Elevation in Serum Irisin Levels after a Single Bout of Exercise do not Modulate Resting Energy Expenditure and Diet-induced Thermogenesis in Healthy Young Adults

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Osaka City Med. J. Vol. 63, 93-104, 2017

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Abstract

Background

The effect of physical activity on diet-induced thermogenesis is still controversial. Irisin is a new protein that is secreted from the muscle into the circulation after acute exercise and induces browning of adipocytes. The purpose of the present study was to examine whether the changes in serum irisin levels following a single bout of exercise could modulate diet-induced thermogenesis.

Methods

Nine normal-weight healthy young volunteers (32±4 years) participated in two experiments performed in a random order. In the exercise trial, 40 min of exercise on a bicycle ergometer at 60% of heart rate reserve were performed on the day before the assessment of energy expenditure. Blood concentrations of irisin, glucose and insulin were measured before and after the intake of a fixed 860 kcal meal. Serum irisin levels were also examined before and immediately after the exercise. In the no exercise trial, energy expenditure was assessed without previous exercise.

Results

Serum irisin levels after the 40-min exercise period increased with borderline significance over baseline levels. Postprandial irisin levels were higher in the exercise trial than in the no exercise trial, whereas dietary intake did not affect serum irisin levels in either trial. Mean diet-induced thermogenesis and resting energy expenditure were not different between the trials. No differences were found in postprandial glucose and insulin levels between the trials.

Received October 24, 2016; accepted January 17, 2017.
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Conclusions

The elevation in serum irisin levels induced by a single bout of exercise did not modulate resting energy expenditure and diet-induced thermogenesis.

Key Words: Irisin; Exercise; Diet-induced thermogenesis; Energy expenditure; Insulin

Introduction

Physical activity is one of the determinants of energy expenditure (EE)[1], and the amount of energy expended during a period of physical activity depends fundamentally on the type and intensity of exercise[2-4]. The energetic budget due to exercise actually accounts for a relatively small percentage of daily EE. Nevertheless, exercise increases whole-body EE beyond the calories used in the actual work performed, and it is generally believed that the increase in lean body mass following physical training increases resting EE (REE). The elevation in REE, however, is observed even after a short-term training session or a bout of exercise, which is not accompanied by the increase in lean body mass. As shown by Speakman et al, REE remained elevated up to approximately 48 h after the cessation of a single bout of exercise[5]. It seems probable that several key hormones (leptin, insulin, and ghrelin), which modulate substrate utilization or energy metabolism, play a role in the prolonged elevation in EE during the post-exercise period because the blood levels of these hormones are influenced by a bout of exercise[6].

The thermic effect of food, also known as diet-induced thermogenesis (DIT), is the energy cost required for the digestion and absorption of nutrients during the postprandial period. DIT corresponds to approximately 10% of the total energy of ingested food. DIT and the two other components (REE and thermogenesis related to physical activity) constitute the total daily EE. Although DIT is the smallest of these three components and only accounts for 3%-10% of daily EE, it can contribute to the regulation of body fatness and the maintenance of obesity[7]. At present, there is a general consensus that the nutritional composition of food is mainly responsible for the amount of DIT, i.e., the intake of a higher protein meal causes greater DIT. In contrast, previous studies have investigated whether habitual exercise could modulate DIT. However, the results relatively differed depending on the type, intensity, and duration of the exercise[8]. Furthermore, there are few reports regarding the effect of acute exercise on thermogenesis during the subsequent dietary intake. Therefore, the issue still remains controversial.

In 2012, Boström et al reported in their *in vitro* and *in vivo* investigation that a novel peptide, irisin, induced the expression of mitochondrial uncoupling protein-1 in white adipose cells, which uncoupled respiratory chain and dissipated chemical energy as heat[9]. This is termed as browning of white adipose cells and resulted in increased thermogenesis in mice[9]. Irisin is transcribed from fibronectin type Ⅲ domain-containing protein 5 (FNDC5) gene, transferred to the extracellular surface of the skeletal muscle cell membrane where it is proteolytically cleaved, and secreted into the circulation. The expression of peroxisome proliferator-activated receptor (PPAR) γ co-activator-1α, which is stimulated by muscle contraction during exercise[10], increases the gene expression of FNDC5[9]. Some reports demonstrated the significant correlation between blood irisin levels and resting or 24h EE[11,12], although they were cross-sectional studies. Therefore, irisin secretion is the probable mechanism linking physical activity to its favorable effects on energy metabolism. Recently, several studies reported significant elevations in plasma irisin levels after acute exercise in mice and humans[13,14]. However, it remains unclear how irisin influences white adipose tissue to increase DIT

and REE in humans.

