Irisin response to downhill running exercise in humans

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INTRODUCTION

Myokines are physiologically active substances released from skeletal muscle. Irisin is a relatively recently discovered myokine that enhances energy expenditure (EE) by increasing UCP1 expression in white and/or beige adipocytes. Several studies have described the irisin response to a single bout of exercise. However, the effect of endurance exercise on the irisin response remains unclear, although the mode of exercise may affect the response. The plasma irisin concentration increased significantly at 54 min during 90 min of running, but not after 60 min of pedaling. In contrast, a single bout of resistance exercise significantly increased the irisin response. Notably, resistance exercise caused a greater increase in irisin concentration than did the same duration of endurance exercise (pedaling), although the EE was much lower during resistance exercise. Differences in the exercise-induced irisin response among various studies may be explained by the different exercise modalities, with varying degrees of eccentric contraction. During eccentric contraction, fast-twitch fibers are more prominently recruited than during concentric contraction. An increase in exercise intensity promotes recruitment of fast-twitch fibers, leading to an augmented irisin response.

Downhill running (DHR) especially highlights eccentric contraction in the quadriceps femoris compared with level running (LR), causing severe exercise-induced muscle damage and inflammation. Huh et al. showed that exercise-induced elevation of irisin was observed with a concomitant increase in creatine kinase (CK) (an indirect muscle damage marker). However, it remains unclear whether the magnitude of exercise-induced muscle damage influences the irisin response. Therefore, we examined the irisin responses in 2 different types of running exercise: DHR and LR under similar EE conditions. We hypothesized that the irisin response would be augmented more after DHR than after LR.

[Received: 2018/03/01, Revised: 2018/04/28, Accepted: 2018/05/27, Published: 2018/06/30]

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METHODS

Subjects

Fifteen healthy, physically active men [mean age ± standard error (SE): 21.6 ± 2.0 y, height: 170 ± 1.3 cm, weight: 64.8 ± 2.7 kg, body mass index: 22.4 ± 0.8 kg/m²] participated in the present study. All participants had undergone several years of exercise training and exercised at least weekly. They were informed of the study purpose, experimental procedures, and possible risks of the study, and they provided written informed consent. All experimental procedures were approved by the Ethics Committee for Human Experiments at Ritsumeikan University (IRB-2014-004) and all complied with the Helsinki Declaration.

Experimental setting

The subjects visited the laboratory twice during the experimental period. On the first day, maximum oxygen consumption (VO2max) during treadmill running was determined. On the second day (main experiment day), all subjects completed either a 30-min LR or a DHR protocol on a treadmill (Valiant Lode B. V., Groningen, the Netherlands). Exercise-induced irisin and metabolic responses were monitored during a 24-h post-exercise period.

Fifteen subjects were randomly divided into either LR (n = 8) or DHR (n = 7) groups to match the VO2 level between the groups. In the LR group, subjects ran for 30 min on a treadmill with a 0% gradient, whereas subjects in the DHR group ran with a −10% gradient. The exercise intensity was set to achieve 70% of VO2max during incremental running on a treadmill (0% gradient). The running velocity in the DHR group was adjusted individually during the initial 5 min of exercise to achieve 70% of the VO2max. The DHR protocol was based on those of previous studies [27, 28] and our pilot study. All subjects were instructed to refrain from caffeine and alcohol and strenuous physical activity for 3 days before the trial day. They were also asked to minimize physical activity for 24 h after the exercise. Prescribed meals were provided at 3 h and 9 h after exercise (Figure 1). All subjects were allowed to consume only water ad libitum on the day of the trial. The sleep duration was set from 22:00 to 07:00.

Blood samples

The subjects presented at 8:00. First, a venous blood sample (i.e., pre-exercise sample) was collected after 30 min of rest. The subjects began prescribed exercise at 8:40. Additional blood samples were collected immediately after exercise (9:10), and at 1 h (10:10), 3 h (13:10), and 24 h (09:10) after exercise (Figure 1). The collected blood samples were centrifuged for 10 min (3,000 rpm, 4°C), and the serum and plasma samples were stored at −60°C under the curve (AUC) of plasma irisin concentrations.

