Irisin is a recently identified exercise-induced myokine. However, the circulating levels of irisin in response to different types of exercise in subjects with metabolic syndrome are unknown.

Objective: This study aimed to study the levels of irisin in healthy males and subjects with metabolic syndrome at baseline and in response to exercise.

Design: Each individual completed high-intensity interval exercise (HIIE), continuous moderate-intensity exercise (CME), and resistance exercise (RE) sessions in a random, crossover design. Percentage change in circulating irisin levels was examined. Two different irisin assays were used to compare the results of the RE study.

Results: Circulating irisin increased immediately after HIIE, CME, and RE and declined 1 hour later. The increase was greater in response to resistance compared with either high-intensity intermittent exercise or CME. Change in irisin in response to exercise did not differ between individuals with and without metabolic syndrome.

Conclusions: Exercise is able to increase circulating irisin levels in individuals with the metabolic syndrome as well as healthy individuals. Whether this increase may contribute to the beneficial effects of exercise on patients with the metabolic syndrome remains to be studied further.
healthy individuals. Therefore, the comparison of different types of exercise in different populations, especially in subjects with metabolic diseases, is warranted. In this study, we have compared the effects of high-intensity interval exercise (HIIE), continuous moderate-intensity exercise (CME), and resistance exercise (RE) on circulating irisin levels. Moreover, a pilot study comparing healthy males and subjects with metabolic syndrome has been conducted.

Research Design and Methods

Subjects and exercise protocols

Twenty sedentary men were divided into healthy individuals (n = 14) and subjects with metabolic syndrome (n = 6) according to the National Heart, Lung, and Blood Institute/American Heart Association criteria, that is, the presence of at least 3 of the following: central obesity (waist circumference >102 cm), high circulating fasting triacylglycerol (≥150 mg/dL), low high-density lipoprotein cholesterol (<40 mg/dL), high fasting glucose (≥100 mg/dL), high blood pressure (systolic ≥130 mm Hg and/or diastolic ≥85 mm Hg) (17). Inclusion criteria were male sex, age 30–50 years, sedentary lifestyle (defined as performing <2 h/wk of regular exercise), and being healthy or with metabolic syndrome depending on group. Exclusion criteria included contraindication for exercising, acute or chronic disease, use of medication or dietary supplements, smoking, and diet change in weight (>2 kg within the last 6 mo). Body fat was measured by bioelectrical impedance analysis using the Bodystat 1500 (Limited, Douglas). Preliminary tests included determination of maximal oxygen uptake (VO2max) and maximum heart rate (HRmax) through a maximal incremental exercise test on a treadmill (h/p/cosmos mercury) at 6% grade and stable environment conditions (26–27°C, 50–60% humidity). Briefly, after a 4-minute warmup by walking at 3 km/h, speed was increased by 1 km/h every 2 minutes. Expiratory gases were collected (quark CPET, Cosmed), and heart rate was monitored continuously. The test was terminated at exhaustion. With respect to muscular strength, because it is not suggested for novice lifters to perform a 1-repetition maximum (1-RM) strength assessment (due to increased risk for injury), 1-RMs were predicted after 7–10-RM tests (21).

Participants completed four sessions in a random, crossover design: 1) no exercise (Rest); 2) HIIE (5 × 4 minutes walking on a treadmill at 3 km/h alternating with 4 × 4 minutes running at 90% HRmax, for a total of 36 min); 3) CME (36 min walking/running on a treadmill at 65% HRmax); and 4) RE (three sets of 8–12 repetitions at 75–80% of 1-RM for six types of exercise targeting all main muscle groups; ie, leg extension, chest press, leg curl, lat. pull down, leg press, biceps; ~2 min per set; total duration, 45 min). Blood was collected 1 hour before the end of exercise, immediately after, and 1 hour after each session for plasma preparation. All participants were informed about the details and potential risks of the experimental procedure after which they provided written consent. The study was approved by the Institutional Review Board at Aristotle University of Thessaloniki in accordance with the Declaration of Helsinki.

Biochemical measurements

Irisin was measured using a previously validated ELISA kit (No. EK-067–52; Phoenix Pharmaceuticals) as described (16). Plasma glucose, triglycerides, total cholesterol, lactate, and creatine kinase (CK) were measured using an automated analyzer (Hitachi cobas c311; Roche Diagnostics). Percentage change in irisin, lactate, and CK was calculated based on the pre-exercise value in each session.