Considering the above background information, we hypothesized that a single bout of exercise increased serum irisin levels in association with an augmentation in DIT during dietary intake in humans. Therefore, in the present study, we examined whether the changes in serum irisin levels following a single bout of exercise could modulate DIT in normal-weight, sedentary young adults.

Methods

Subjects

We recruited sedentary, healthy young adult volunteers between the ages of 25 and 40. We excluded subjects who had a history of ischemic heart disease, hypertension (resting systolic blood pressure [BP] ≧140 and/or diastolic BP ≧90), diabetes, or obesity (body mass index [BMI] ≧30). The eligible applicants who met the inclusion criteria participated in this investigation after familiarizing themselves with the experimental protocol. The protocol was approved by the Institutional Review Board of Osaka City University Graduate School of Medicine (approval No. 2630) and registered with the University Hospital Medical Information Network-Clinical Trial Registry (study ID: UMIN 000011735). This study also conformed to the standard set by the Declaration of Helsinki, and we obtained informed written consent from all participants.

Experimental protocol

The study design was a randomized, unblinded crossover study. The subjects were required to attend the laboratory on two consecutive days and again on another day for two trials, the exercise (EX) and the no exercise (NOEX) trials. These were conducted in a random order with at least one week apart but within two weeks between the first and second experiments. In the three days prior to the experimental session, all subjects were instructed to consume a normal diet but to take a fixed amount of carbohydrate (18 kcal×individual ideal body weight [(height, m)^2×22, kg]) in accordance with a food guide. Adherence to the food guide was confirmed by the diet records. Subjects were also instructed to abstain from exercise, caffeine, and alcohol for 24 h prior to each experimental day.

In each experimental session, we used indirect calorimetry to continuously assess EE before and for 180 min after consuming the fixed meal. Blood samples were also collected immediately before and every 30 min after the start of dietary intake for measurements of glucose, insulin, and irisin levels. In the EX trial, the subjects engaged in an exercise session on the day previous to the experimental session. In contrast, no exercise session was performed in the NOEX trial (Fig. 1).

Indirect calorimetry

Whole-body indirect calorimetry was performed using an electronic spirometry system integrated with a gas analyser (AE-310S, Minato Medical Science Co., Osaka) to estimate EE (kcal/min). After an overnight fast, experimental session was started at 0900 h. Subjects quietly rested on a hospital bed in a supine position at a constant temperature of approximately 22℃ and a relative humidity of approximately 45%-50% of the laboratory. After taking adequate rest, the baseline assessment of EE was performed for 25 min, and REE was determined by averaging EE in the last 10 min of this baseline period. Within 15 min, the subjects consumed a fixed test meal and 100 mL of water. The test meal consisted of a food box including chicken stew, a pudding, and crackers (460 kcal, E460f18, Kewpie Co., Japan) and a jelly-type supplement flavored yogurt (400 kcal, ISOCAL® Semi-Solid, Nestle Health Science, Japan). The test meal amounted to a total of 860 kcal with 56.5g of carbohydrate, 34.0g of fat, and 32.4g of protein. After the meal, the EE assessment was restarted and