Statistical analysis

All experimental data are shown as means ± SE. An unpaired t-test was used to explore the significance of differences between groups for respiratory gas data and the area under the curve (AUC) of plasma irisin concentrations. Moreover, two-way (group × time) repeated analysis of variance (ANOVA) was applied to identify significant in-

Respiratory gas samples during exercise were collected with breath-by-breath method using an automatic gas analyzer (AE300S Minato Medical Science, Osaka, Japan). All data were averaged every 30 s. The treadmill gradient during the VO2max test was set at 0%. The VO2max test commenced with running at 6.0, 8.0, and 10.0 km/h for 4 min each. The running speed was then increased by 2.0 km/h every 3 min to 12.0 km/h and 14.0 km/h. When treadmill running at 14.0 km/h was completed, the speed was progressively increased every minute by 0.6 km/h to exhaustion. Exhaustion was defined as 1) VO2max plateau; 2) a heart rate equal to the maximum age-predicted value (220 minus age); or, 3) a respiratory exchange ratio (RER) of 1.1. When a subject met at least 2 of these 3 criteria, the test was concluded.

VO2max

Approximately 1 week prior to the main experiment, the VO2max was evaluated to determine running velocity.

Respiratory gas sampling during exercise

On main experiment days, respiratory gas samples during 30 min (8:40-9:10) of treadmill running were evaluated with the breath-by-breath method using an automatic gas analyzer (AE300S Minato Medical Science, Osaka, Japan). Respiratory gas samples were used for continuous determination of VO2, carbon dioxide output (VCO2), minute ventilation (VE), and the RER. All data were averaged every 30 s.

Statistical analysis

All experimental data are shown as means ± SE. An unpaired t-test was used to explore the significance of differences between groups for respiratory gas data and the area under the curve (AUC) of plasma irisin concentrations. Moreover, two-way (group × time) repeated analysis of variance (ANOVA) was applied to identify significant in-
RESULTS

Respiratory gas variables during exercise
Table 1 presents the respiratory gas variables during the 30 min of exercise in each group. The average VO₂, VCO₂, VE, and energy expenditure (EE) during exercise did not differ significantly between the groups (P = 0.35, d = 0.50 for VO₂; P = 0.60, d = 0.28 for VCO₂; P = 0.62, d = 0.26 for VE; and P = 0.39, d = 0.46 for EE, respectively). However, the average RER during exercise was significantly higher in the DHR group (P = 0.03, d = 1.23).

Blood variables
Figure 2 presents the time-course changes in plasma irisin concentrations. No significant interaction (group × time, P = 0.159, η² = 0.07) or main effect for group (P = 0.25, η² = 0.04) and time (P = 0.30, η² = 0.04) was evident. In the DHR group, and the plasma irisin concentration increased approximately 3-fold by 3 h after exercise. However, the difference did not reach significance (P = 0.20, d = 0.74). When the exercise-induced irisin responses over the 3-h post-exercise period were compared, the AUC was significantly greater in the DHR group than in the LR group (P = 0.04, d = 0.99; Figure 2b).

As shown in Figure 3a, there was no significant interaction (group × time; P = 0.122, η² = 0.06) or main effect for group (P = 0.105, η² = 0.10) for the plasma IL-6 concentration. Plasma IL-6 concentrations increased significantly immediately (0 h) and 1 h after exercise in both groups (main effect for time; P = 0.001, η² = 0.37). Moreover, the DHR group showed a significant increase at 3 h after exercise (P = 0.002, d = 1.51). The AUC over the 3-h post-exercise period for plasma IL-6 tended to be greater in the DHR group than in the LR group (P = 0.061, d = 1.06, Figure 3b).

No significant interaction (group × time) was evident for blood glucose (P = 0.07, η² = 0.09), lactate (P = 0.16, η² = 0.10), CK (P = 0.061, d = 1.06, Figure 3b). The blood lactate concentration increased significantly immediately (0 h) and 1 h after exercise in both groups (main effect for time; P = 0.001, η² = 0.37). Moreover, the DHR group showed a significant increase at 3 h after exercise (P = 0.002, d = 1.51). The AUC over the 3-h post-exercise period for plasma IL-6 tended to be greater in the DHR group than in the LR group (P = 0.061, d = 1.06, Figure 3b).

