Variations in leptin, nesfatin-1 and irisin levels induced by aerobic exercise in young trained and untrained male subjects

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ABSTRACT: The aims of this study were to investigate the impacts of acute aerobic exercise on circulating levels of hormones associated with energy metabolism, namely leptin, nesfatin-1 and irisin, in trained and untrained male subjects and to determine whether the timing of the exercise (i.e. morning or night) amplified these impacts. Thirty trained (19.2±0.7 years) and 30 untrained (19.5±0.6 years) male subjects performed two aerobic running exercises (3 days between tests) to 64-76% of the subjects' maximal heart rate for about 30 min. Pre- and post-exercise venous blood samples were taken and analysed for leptin, nesfatin-1 and irisin using enzyme-linked immunosorbent assay (ELISA). Paired samples and independent samples t-tests were used to analyse data. Irisin levels increased in all the subjects ($p < 0.001$). In both groups, nesfatin-1 levels increased significantly after the night-time exercise ($p < 0.05$). Importantly, leptin and nesfatin-1 levels varied among the trained and untrained groups: Both leptin and nesfatin-1 levels increased in 4 (13%) and 12 (40%) subjects, respectively, after the morning exercises, and they increased in 9 (30%) and 10 (33%) subjects, respectively, after the night-time exercise. They decreased in 5 (16%) and 7 (23%) subjects, respectively, after the morning exercise and in 6 (20%) and 3 (10%) subjects, respectively, after the night-time exercise. Exercise may result in increased energy consumption by altering irisin levels. However, due to variations among individuals, increasing leptin and nesfatin-1 levels by reducing food intake may not be applicable.

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INTRODUCTION

It is generally accepted that exercise has important beneficial effects on metabolic disorders associated with impaired energy homeostasis [1]. It is also known that there is a delicate balance in the energy consumption to energy intake ratio and that the molecular mechanism underlying this balance is complex [2]. Many critical processes, including those related to hormonal factors and the nervous system, play a role in maintaining this balance [2]. The importance of skeletal muscle and adipose tissue as secretory organs of many energy-regulating hormones is well known [3]. However, the exact mechanisms by which exercise regulates energy homeostasis via energy-related hormones are unclear.

The effect of exercise on altering the levels of hormones involved in energy regulation is an interesting topic, which has attracted a lot of research attention, with a particular focus on leptin, nesfatin-1 and irisin [4-7]. Leptin plays an important function in energy homeostasis by suppressing food intake and increasing energy expenditure [5]. The results of studies on the relationship between acute exercise and leptin levels have been inconclusive, with some reporting decreases [8, 9], others reporting increases [10] and some finding no change [11].

Previous research identified nesfatin-1 as a new hypothalamic anorectic peptide, which is involved in metabolic regulation and food intake via a leptin-independent mechanism [6]. However, studies of the effects of exercise on nesfatin-1 levels have described varied findings, with no changes [12], increases [13] and decreases [14] reported.

In recent years, a new myokine hormone, irisin, was identified [7]. Irisin is an exercise-related hormone, which increases the body's energy consumption rate and reflects subjects' metabolic status. Irisin has shown promise as an agent for the monitoring and treatment of diseases, including obesity and diabetes, associated with metabolic impairment.

The functions of hormones in increased energy consumption are well known. It is important to also clarify the effects of exercise on hormones, such as leptin, nesfatin-1 and irisin, that are involved in the regulation of energy homeostasis [4, 15] because aerobic exercise may be used as a non-pharmacological treatment to stimulate the levels of these hormones and improve the energy balance. Exercise-induced increases in leptin, irisin and nesfatin-1 levels may infer reduced food intake and increased energy consumption. Thus far, no

studies have examined the effects of acute exercise on variations in leptin, nesfatin-1 and irisin levels in individual subjects while considering whether the timing of the exercise (i.e. morning or night) augmented these effects.