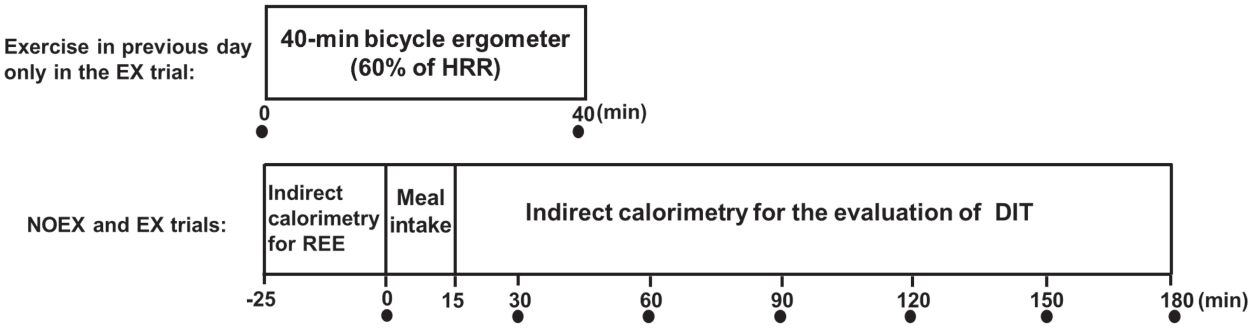

Figure 1. Nine subjects participated in two experiments, the EX and NOEX trials. In the EX trial, they performed a 40-min exercise on bicycle ergometer at 60% of HRR at the day before the assessment of EE. Blood samples indicated by solid circles were collected before and immediatelly after the 40-min exercise session. No previous exercise was performed in the NOEX trial. Blood samples were also collected immediatlly before and every 30 minute after food intake in both trials. Abbreviations: EX, exercise; NOEX, no exercise; HRR, heart rate reserve; EE, energy expenditure; and DIT, diet induced thermogenesis.

continued up to 180 min. We averaged EE in the last 10 min of each 30-min period during the 180-min assessment. The elevation in EE due to dietary intake than that observed at fasting baseline reflected DIT. We calculated the mean DIT during the postprandial period of 180 min by subtracting REE from the average EE in each 30-min period.

Exercise protocol

In the EX trial, the subjects participated in a 40-min exercise session by cycling ergometery between 1500 h and 1800 h on the day prior to DIT evaluation. The protocol was adopted because the similar ergometry exercise was reported to immediately increase serum irisin levels[15] and our sedentary subjects were also expected to complete the protocol. Before the session, resting heart rate (RHR) was assessed, and 60% of heart rate reserve (HRR) for each subject was determined as the target heart rate using Karvonen's formula[16] as follows:

$$\text{Maximum heart rate (MHR)} = 220 - \text{Age}$$
$$60\% \text{ of HRR} = (\text{MHR} - \text{RHR}) \times 0.6 + \text{RHR}$$

Subjects completed 40 min of exercise including a 5-min warm-up period on a bicycle ergometer (Monark, Stockholm, Sweden), in which the load was appropriately adjusted to maintain the target heart rate while under continuous monitoring of heart rate by electrocardiography (Life Scope BSM-7201, Nihon Kohden, Tokyo, Japan). Blood samples were collected before and immediately after 40-min exercise period to assess serum irisin levels.

Anthropometrical measurements

The percentage of body fat was estimated by bioelectrical impedance analysis using the body composition analyser (Body Fat Analyzer Tbf-102, Tanita) before the first experiment. BMI (kg/m^2) was calculated as body weight (kg) divided by height $(m)^2$.

Laboratory measurements and insulin sensitivity evaluation

The collected blood samples were centrifuged for 15 min at 3000 rpm, and serum and plasma were stored at −80℃ until assays were performed. Plasma glucose levels were measured by the hexokinase UV method, and serum immunoreactive insulin (IRI) levels were measured by the chemiluminescent enzyme immunoassay. We calculated the homeostasis model assessment of insulin resistance (HOMA-IR), an established surrogate index of insulin resistance[17], from fasting blood samples in the

NOEX trial. The HOMA-IR was obtained from fasting plasma glucose (FPG) and fasting IRI (FIRI) levels according to the original method developed by Matthews et al[18] with the following formula:

$$\text{HOMA-IR} = \text{FPG (mg/dL)} \times \text{FIRI (}\mu\text{U/mL)}/405$$

A greater HOMA-IR value represents a higher insulin resistance. Serum irisin levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (#EK-067-52, Phoenix Pharmaceuticals, Inc., CA, United States) according to the manufacturer's protocol. Regarding the accuracy of the kit, the coefficients of variation for inter- and intra-assay were less than 10%.

The area under the curve (AUC) for glucose, insulin, and irisin were also calculated from their blood levels during the postprandial period for 180 min.