Table 1. Respiratory gas variables during exercise in each group.

| Variables | LR(n=8) | DHR(n=7) | P Value |
|-----------|---------|----------|---------|
| VO₂ (mL/min) | 2.44 ± 0.192 | 2.205 ± 0.193 | 0.35 |
| VCO₂ (mL/min) | 2.28 ± 0.188 | 2.143 ± 0.129 | 0.60 |
| VE (mL/min) | 66.3 ± 7.5 | 71.4 ± 6.5 | 0.62 |
| RER | 0.93 ± 0.01 | 0.97 ± 0.02 | 0.03 |
| EE (kcal) | 361 ± 29 | 329 ± 20 | 0.39 |

Values are means ± SE. *: P < 0.05 vs. LR group.

Table 2. Time-course changes in blood variables in each group. Values are means ± SE. #: P < 0.05 vs. Pre. †: P < 0.05 vs. LR group.

| Variables | Pre | 0 h | 1 h | 3 h | 24 h |
|-----------|-----|-----|-----|-----|------|
| Glucose (mg/dL) | LR | 91 ± 1 | 108 ± 10 | 86 ± 2 | 86 ± 2 | 90 ± 1 |
| | DHR | 87 ± 2 | 133 ± 8 | 81 ± 3 | 87 ± 2 | 91 ± 2 |
| Lactate (mmol/L) | LR | 1.5 ± 0.2 | 3.5 ± 0.8 | 1.5 ± 0.1 | 1.5 ± 0.2 | 1.4 ± 0.1 |
| | DHR | 1.8 ± 0.2 | 6.1 ± 1.4 | 2.1 ± 0.1 | 1.6 ± 0.1 | 1.6 ± 0.1 |
| Cortisol (μg/dL) | LR | 17.2 ± 2.2 | 18.1 ± 2.4 | 16.4 ± 2.5 | 11.8 ± 1.9 | 17.5 ± 1.5 |
| | DHR | 21.1 ± 3.0 | 28.0 ± 2.3 | 26.7 ± 2.8 | 13.9 ± 1.5 | 19.6 ± 2.5 |
| Myoglobin (ng/mL) | LR | 39.3 ± 5.6 | 63.3 ± 9.0 | 87.3 ± 9.9 | 79.1 ± 10.1 | 40.9 ± 3.3 |
| | DHR | 60.0 ± 29.7 | 207.7 ± 62.1 | 564.0 ± 115.9 | 483.7 ± 109.5 | 68.2 ± 23.4 |
| CK (U/L) | LR | 220 ± 55 | 241 ± 62 | 275 ± 54 | 261 ± 53 | 246 ± 30 |
| | DHR | 606 ± 467 | 712 ± 514 | 751 ± 525 | 877 ± 505 | 1070 ± 307 |
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DISCUSSION

This is the first study to explore the effects of exercise-induced muscle damage on the irisin response. As expected, exercise-induced elevation of serum myoglobin (an indirect muscle damage marker) and plasma IL-6 (an inflammatory cytokine) were significantly greater in the DHR group than in the LR group, although EE during 30 min of exercise was not significantly different. Consequently, the plasma irisin concentration increased approximately 3-fold 3 h after completion of DHR (Pre: 466.1 ± 78.8 ng/mL, 3 h after exercise: 1,358.0 ± 666.7 ng/mL). Additionally, the exercise-induced irisin response over 3 h after DHR was significantly greater than that after LR, suggesting that the augmented irisin response may be associated with magnitude of muscle damage.

Exercise intensity is a primary factor in irisin production\textsuperscript{19,26}, and increased recruitment of fast twitch fibers may play an important role in the irisin response. During DHR, eccentric muscle contraction (which preferentially recruits fast twitch fibers) is highlighted during the landing phase\textsuperscript{15-18}. Furthermore, running velocity was significantly higher in the DHR group (14.0 ± 0.2 km/h) than in the LR group (10.8 ± 0.3 km/h), to match EE during exercise. We previously showed that higher-velocity running was associated with a greater irisin response than lower-velocity running under matched EE conditions\textsuperscript{19}. Therefore, enhanced irisin response (revealed by the AUC over 3 h) after DHR might be, at least in part, explained by higher running velocity with increased recruitment of fast twitch fibers.