The aim of this study was to evaluate the effects of acute aerobic exercise performed at two different time points (daytime and nighttime) on the regulation of three different energy regulatory hormones (leptin, nesfatin-1 and irisin) in trained and untrained subjects.

MATERIALS AND METHODS

Subjects

The study was carried out in accordance with the Declaration of Helsinki, and it was approved by the local ethics committee. All the participants provided signed written informed consent prior to the study.

Sixty young healthy male subjects (trained, $n=30, 19.2\pm0.7$ years; untrained $n=30$, 19.5 ± 0.6 years) volunteered to participate in this study. The demographic characteristics of the subjects at baseline are shown in Table 1. The trained subjects had a history of regular exercise for at least 5 years, exercising at least 3 days per week in a local amateur soccer team. The untrained subjects had no more than 1 h/week of regular activity for at least 1 year.

All the subjects completed a medical questionnaire and a medical examination to ensure that they were non-smokers; were not taking any medication; were free of cardiac, respiratory, renal and metabolic diseases; were not using steroids; were not currently on a weight gain/weight loss diet (body mass index between 18 kg/m^2 and 25 kg/m^2]; and were in good health. Other than body fat mass, there were no differences in the anthropometric data of the trained and untrained groups.

Body composition was assessed using leg-to-leg bioelectrical impedance (Tanita Body Fat Analyser, TBF 300 M, Tanita, Tokyo, Japan). The measurements of body composition were standardized, and the same investigator performed all the measurements in the morning, using the same equipment. The subjects were asked to avoid liquid and food intake before the measurements. The validity of bioelectrical impedance in the measurement of body composition has been documented [16].

Prior to data collection and during the protocol period, the trained and untrained subjects were instructed not to change their normal eating habits and to refrain from additional vitamin or antioxidant dietary supplementation. The subjects were also instructed to abstain from exhaustive exercise during the 72-h pre- and post-exercise period, except for the functional tests. Furthermore, they were asked to maintain their normal dietary programmes and to avoid meals with a high fat content throughout the duration of the study. In addition, the subjects were asked to refrain from consuming alcohol and caffeine 24 h before testing.

Exercise intervention

The subjects reported to the exercise field between 08:00 and 10:00 h after an overnight fast and at 20:00 and 22:00 h, having had a light meal at least 3 h before exercising. Each participant undertook the exercises at the same time each day.

All the individuals randomly performed two aerobic running exercises in about 30-min bouts on the field at an intensity corresponding to 64–76% of their predicted maximal heart rate [17].

Tests were carried out at intervals of 72 h. Each subject's heart rate was monitored throughout every 30-min session using a Polar heart-rate monitor to ensure that the exercise intensity remained at the prescribed level.

Blood collection and biochemical analysis

Venous blood samples were taken from the antecubital vein in aprotinin-containing tubes before the exercise (used as the baseline) and again immediately after the end of the exercise. Serum was separated immediately by centrifuging at 4000 rpm at 4°C for 5 min. It was then frozen and stored at –80°C for subsequent analyses, which were performed within 4 weeks. The levels of leptin, nesfatin-1 and irisin in the samples were analysed in a double-blind fashion.

Serum levels of leptin (range: 62.5–4000 pg/ml) were measured using a commercial enzyme linked-immunosorbent assay (ELISA) kit (Boster Biological Technology Co Ltd., USA; Cat. no: EK0437). The intra- and inter-assay coefficients of variation and sensitivity for leptin were lower than 10% and 15%, respectively.

Serum levels of nesfatin-1 (range: 31.2–2000 pg/ml) were measured using a commercial ELISA kit (Boster Biological Technology Co Ltd., USA; Cat. no: EK1138). The intra- and inter-assay coefficients of variation and sensitivity for nesfatin-1 were lower than 10% and

TABLE I: Physical characteristic of the subjects, body mass index (BMI), fat free mass (FFM) and fat mass (FM).