Statistical analysis

All analyses were performed using the SPSS statistical software package (version 21.0). Data are provided as the mean±standard error of mean (SEM) unless otherwise indicated. The correlation between serum irisin levels and BMI, body fat, or HOMA-IR was examined by simple linear regression analysis. The analysis was also used to examine the correlation between AUC for postprandial irisin levels and AUC for DIT. The effects of exercise and dietary intake on postprandial glucose, insulin, and irisin levels as well as mean EE were examined by two-way (time×trial) analysis of variance (ANOVA) with repeated measurements. Post-hoc multiple pairwise comparisons (Dunnett's method) were performed in the case of significant time effects. In the case of significant trial effects, subsequent pairwise comparisons were performed by paired t-test. The test was also used for the comparison of serum irisin levels before and after exercise and for the comparisons of the AUC for postprandial glucose, insulin, and irisin levels, REE, and mean DIT between the trials. p-values of <0.05 were considered statistically significant.

Results

Nine applicants, seven males and two females, who met the inclusion criteria were enrolled in the present study. The clinical characteristics of all subjects are summarized in Table 1. There was no significant correlation between serum irisin levels at baseline and BMI ($r=0.038$, $p=0.922$, 95%

Table 1. Clinical characteristics of the subjects

n	(female/male)	9 (2/7)
Age	yrs	32±4.2
Weight	kg	65.1±7.6
BMI	kg/m^2	22.8±2.3
Body fat	%	21.3±6.9
(Laboratory data from the baseline measurement in the NOEX trial)		
FPG	mmol/L	5.1±0.3
Fasting insulin	pmol/L	26.3±12.5
HOMA-IR		0.88±0.42
Irisin	ng/mL	165.7±39.8

All values are presented as n or mean±standard deviation (SD). Abbreviations: BMI, body mass index; FPG, fasting plasma glucose; and HOMA-IR, homeostasis model assessment of insulin resistance.

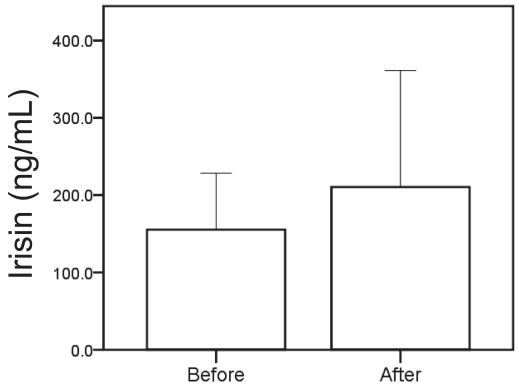

Figure 2. Changes in serum irisin level (ng/mL) before and after 40-min exercise. Serum irisin level was increased following 40-min exercise with borderline significance (p=0.054). Values are presented as mean±SD.

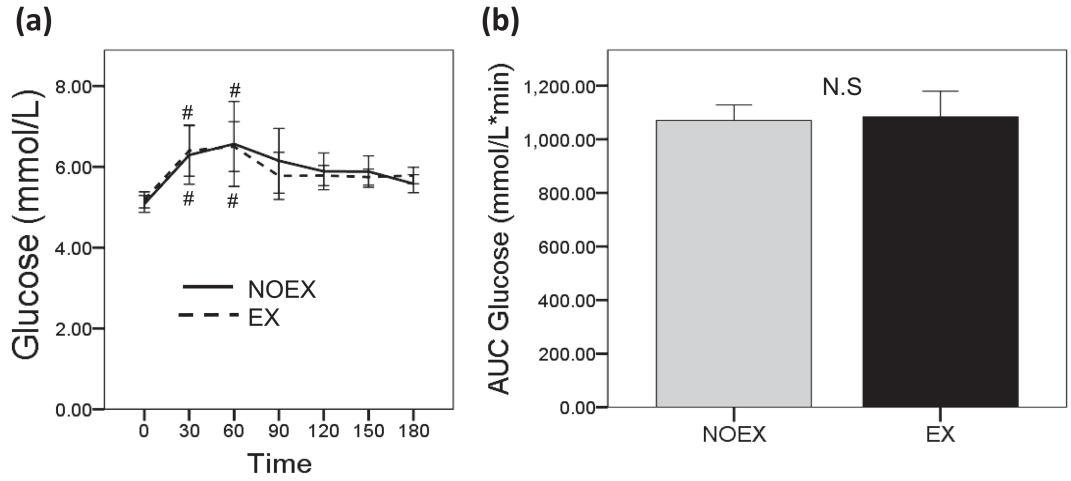

Figure 3. Comparison of postprandial glucose levels between the EX and NOEX trials. (a) Time course of plasma glucose level. Although glucose levels were increased during 1 h after the diet intake compared with those at baseline in both trials, there was no difference in glucose levels between the trials throughout the 3-h observations. (b) AUC for postprandial glucose level. No difference was found between the trials throughout 3 h observations. Data are presented at mean±SEM. #p<0.05 vs baseline. Abbreviations: EX, exercise; NOEX, no exercise; and AUC, area under the curve.

