High-Intensity Exercise Causes Greater Irisin Response Compared with Low-Intensity Exercise under Similar Energy Consumption

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Irisin is mainly released from skeletal muscle (myocytes) and promotes thermogenesis by browning of the white adipose tissue. Although exercise has been shown to increase irisin concentration in blood and myocytes via up-regulation peroxisome proliferator receptor γ coactivator-1α (PGC-1α) expression, the influence of exercise intensity on irisin secretion remains unclear. Therefore, we determined circulating irisin responses following a single bout of running at different intensities. Six sedentary males underwent treadmill running under two different conditions: a low-intensity (40% of VO2max) exercise trial (LIE) or a high-intensity (80% of VO2max) exercise trial (HIE). The exercises in LIE and HIE were lasted for 20 and 40 min, respectively. All subjects underwent the two trials on separate days, and a randomized cross-over design was used. Blood samples were collected before (Pre) and immediately after exercise, at 3, 6, and 19 h after exercise. Energy consumption during exercise did not significantly differ between the two trials. HIE significantly increased blood lactate and serum lactate dehydrogenase levels (P < 0.05). Compared with pre-exercise levels, the irisin concentrations were elevated at 6 h (18% increase) and 19 h (23% increase) after HIE, but significantly decreased after LIE. The relative irisin concentrations (compared with pre-exercise levels) were significantly greater in HIE than in LIE immediately after exercise, and at 6 and 19 h after exercise (P < 0.05). These findings suggest that irisin secretion after acute running exercise is affected by exercise intensity, independent of energy consumption.

Keywords: exercise intensity; fibronectin type III domain-containing 5; irisin; myokine; peroxisome proliferator receptor γ coactivator-1α

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expression and oxygen consumption, and facilitated weight loss and improvement of high-fat-diet-induced insulin resistance compared with the non-treated group. Additionally, they demonstrated that plasma irisin concentration was decreased by 72% in PGC-1α-knockout mice, whereas the value was significantly elevated (65%) after 3 weeks of freewheel running. Although data from human studies are limited, exercise has been shown to significantly increase irisin concentrations (Boström et al. 2012; Huh et al. 2012; Kraemer et al. 2014). In the exercise-induced irisin secretion mechanism, up-regulation of PGC-1α could be a primary factor. Egan et al. (2010) reported that endurance high-intensity running exercise [80% of maximal oxygen uptake (VO₂max)] caused greater increase in PGC-1α compared with isocaloric low intensity exercise (40% of VO₂max) at 3 h after exercise, although the exercise-induced irisin response was not measured in this study. Based on these findings, irisin response to acute exercise may be regulated in an exercise intensity-dependent manner.

In the present study, we determined the levels of circulating irisin after single bouts of treadmill running at two different intensities. We hypothesized that the irisin response to acute running exercise would peak within 3 to 6 h after conclusion of such exercise. We also hypothesized that irisin concentrations would be higher following high-intensity exercise trial (HIE) than low-intensity exercise trial (LIE).

**Methods**

**Subjects**

Six healthy sedentary young males [mean ± standard error (SE): age 22.5 ± 1.1 years] participated in this study. Their physical characteristics were height: 174.8 ± 2.8 cm, body weight: 67.1 ± 2.2 kg and body mass index (BMI: 22.1 ± 1.1). Exclusion criteria were subjects with coronary heart disease and addictive or prescribed drug or tobacco use. None of the subjects had performed any exercise training during the 6 months prior to the present study. Each subject was informed of the experimental procedures and the possible risks involved in the study, and informed consent was subsequently obtained. All experimental procedures were approved by the Human Research Ethical Committee of the University of Yamanashi, in accordance with the dictates of the Helsinki Declaration.

**Experimental protocol**

The present study used a randomized cross-over design and each trial was conducted on two different days at intervals of more than 1 week. In a preliminary experiment, individual VO₂max was determined. On the second and third visits, the experimental trials were conducted. In the main experiment, the subjects performed treadmill running at 40% of VO₂max for 40 min (LIE) and at 80% of VO₂max for 20 min (HIE). Both trials were conducted at the same time of day, thus from 10:30-13:00. Blood samples were collected before exercise (Pre), immediately after exercise (0 h) and at 3, 6 and 19 h after exercise. Time course changes in concentrations of serum irisin and other blood parameters were determined (Fig. 1).

