable difference in absolute values. Additional research for comparison and validation of commercial irisin ELISA kits is warranted. However, the two assays used herein, including the previously validated Aviscera kit, which has been applied in most previous studies and suggested as an appropriate ELISA method (37), showed the same primary outcomes in our study.

In conclusion, the present study demonstrated that circulating irisin concentrations were not different in individuals with BAT or with sarcopenia compared with control subjects. Furthermore, SMMI measured using DXA were not associated with irisin levels in Asian men and women. Further studies might be needed to explore the role of irisin on human body composition and metabolic phenotypes.

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Address all correspondence and requests for reprints to: Dr. Kyung Mook Choi, Division of Endocrinology and Metabolism, Department of Internal Medicine, Korea University Guro Hospital, 80 Guro-Dong, Guro-Gu, Seoul 152–050, Korea. E-mail: medica7@gmail.com.

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