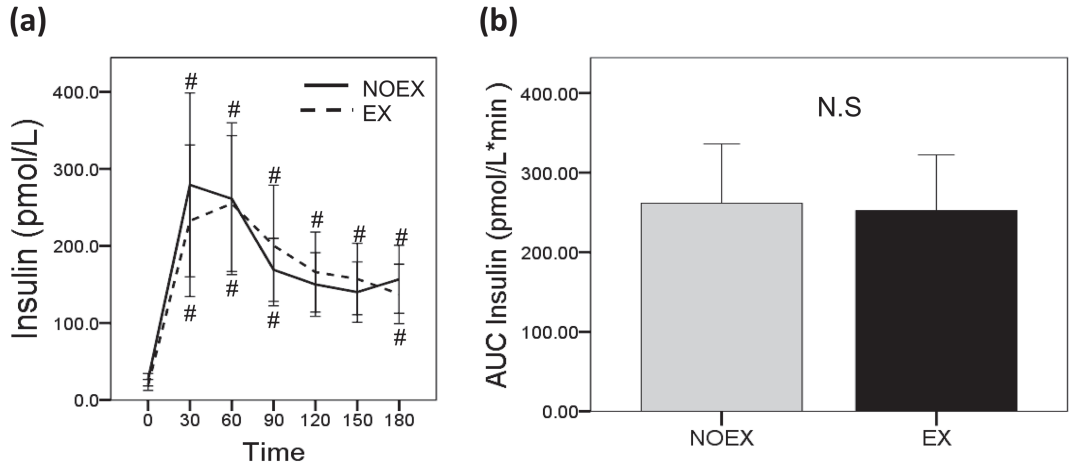

Figure 4. Comparison of postprandial insulin levels between the EX and NOEX trials. (a) Time course of plasma insulin level. Although insulin levels were increased during 3 h after the diet intake, except for at 120 and 150 min in the NOEX trial, compared with those at baseline in both trials, there was no difference in insulin levels between the trials throughout the 3-h observations. (b) AUC for postprandial insulin level. No difference was found between the trials. Data are presented at mean±SEM. #p<0.05 vs baseline. Abbreviations: EX, exercise; NOEX, no exercise; and AUC, area under the curve.