Comparison of two irisin assay

Given that there is an ongoing debate on different results from different irisin assays, we have compared two of the most used ELISA kits regarding the irisin response to exercise. RE samples were also analyzed with a newly developed irisin kit from the same company (No. EK-067–29l; Phoenix Pharmaceuticals) for comparison. The sensitivity of the assay was 1 ng/mL and the linear range of the standard curve was 1–50 ng/mL. Intra- and interassay variations were <10% and <15%, respectively. The samples were diluted 1/40 with assay buffer so that the data would be in the linear range of the standard curve.

Statistical analysis

Data are expressed as means ± SE unless stated otherwise. Bio-marker concentrations that were not normally distributed (according to the Shapiro-Wilk test) were logarithmically transformed. Baseline differences between groups in anthropometric and biochemical characteristics were determined with Student t or Mann-Whitney U test, as appropriate. Repeated-measures ANOVA was performed to compare the dependent variables over time and to determine the interaction between time, session, and group factors. Paired t test or Wilcoxon signed rank test was used to compare the differences in percentage changes in irisin, lactate, and CK levels at the same time point. Spearman’s correlation coefficients were calculated to correlate irisin concentration between two irisin assays. Analyses were performed with the SPSS, and P < .05 was considered significant.

Results

Biochemical responses to different exercise types in healthy individuals and individuals with metabolic syndrome

Characteristics of the participants are summarized in Table 1. There were no differences in age, waist circumference, body mass index, or percent body fat between groups, but plasma triglycerides and glucose levels were significantly higher in subjects with metabolic syndrome compared with healthy subjects. Circulating irisin levels were significantly different over time (P < .001). There was a significant interaction between exercise session and time with regard to irisin concentration (P < .001). In contrast, neither two-factor (time × group) nor three-factor (time × group × session) ANOVA with repeated measures over time showed significant differences (P = .41 and .97, respectively), implying that exercise-induced irisin response was similar between subjects with and without
metabolic syndrome. Comparison among exercise regimens in terms of percentage change revealed that RE, HIIE, and CME significantly increased irisin levels immediately after exercise (Figure 1A). In addition, RE was most effective in increasing circulating irisin, with significantly higher change than either HIIE or CME immediately after exercise. The changes in lactate and CK levels were similar to irisin (Figure 1, B and C), implying that exercise-induced irisin changes could be related not only to exercise intensity but also to muscle disruption. Percentage change in irisin levels confirmed that the irisin response to RE was not significantly different between healthy subjects and subjects with metabolic syndrome (Figure 1D).

Comparison of commercially available irisin assays

We compared the RE results, which showed the most dynamic irisin changes (measured using the EK-067–52 kit), with results using the EK-067–29 kit. Results with the latter kit showed approximately 10-fold higher irisin levels.

Table 1. Characteristics and Plasma Biochemical Parameters in Healthy Individuals and Subjects with Metabolic Syndrome at Baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Healthy (n = 14)</th>
<th>Metabolic Syndrome (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>41.1 ± 6.7</td>
<td>44.5 ± 8.5</td>
<td>.34</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>102.1 ± 11.2</td>
<td>108.3 ± 8.3</td>
<td>.24</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.1 ± 4.2</td>
<td>30.1 ± 3.7</td>
<td>.33</td>
</tr>
<tr>
<td>Fat, %</td>
<td>22.7 ± 5.0</td>
<td>24.6 ± 4.7</td>
<td>.44</td>
</tr>
<tr>
<td>VO₂max, mL/min/kg</td>
<td>37.0 ± 3.9</td>
<td>31.1 ± 2.9</td>
<td>.01</td>
</tr>
<tr>
<td>HRmax, bpm</td>
<td>179.0 ± 13.2</td>
<td>178.7 ± 13.0</td>
<td>.96</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.80 (4.22–4.99)</td>
<td>6.13 (5.48–6.83)</td>
<td>.11</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.99 (0.63–1.10)</td>
<td>2.63 (2.24–3.33)</td>
<td>.02</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.27 (5.11–5.49)</td>
<td>5.67 (5.33–6.19)</td>
<td>.01</td>
</tr>
<tr>
<td>Lactate, mmol/L</td>
<td>3.01 (2.69–3.19)</td>
<td>3.15 (2.90–3.23)</td>
<td>.17</td>
</tr>
<tr>
<td>Creatine kinase, U/L</td>
<td>105 (92–127)</td>
<td>139 (113–157)</td>
<td>.79</td>
</tr>
<tr>
<td>Irisin, ng/mL</td>
<td>80.1 (73.5–89.5)</td>
<td>94.6 (93.1–95.7)</td>
<td>.29</td>
</tr>
</tbody>
</table>