We sought to identify associations between the levels of exercise-induced muscle damage markers (myoglobin and CK) in blood and the irisin response. Several studies suggested a relationship between these 2 factors in both humans\textsuperscript{28} and rats\textsuperscript{29}. However, the above studies involved limitations, including low frequencies of blood collection. In the present study, we monitored the time course of changes in the levels of plasma irisin and muscle damage markers over a 24-h post-exercise period. The serum myoglobin level was markedly higher in the DHR group than in the LR group, indicating that exercise-induced muscle damage was profound in the DHR group. Moreover, exercise-induced plasma IL-6 elevation was profound in the DHR group (until 3 h post-exercise). The AUC value over a 3-h post-exercise period tended to be greater in the DHR group than in the LR group (P = 0.06). Similarly, Vyver et al. showed that both serum myoglobin and IL-6 concentrations increased concomitantly after completion of DHR\textsuperscript{20}. Therefore, significantly higher plasma IL-6 concentration during the post-exercise period in the DHR group may be related to proinflammatory action following ultrastructural muscle damage. The exercise-induced elevation of lactate was also greater in the DHR group than in the LR group, suggesting that DHR promoted muscle glycogen utilization. Previous studies suggested that a high level of IL-6 was seen with low glycogen levels, and that the lower glycogen level induces PGC-1α (an upstream factor in irisin production) gene expression\textsuperscript{31-32}. Additionally, Huh et al.
found that the mRNA levels of PGC-1α (an upstream factor in irisin production) and FNDC5 (the precursor of irisin) were increased by IL-6 addition in vitro. Thus, further reduction of muscle glycogen levels caused by DHR may promote the PGC-1α-FNDC5-irisin axis via IL-6 production. However, it is difficult to clarify completely the association between IL-6 and irisin, since IL-6 has multiple sources (e.g., leucocytes, myocytes) and actions (pro- and anti-inflammatory). Taken together, the present data suggest that the exercise-induced irisin response may be associated with muscle damage (reflected by the elevation in myoglobin concentrations) and/or inflammation (reflected by the elevation in IL-6 concentrations).

In the present study, post-exercise increases in irisin levels were modest compared to those of previous studies. Although the intensity (70% of VO2max) and duration (30 min) during DHR exercise appeared to be sufficient to stimulate irisin production, strenuous muscle contraction can trigger myokine proteolysis. Therefore, it is possible that excessive muscle damage caused by DHR attenuated the transient irisin response.

Several limitations should be noted when interpreting the results. First, we did not use a crossover design, because we attempted to avoid “repeated bout effects” for exercise-induced muscle damage. Thus, we divided the 15 subjects into DHR and LR groups, and inter-individual variation in the irisin response should be considered. Second, our results may be specific to healthy subjects. Indeed, obese subjects and patients with type 2 diabetes exhibited irisin responses different from those of healthy individuals.

Third, the existence of irisin and its role in humans is controversial. However, Jedrychowski et al. used quantitative mass spectrometry to precisely measure plasma irisin levels in healthy humans in both the resting and post-exercise state. Furthermore, the ELISA that we used in the present study was the most commonly employed in previous studies. From a practical viewpoint, our findings will aid in the design of exercises that efficiently reduce the risk of metabolic disorder and associated disease. We speculate that exercise-induced irisin secretion would be influenced by muscle damage and inflammation. A future study should address the physiological implications of transient increases in irisin levels on the resting metabolic rate, insulin sensitivity, and body composition in various populations.

In conclusion, we found that the exercise-induced irisin response over the 3 h period after DHR was significantly greater than that after LR, although the EE was similar.

ACKNOWLEDGEMENTS

We gratefully thank the subjects for commitment in the present study. We also thank laboratory members for help with the experiment and analyses of blood samples.

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