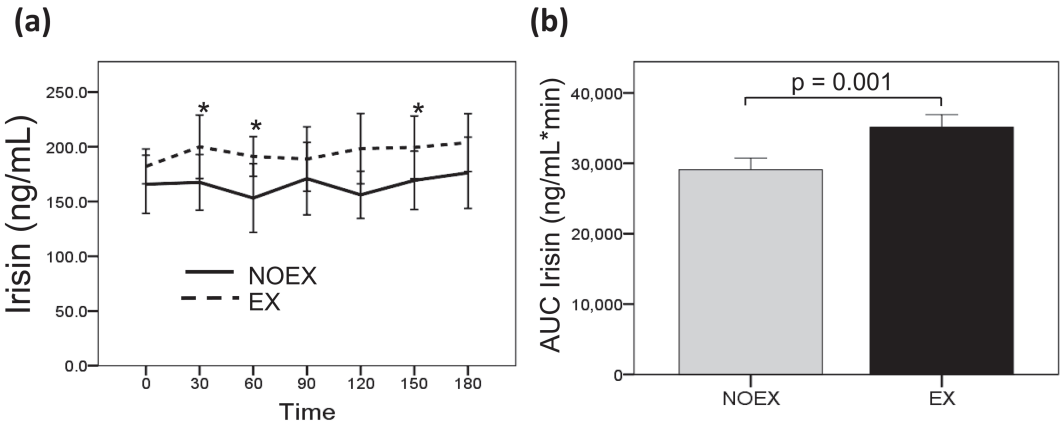

Figure 5. Comparison of postprandial irisin levels between the EX and NOEX trials. (a) Time course of plasma irisin level. Although serum irisin levels were higher before and throughout 3 h after the diet intake in the EX trial than those in the NOEX trial, diet intake itself affected serum irisin levels in neither trial. (b) AUC for postprandial irisin level. A greater AUC for postprandial irisin level was found in the EX trial. Data are presented at mean±SEM. $^{*}p<0.05$ compared with the NOEX trial. Abbreviations: EX, exercise; NOEX, no exercise; and AUC, area under the curve.

confidence interval (CI) −0.082 to 0.089), body fat (r=−0.240, p=0.604, 95% CI −0.412 to 0.266), or HOMA-IR (r=0.019, p=0.962, 95% CI −0.015 to 0.016) in the NOEX trial.

As shown in Figure 2, serum irisin levels increased following the 40-min exercise period in the EX trial with borderline significance (155.4±36.5 [SD] to 210.5±75.3 ng/mL, p=0.054).

The time courses for postprandial plasma glucose and IRI levels are shown in Figures 3 and 4, respectively. Although glucose levels increased during the 1-h period after the dietary intake compared with those at baseline in both trials, there was no difference in glucose levels between the trials throughout the 3-h observations (main effect of time: p<0,001, main effect of trial: p=0.787, interaction effect of time×trial: p=0.952, Fig. 3a). Insulin levels increased during the 3-h period after the dietary intake (except at 120 and 150 min in the NOEX trial) when compared with those at baseline in both trials (main effect of time: p<0.001, Fig. 4a). However, neither the main effect of trial (p=0.979) nor the interaction effect of time×trial (p=0.898) was significant. No differences were found in the AUC for postprandial glucose and insulin levels (Figs. 3b and 4b, respectively) between the trials.

Serum irisin levels were higher before and throughout the 3-h period after dietary intake in the EX trial compared with those in the NOEX trial (main effect of trial: p<0.001, Fig. 5a). A greater AUC for postprandial irisin levels was found in the EX trial compared with that found in the NOEX trial (35121±1799 vs 29080±1664 ng/mL·min, p=0.001, Fig. 5b). In contrast, dietary intake itself did not affect serum irisin levels in either trial (main effect of time: p=0.859, interaction effect of time ×trial: p=0.960, Fig. 5a).

The postprandial changes in mean EE in both trials are shown in Figure 6a. A significant effect of time was found (p=0.031) for EE as determined by two-way ANOVA, and the post-hoc test showed significant increase in EE at 180 min when compared with that at baseline in the EX group. However, the mean DIT (0.18±0.02 kcal/min in the NOEX trial vs 0.18±0.03 kcal/min in the EX trial, p=0.907, Fig. 6b) and REE (0.91±0.07 kcal/min in the NOEX trial vs 0.88±0.06 kcal/min in the EX trial, p=0.445) were not different between the trials. No significant correlation was found between the AUC for postprandial irisin levels and the AUC for DIT in all trials as well as in each trial.