**Maximal oxygen uptake (VO₂max)**

VO₂max during treadmill running was assessed by a multi-stage test modified from the protocol proposed by the American College of Sports Medicine Position Stand (1998). After 2 min of warm-up (6.0 km/h), running speed was progressively increased by 1.2 km/h every 1 min until exhaustion. The criteria for exhaustion were as follows: 1) VO₂ reached a plateau, 2) heart rate achieved age-predicted maximal values (220-age), 3) respiratory exchange ratio (RER) increased up to 1.1, 4) rating of perceived exertion (Borg’s) scale reached 19. When the subjects met at least two of the above four criteria, the exercise was terminated.

**Dietary control**

On the experimental trial day, all subjects consumed a control meal at the same time of day to avoid the influence of diet on irisin secretion, as described previously (Sharma et al. 2012). The macronutrient composition of breakfast (by weight) was as follows: protein 0%, fat 0%, and carbohydrate 100%. The macronutrient composition of lunch was as follows: protein 10%, fat 28%, and carbohydrate 62%. For supper, the macronutrient composition was as follows: protein 12%, fat 31%, and carbohydrate 57%. Each meal was designed to provide the estimated energy requirements. Breakfast, lunch, and supper contained 688 ± 26, 920 ± 34, and 774 ± 29 kcal, respectively. Snacks containing 188 ± 7 kcal were also provided between meals. All subjects ate at 08:30, 13:00, and between 18:00-20:00. Subjects were instructed to refrain from consuming caffeine and alcohol and performing physical activity 24 h prior to the first blood sampling and to record dietary intake content and time.

**Measurements of respiratory gas parameters and blood parameters**

During the treadmill running test, VO₂, carbon dioxide output (VCO₂), respiratory minute ventilation (VE) and RER were deter-

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**Figure 1.** Experimental protocol.
mined by an expired gas analyzer (AE300S, MINATO Medical Science, Osaka, Japan). Energy consumption during exercise was determined from the \( \dot{V}_\text{O}_2 \) and \( \dot{V}_\text{CO}_2 \) measurements (Weir 1949).

Blood samples were collected from an antecubital vein before and immediately after exercise and at 3, 6, and 19 h after exercise. Serum samples were centrifuged for 15 min (3,000 rpm, 4°C) and stored at −80°C. Before and immediately after exercise, blood lactate concentration was measured using an automatic lactate analyzer (Lactate Pro LT-1710, Arkray, Kyoto, Japan). Immediately after exercise, hematocrit (Hct) was measured with a Hct reader (Kubota Corporation, Tokyo, Japan). Serum irisin concentrations were determined using a commercially available ELISA kit (EK-067-52, Phoenix Pharmaceuticals, Germany). The concentrations of lactate dehydrogenase (LDH) and high-sensitivity C-reactive protein (hs-CRP) were measured at a clinical laboratory (Kofu Medical Association, Medical Technology Center, Kofu, Japan) via nephelometry and a method approved by the Japan Society of Clinical Chemistry (JSCC), respectively.

Statistical analysis

Experimental data are shown as means ± SE. For comparisons of exercise-induced changes in blood lactate, serum irisin, LDH and hs-CRP concentrations, a two-way (trial × time) ANOVA with repeated measures was used to identify significant interaction (trial × time) and main effects for trial and time. For comparison of Hct and respiratory gas parameters, a paired \( t \)-test was performed to identify differences between trials for variables within a single measurement. The significance level was set at \( \alpha = 0.05 \) for all statistical tests.

Results

Respiratory gas parameters

Table 1 shows respiratory gas parameters during exercise in each trial. The average values of \( \dot{V}_\text{O}_2 \), \( \dot{V}_\text{CO}_2 \), VE and RER during the exercise test were significantly higher during HIE than LIE \( (p < 0.05) \). However, energy consumption during exercise was not significantly different between the trials.