	Age (yr)	Height (cm)	Weight (kg)	BMI (kg/m^2)	FFM (kg)	FM (kg)
Trained	19.2±0.7	176.6±1.3	66.9±1.6	21.3±0.4	60.44±1.41	6.43±0.46*
Untrained	19.5±0.6	175.8±0.8	67.3±1.6	21.7±0.4	59.09±1.04	8.39±0.64

Note: *represent significantly difference compared to the untrained subjects

15%, respectively. The ELISA assays of leptin and nesfatin-1 had a sensitivity of <10 pg/ml.

Irisin levels were also measured using a commercial ELISA kit (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). The intra- and inter-assay coefficients of variation and sensitivity were 5.61% and 14.56% and 0.78 ng/mL, respectively.

Statistical analysis

The data are expressed as means (± standard error [SE]). A paired *t*-test was used to analyse the significance of within-group comparisons of the data. The statistical analyses of between-group data were performed using an independent *t*-test. A linear regression analysis was used to analyse the correlation of body weight and fat mass with baseline hormone levels. A value of $p < 0.05$ was accepted as statistically significant.

RESULTS

There were no statistically significant changes in pre- and post-exercise (mean ± SE) leptin levels between the daytime and night-time exercises in the trained (3675±385 pg/ml vs. 3502±360 pg/ml $p=0.7$ and 3737±315 pg/ml vs. 3899±405 pg/ml $p=0.7$) and untrained (5237±585 pg/ml vs. 5193±557 pg/ml $p=0.06$ and 5020±547 pg/ml vs. 5102±595 pg/ml $p=0.3$) groups.

However, when the data were analysed for each individual, the leptin levels varied after the daytime and night-time exercises (Fig. 1). In the trained group, after the daytime and night-time exercises, leptin levels increased in 8 (26.7%) and 14 (46.7%) subjects, respectively. However, they decreased in 22 (73.3%) and 16 (53.3%) subjects, respectively. In the untrained group, after the daytime and

night-time exercises, leptin levels increased in 16 (53.3%) and 15 (50%) subjects, respectively, and they decreased in 14 (46.7%) and 15 (50%) subjects, respectively. Leptin levels increased in only 3 (10%) subjects in the trained group and in 11 (36.7%) subjects in the untrained group. Decreases in leptin levels occurred in 11 subjects (36.7%) in the trained group and in 10 subjects (33.3%) in the untrained group.

Basal leptin levels normalised to total body weight in the morning (54.71±5 pg/ml/kg vs. 75.56±7 pg/ml/kg, $p=0.02$) and at night (55.68±4 pg/ml/kg vs. 72.59±6 pg/ml/kg, $p=0.03$) were significantly lower in the trained group compared to those of the untrained group. However, basal leptin levels normalised to fat mass in the morning (595.17±50 pg/ml/kg vs. 642.12±45 pg/ml/kg, $p=0.4$) and at night (617.68±43 pg/ml/kg vs. 619.53±40 pg/ml/kg, $p=0.9$) were not significantly different in the trained group compared to those of the untrained group.

In addition, the linear regression analysis showed significant relationships between fat mass and baseline leptin levels in the morning and at night in the trained group ($R=0.66242$ and $p<0.0001$ vs. $R=0.68329$ and $p<0.0001$) and untrained group ($R=0.60355$ and $p<0.0001$ vs. $R=0.63572$ and $p<0.0001$).