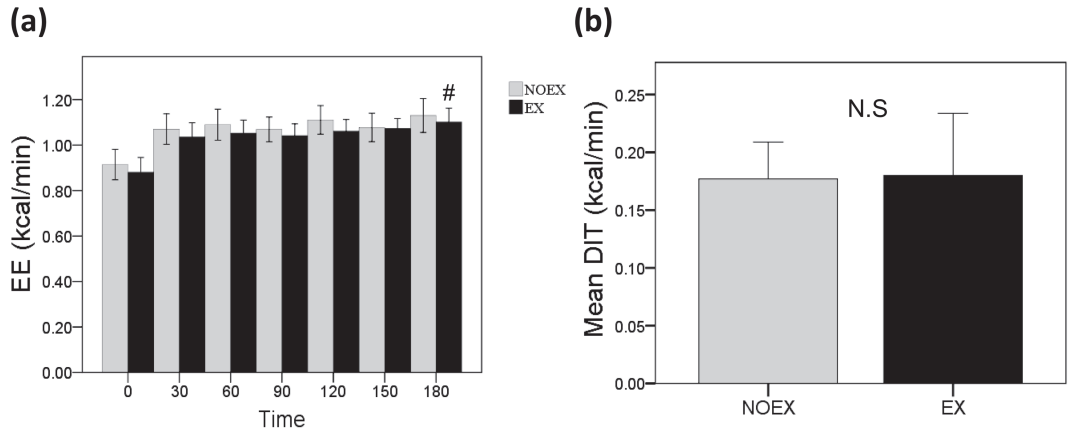

Figure 6. Comparison of postprandial changes in EE (a) and mean DIT (b) between the EX and NOEX trials. Mean DIT as well as REE was not different between the trials. All data are presented as mean±SEM. #$p<0.05$ vs 0 min. Abbreviations: EE, energy expenditure; DIT, diet induced thermogenesis; EX, exercise; NOEX, no exercise; and REE, resting energy expenditure.

Discussion

In the present study, we demonstrated that serum irisin levels increased after a 40-min exercise on a bicycle ergometer and that this increase in irisin levels did not influence DIT and REE measured on the following day in healthy subjects.

We observed that serum irisin levels showed a 36% increase in response to a single bout of exercise, and the elevation persisted at least until the next day in spite of the tendency to return to baseline. Our results were consistent with previous findings[14,19,20], most of which demonstrated a 1.2-1.3 fold increase in blood irisin levels following acute endurance or strength training. Some reports have shown a 24-h time course changes in blood irisin levels after a single bout of exercise[21,22]. According to these reports, blood irisin levels peaked immediately or approximately 1 h after acute exercise. The elevation persisted for several hours and then gradually returned to baseline in 24 h. In our study, the increased serum irisin levels were maintained during the postprandial period of the following day (15-18 h after a bout of exercise) in the EX trial, although they were lower than that immediately after the exercise. Taking these results into consideration, it seems that the elevation in blood irisin levels following a single bout of exercise persisted for approximately 24 h. Nygaard et al reported that transient increases in blood irisin levels after single bouts of intense endurance or heavy strength training was not accompanied by the increase in FNDC5 expression in the skeletal muscle[22]. Therefore, the release of irisin into the circulation in response to acute exercise seems to occur not from the transcriptional regulation of FNDC5 but from the cleavage of the ectodomain of FNDC5 at the cell surface.

To the best of our knowledge, this is the first report regarding the effect of irisin on DIT in humans. A few reports previously demonstrated that dietary intake alone did not affect blood irisin levels[14,15], although the caloric content of the standardized meal used in their experiments were considerably lower than that of our test meal. However, because irisin induces browning of subcutaneous adipose cells and increases thermogenesis in rodents[9], we hypothesized that irisin mediated the thermogenic effect of a bout of exercise in which increased whole-body EE was sustained for a few days even after the cessation of exercise. However, in contrast to our hypothesis, the elevation in serum irisin levels in the present study did not modulate DIT and REE.

The potential thermogenic effect of irisin is still controversial in humans. A cross-sectional study reported a significant correlation between plasma irisin levels and 24-h EE in the subjects whose EE was greater than that predicted from fat-free mass[12]. However, in another study, it was reported that there were no differences in serum irisin levels between brown adipose tissue (BAT)-detected women and BAT-undetected counterparts[23]. As recently reported by Wu et al, endurance training increased FNDC5 content in subcutaneous white adipose tissue (WAT) and whole-body EE; however, no increase in skeletal muscle FNDC5 content and circulating irisin levels was observed in rats[24]. The authors suggested that locally produced FNDC5 in WAT rather than circulating irisin mediated the exercise-induced browning of subcutaneous adipose cells and thermogenesis. The mechanism that mediates exercise-induced browning of subcutaneous adipose cells in humans has to be further examined. Huh et al suggested that irisin may have regulated muscle energy metabolism through the activation of adenosine monophosphate-activated protein kinase in conjunction with adenosine triphosphate (ATP) depletion in skeletal muscle following exercise[25]. The physiological role of irisin cleaved in muscle cells and secreted into the circulation in response to acute exercise needs to be determined.