Blood parameters

Table 2 shows time course changes in blood parameters in each trial. Serum irisin concentrations were significantly higher in LIE than HIE \( (p < 0.05) \). Although the irisin concentration did not change significantly after HIE, a significant reduction was observed immediately \( (0 \text{ h}) \) after LIE \( (p < 0.05) \). Serum irisin concentrations 3 and 6 h after exercise were significantly lower in HIE than in LIE, attributable to differences in pre-exercise values before exercise \( (p < 0.05) \). Fig. 2 shows percent changes in serum irisin concentrations relative to values before exercise. Relative irisin concentrations immediately after exercise \( (0 \text{ h}) \) and at 6 and 19 h after exercise \( (p < 0.05) \), Fig. 2 were significantly higher in HIE than in LIE.

Serum LDH concentrations significantly increased after HIE \( (0, 3 \text{ h}; p < 0.05) \). The LDH concentrations immediately and 3 h after HIE were significantly higher than after LIE \( (p < 0.05) \). Serum hs-CRP concentrations

| Table 1. Respiratory gas parameters and energy consumption during exercise in each trial. |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| \( \dot{V}_\text{O}_2 \) (ml/min) | LIE 1,665 ± 27 \( \text{†} \) | HIE 2,964 ± 34 \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) |
| \( \dot{V}_\text{CO}_2 \) (ml/min) | LIE 1,629 ± 23  | HIE 3,146 ± 42 \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) |
| VE (ml/min)                       | LIE 47 ± 4      | HIE 93 ± 18 \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) |
| RER                               | LIE 0.99 ± 0.01 | HIE 1.06 ± 0.01 \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) |
| Energy consumption (kcal)         | LIE 325 ± 34    | HIE 295 ± 14    | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) | \( \text{†} \) |

Values are means ± SE. \( \text{‘} \) \( p < 0.05 \) between trials.

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<th>Table 2. Time-course changes in blood parameters in each trial.</th>
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<td>Variables</td>
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<td>Irisin (ng/ml)</td>
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<td>LDH (U/L)</td>
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<td>hs-CRP (ng/mL)</td>
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Values are means ± SE. \( *p < 0.05 \) vs. Pre. \( \text{‘} \) \( p < 0.05 \) between trials.
did not change significantly over the post-exercise period in either trial.

Blood lactate concentrations significantly increased immediately after HIE (Pre: 0.9 ± 0.1 mmol/L, 0 h: 6.1 ± 0.1 mmol/L, \( p < 0.05 \)), whereas no significant change was observed after LIE (Pre: 1.2 ± 0.1 mmol/L, 0 h: 1.2 ± 0.1 mmol/L).

Exercise-induced hemoconcentration due to dehydration was evaluated by Hct level, which did not differ between the trials immediately after exercise (LIE: 49.0 ± 1.2%, HIE: 50.6 ± 0.9%), suggesting that the extent of exercise-induced plasma volume shift did not differ significantly between trials.

**Discussion**

In the present study, HIE caused significantly greater responses of blood lactate and serum LDH, indicating that metabolic and mechanical stimuli for skeletal muscles were higher during HIE than LIE. Notably, in the present experimental setting, energy consumption during exercise was similar between the two trials. Therefore, we could identify the influence of exercise intensity on exercise-induced irisin secretion. In previous studies, exercise was shown to increase FNDC 5 significantly, a precursor of irisin (Boström et al. 2012) and irisin concentrations (Huh et al. 2012; Kraemer et al. 2014). However, to date, no study has been explored the influence of exercise intensity on irisin concentrations. Additionally, no information was available regarding detailed time course changes in irisin response after acute exercise. The present study revealed that exercise intensity affected time course changes in irisin response to acute exercise.