In the morning, there were no significant changes in pre- and post-exercise (mean±SE) nesfatin-1 levels in the trained (112.2±5 pg/ml vs. 120.6±5 pg/ml) and untrained (194.5±12 pg/ml vs. 202.9±13 pg/ml) groups. However, statistically significant increases in nesfatin-1 levels were observed after the night-time exercise in both the trained (109.6±6 pg/ml vs. 124.5±6 pg/ml, $p=0.03$) and untrained (172.5±12 pg/ml vs. 187.9±12 pg/ml, $p=0.01$) groups.
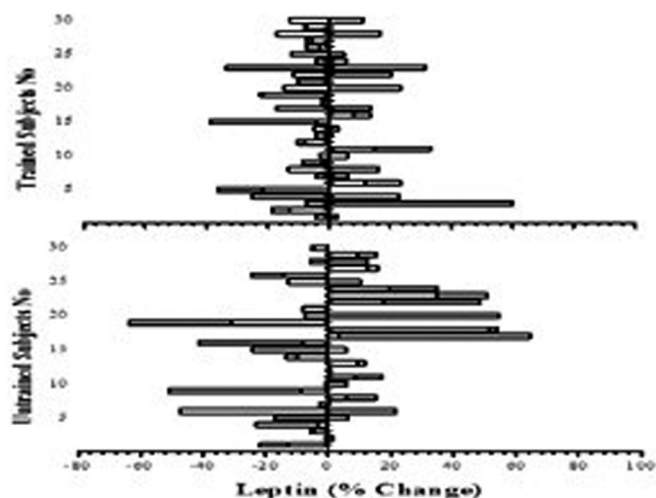
FIG. 1. Percentage change in the leptin levels of each subject in the trained and untrained groups ($n=30$ in each) during the exercise performed in the morning (white column) and at night (grey column).
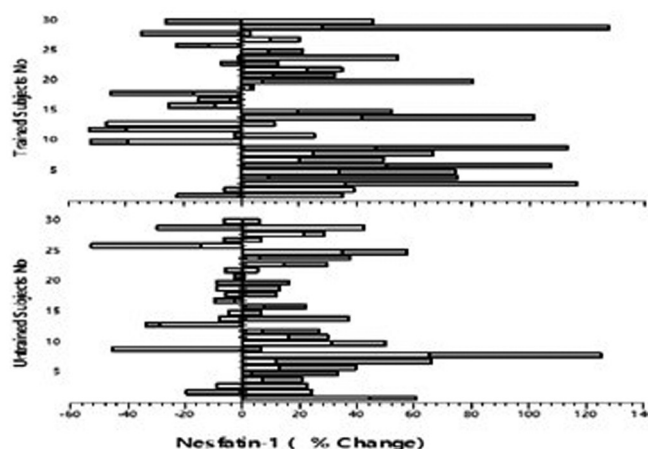
FIG. 2. Percentage change of nesfatin-1 levels of each subject in the trained and untrained groups ($n=30$ in each) during the exercise performed in the morning (white column) and at night (grey column).
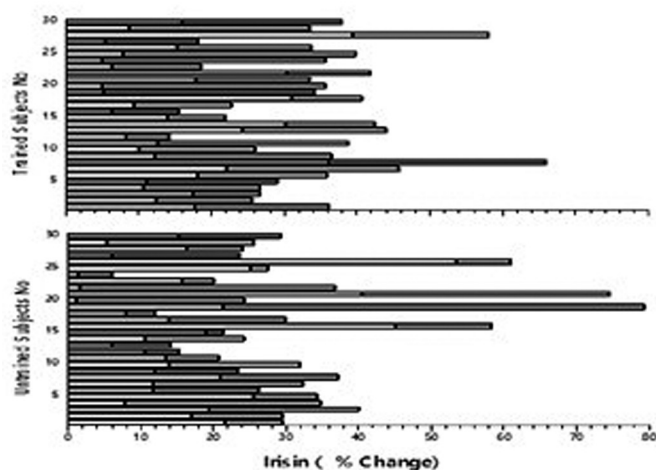
FIG. 3. Percentage change of irisin levels of each subject in the trained and untrained groups ($n=30$ in each) during the exercise performed in the morning (white column) and at night (grey column).

The nesfatin-1 levels of each subject in both exercise groups are shown in Figure 2. In the trained group, nesfatin-1 levels increased in 21 (70%) subjects after the daytime exercise, and they increased in 19 subjects (63.3%) after the night-time exercise. In 16 (53.3%) subjects, nesfatin levels increased after both exercise times. In contrast, nesfatin-1 levels decreased in 9 (30%) subjects after the morning exercise, and they decreased in 11 (36.7%) subjects after the night-time exercise. They decreased in 6 (20%) subjects after both exercise times.