In the present study, a single bout of exercise did not increase DIT or REE measured on the following day. Whether exercise training or physical activity favorably increases DIT is also a conflicting issue. Previous reports have demonstrated the correlation between high physical activity level or habitual exercise and increased DIT[26,27]. An interventional study has also reported that endurance training augmented DIT[28]. However, other studies including a relatively recent one have found no effect of habitual exercise on DIT[29]. DIT consists of two components: obligatory thermogenesis and facultative thermogenesis. Obligatory thermogenesis is an essential element for the digestion, absorption, and storage of nutrients, and facultative thermogenesis is derived from the stimulation of sympathetic nerve activity[30], which consequently increases the lipolytic responsiveness of adipocytes to catecholamines and cellular ATPase activity[31]. Therefore, it may be possible that exercise-enhanced sympathetic activity has a synergistic effect on the thermogenic response to dietary intake. The lack of the effect of exercise on DIT, particularly in elderly people or women, as shown in the previous reports, may be derived from a blunted responsiveness to β-sympathetic stimulation. Ohnaka et al has demonstrated that 1-h bicycle ergometer exercise preceding dietary intake did not change DIT[32]; our results were consistent with those. This may also be because it takes a certain time period to enhance responsiveness to β-sympathetic stimulation by exercise training. In the present study, we examined the acute effect of exercise on serum irisin levels and energy expenditure because a certain duration of training rather decreases irisin levels if it is accompanied by body weight loss[14]. However, a shorter period of training without the change in body weight or body fat may have resulted in augmentation of DIT in relation to the increase in blood irisin levels. In contrast to our results and those by Ohnaka et al, Denzer et al found that a bout of resistance exercise increased DIT[33]. The differences in the type of exercise and composition of the standardized meal may explain the discrepancy. A high carbohydrate meal as opposed to a high protein meal was used in their study. Thus, many factors are involved in determining DIT, and the issue warrants additional investigation.

Many previous studies have suggested that a single bout of exercise improved insulin sensitivity and that the improvement lasted for 12-48 h[34-36]. In the present study, however, postprandial plasma glucose and FIRI levels were not different between the trials, indicating that a single bout of exercise

did not influence insulin sensitivity. This may be because the young, healthy subjects in the present study originally presented with good insulin sensitivity, and thus, further improvements in insulin sensitivity following exercise were not observed. In addition, we found no significant correlation between serum irisin levels and HOMA-IR, BMI, or fat mass. Cross-sectional studies have investigated the correlation between blood irisin levels and insulin resistance. There was a significant discrepancy between their results according to the characteristics of study population, including age, gender, anthropometry, and the presence of diabetes[37-39]. Nevertheless, most of these reports concluded that muscle mass is the main predictor of irisin production. Unfortunately, we did not directly evaluate fat-free mass of the subjects. Further studies are needed to clarify the effect of circulating irisin on glucose metabolism.

There are limitations to the present study. First, several recent investigations have questioned the validity of the commercial ELISA kits to evaluate serum irisin levels. Polyclonal antibodies used in those kits were reported to have cross-reactivity with non-specific proteins[40]. Therefore, mass spectrometry is now becoming the gold standard for the identification of irisin. Although we used the most common ELISA kit (#EK-067-52) on which many reports concerning irisin to date were based, the method may possibly have affected the quantification of exercise-induced elevation in serum irisin levels. Second, the small number of subjects may not be enough to verify the effect of a bout of exercise on DIT because of the high intra-individual variation of DIT. In the cases when DIT was evaluated by the method used in the present study, the intra-individual coefficients of variance (CVs) for DIT were reported to reach as high as 15%-23%, particularly in women[41,42], although the results were the same when we analyzed only in male subjects (data not shown). The high variation is mainly due to the small magnitude of thermogenic response of DIT assessed over a prolonged period as well as daily biological variations. Therefore, a small sample size could contribute to inaccurate statistics. Furthermore, our 180-min assessment of DIT may not be long enough because EE remained elevated 180 min after the dietary intake compared to baseline in the EX trial. Longer assessment period possibly brought about different results.

We demonstrated that serum irisin levels were increased in response to a single bout of exercise, and the increased levels persisted at least until the next day. In contrast, DIT and REE was not affected by exercise performed the previous day in spite of the prolonged elevation in serum irisin levels. Further studies are required to address the mechanism by which exercise causes prolonged elevation in EE even after the cessation of exercise, with emphasis on hormonal effects including irisin.

Acknowledgments

We would like to thank our volunteers for their time and participation in this investigation.

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