Serum irisin concentrations at 3 and 6 h after exercise were significantly higher when subjects engaged in LIE rather than HIE (\( p < 0.05 \)). Caution is needed when interpreting the results because baseline concentrations before exercise were significantly higher in subjects commencing LIE than HIE. The difference in baseline values may be attributable to the relatively small number of study subjects or differences in meals consumed in the 2 days prior to the day of exercise. However, no plausible explanation is evident, and the order of each trial was counterbalanced. Therefore, we focused on the percentage changes from pre-exercise values to compare exercise-induced irisin responses between the two trials.

Relative values of irisin response were greater 6 and 19 h after HIE (18 and 23% higher than baseline levels, respectively), but not after LIE. A previous study reported that PGC-1α mRNA, an upstream factor of irisin, was significantly elevated at 3 h after exercise (Egan et al. 2010; Stepto et al. 2012). Hence, we speculated that PGC-1α mRNA expression signal would stimulate irisin production through FNDC 5 in skeletal myocytes 3 h after exercise. Although detailed times required for irisin secretions via exercise-induced PGC-1α mRNA expression remain unclear, present results indicated that high intensity exercise caused greater exercise-induced irisin response than low intensity exercise, regardless of similar energy consumption.

Relative values of irisin concentrations were significantly greater at 0, 6 and 19 h after HIE than after LIE (\( p < 0.05 \)). Egan et al. (2010) reported that PGC-1α mRNA expression after high intensity exercise was significantly greater than it was after low intensity exercise. Furthermore, Huh et al. (2012) demonstrated that a single bout of sprint interval exercise significantly increased irisin concentration (18% higher than pre-exercise values). Therefore, we speculated that high intensity exercise would cause greater irisin response than low intensity exercise. The present results partially support our hypothesis, that exer-
exercise intensity has an effect on exercise-induced irisin responses.

Unexpectedly, low intensity exercise showed a significant reduction of irisin concentration immediately after exercise (0 h) compared with pre-exercise values ($p < 0.05$). Only two studies have reported irisin responses after a single bout of exercise. Pekkala et al. (2013) suggested that a single bout of resistance exercise did not affect serum irisin concentrations immediately after exercise. Kraemer et al. (2014) also reported that a significant increase in irisin concentration was observed in the middle of a 90-min endurance exercise. However, the irisin concentration tended to decrease during the second half of the exercise and a significant increase relative to pre-exercise values disappeared at the end of 90 min of exercise. Thus, it appears that time course changes of irisin concentrations in response to acute exercise are complex. Recent studies suggested that multiple sources (e.g., skeletal myocytes, adipocytes, cardiomyocytes and purkinje cells of cerebellum) for irisin production exist (Roca-Rivada et al. 2013; Raschke and Eckel 2013; Dun et al. 2013) and interactions with other cytokines and hormones (e.g., IL-6, BDNF, adiponectin) are involved (Pedersen and Febbraio 2012; Wrann et al. 2013; Park et al. 2013). These factors may explain, partially, the rapid reduction of irisin concentration immediately after LIE. Currently, the details of the mechanism underlying the rapid reduction remain unclear.

Several limitations exist in the present study. First, we could not draw definite conclusions regarding the potential influence of exercise duration on exercise-induced irisin responses. Second, more frequent blood samplings with a larger number of subjects may be necessary to show detailed time course changes of irisin concentrations. Recently, we have discovered that irisin concentrations were maximized at 1 h after acute exercise in healthy humans (unpublished observation). Therefore, a possibility exists that we could not detect peak values of irisin after exercise in the present study. Additionally, it is possible that irisin was secreted independent of PGC-1α up-regulation. Third, the samples used in the present study were blood only, and muscle tissue samples are essential to determine PGC-1α protein and mRNA levels using western blot and polymerase chain reaction (PCR) analysis. Finally, we did not control the meals consumed on the day before the exercise day. It is likely that meals should be controlled for several days prior to exercise. Nevertheless, this is the first study to show that exercise intensity influences the human irisin response, and our findings may aid in the design of exercises tackling obesity, by stimulating formation of the novel myokine, irisin.

In summary, irisin responses to a single bout of running exercise were greater for high intensity compared with low intensity exercise in young healthy males, regardless of similar energy consumption.

Conflict of Interest
We declare no conflict of interest.

References