Data are means ± sd or median (interquartile range). P values are from Student t test or Mann-Whitney U test, as appropriate. Numbers in bold indicate statistically significant differences.

Figure 1. Percentage change in circulating irisin (A), lactate (B), and CK (C) levels in response to HIIE (running), CME (walking/running), and RE in 20 subjects (14 healthy and 6 with metabolic syndrome). *, P < .05 vs rest; †, P < .05 vs CME; ‡, P < .05 vs HIIE at a given time point. D, RE-induced change in irisin levels in healthy subjects vs subjects with metabolic syndrome. E, Changes in circulating irisin levels in response to RE measured by two different irisin ELISA kits from Phoenix Pharmaceuticals. Values are means ± SE.
els than with the former, and there was low correlation between the results from the two kits \((r = 0.233, P = .191)\). Nonetheless, both kits were able to detect the significant increase in irisin levels immediately after RE (Figure 1E). These data are consistent with recently published data \((22)\) indicating that different commercially available kits bind to different epitopes of the irisin assay. Importantly, the kit that we have been using in our studies \((EK-067–52 \text{ kit})\) and which showed the most dynamic change herein is the one that measures exclusively circulating irisin because the epitopes do not contain any part of the transmembrane or intracellular parts of the molecule.

**Discussion**

We compared the effect of three different types of exercise on circulating irisin levels in humans. Our results suggest that RE was more effective in increasing the circulating irisin concentration than the two types of endurance exercise \((\text{high and moderate intensity})\). Others have also reported similar increase \((\sim 20\%)\) in circulating irisin levels in response to acute exercise, including 45-minute bicycle test at \(70\% \text{ VO}_2\text{max} \) \((14)\) and 90-minute treadmill exercise protocol at \(60\% \text{ VO}_2\text{max} \) \((15)\). The highest increase in CK levels by RE raises a possibility that muscle damage evokes the release of irisin into the circulation.

The effect of exercise on circulating irisin concentration was then compared in subjects with and without metabolic syndrome, which may be of value in determining whether patients with the metabolic syndrome would benefit from exercise regimens through increased irisin secretion. We found that the exercise-induced change in irisin levels did not differ between healthy individuals and subjects with metabolic syndrome regardless of exercise type. Therefore, with the reservation of the small sample size in this pilot study, the ability of exercise to release irisin into the circulation seems to be unaltered in the metabolic syndrome state. Thus, exercise could alleviate the metabolic syndrome by normalizing glucose and lipid levels partly through the action of irisin on adipose tissue \((4, 18, 19)\) and/or muscle \((18, 20)\).

It is possible that although the irisin response to exercise seems normal in individuals with the metabolic syndrome, a state of irisin resistance may exist in target organs. Such a state is suggested by observational studies including ours where circulating irisin levels correlate positively with body mass index and patients with metabolic syndrome have higher levels of irisin than healthy controls \((13, 16)\). Of note, the baseline irisin levels were not significantly different between groups in the present study but showed a trend of being higher in the subjects with metabolic syndrome. Power calculations based on data presented herein would suggest that a sample size of 31 in each group would provide \(80\%\) power to detect a significant difference at the conventional \(0.05\) level. Thus, a larger-scale study, possibly with more severe metabolic disorders, would be needed to assess whether irisin levels in response to exercise are different between healthy subjects and those with metabolic syndrome, and whether there is irisin resistance in the metabolic syndrome.

This study has the apparent limitation of the relatively small sample size, but is powerful in that all individuals performed all exercise regimens, which enabled pairwise comparison of data within subjects, which reduces variability. Larger trials involving both acute and chronic exercise in subjects with metabolic syndrome and possibly inclusion of muscle or fat biopsies would greatly enhance our knowledge of the regulation and role of irisin and/or its resistance in the metabolic syndrome.

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