In the untrained group, nesfatin-1 levels increased in 19 (63.3%) subjects after the morning exercise, and they increased in 22 subjects (73.3%) after the night-time exercise. Nesfatin-1 levels increased in 14 (46.7%) subjects after both exercise times. In contrast, nesfatin-1 levels decreased in 11 (36.7%) and 8 (26.7%) subjects after the daytime and night-time exercises, respectively. They decreased in 3 (10%) subjects after both exercise times.

Basal nesfatin-1 levels normalised to total body weight were significantly lower in the trained groups compared to those of the untrained group in the morning (1.687 ± 0.08 pg/ml/kg vs. 2.915 ± 0.20 pg/ml/kg, $p<0.0001$) and at night (1.657 ± 0.09 pg/ml/kg vs. 2.585 ± 0.18 pg/ml/kg $p<0.0001$). There was no significant correlation between nesfatin-1 levels and body fat mass during exercise performed in the morning or at night in the trained group ($R=0.07$ and $p=0.6$ vs. $R=0.03$; $p=0.8$) and untrained group ($R=0.2$ and $p=0.2$ vs. $R=0.1$; $p=0.4$).

In both the trained and untrained groups, irisin levels increased significantly after the morning exercise (254.5 ± 6 ng/ml vs. 292.7 ± 6 ng/ml, $p<0.0001$; 116.0 ± 2 ng/ml vs. 139.7 ± 3 ng/ml, $p<0.0001$) and night-time exercise (266.8 ± 4 ng/ml vs. 314.5 ± 5 ng/ml, $p<0.0001$; 116.9 ± 2 ng/ml vs. 137.3 ± 3 ng/ml,

$p<0.0001$). As shown in Figure 3, irisin levels were elevated in all the subjects in both groups after both exercise times.

In contrast to the findings on leptin and nesfatin-1, baseline irisin levels normalised to total body weight were significantly higher in the trained group compared to those of the untrained group in the morning (3.858 ± 0.11 ng/ml/kg and 1.750 ± 0.05 ng/ml/kg, respectively; $p<0.0001$) and at night (4.056 ± 0.12 ng/ml/kg and 1.763 ± 0.04 ng/ml/kg, respectively; $p<0.0001$). There were no correlations between baseline irisin levels and body weight during the exercise performed in the morning or at night in the trained group ($R=0.2$ [$p=0.1$] vs. $R=0.1$ [$p=0.3$]) or untrained group ($R=0.02$ [$p=0.8$] vs. $R=0.09$ [$p=0.6$]).

DISCUSSION

This investigation aimed to evaluate the effects of two bouts of moderate-intensity exercise, performed in the morning and at night, on individual responses of circulating levels of the appetite-regulatory hormones leptin, nesfatin-1 and irisin in trained and untrained participants.

Leptin response

Based on the results of the statistical analyses of the pre- and post-exercise mean leptin values, acute aerobic exercise performed in the morning and at night seemed to have no significant effects on leptin levels in either the trained or untrained groups. The absence of any change in leptin levels during exercise is in accordance with the results of some previous studies [11, 18], but it contrasts with findings of exercise-induced decreases in leptin levels [9]. Based on the finding of individual-based variations in leptin levels (i.e. increase, decrease or both) at both exercise times (morning and night), it appears reasonable to question whether leptin is an exercise-related hormone (Fig. 1). It should be emphasised that the observed individual-based variations in leptin levels following the exercises were not associated with increased stress-related exercise. Exercise-induced changes in energy balance may have a greater effect on leptin levels [19]. Regarding possible circadian regulation, the present study, which measured leptin levels in the morning and at night, did not support the findings of a previous study [20], which reported the lowest leptin levels in the morning.

Nesfatin-1 response

Interestingly, we found statistically significant increases in nesfatin-1 levels after the night-time exercise in both groups. Despite the non-significant change in the mean values of nesfatin-1, these values varied widely among the subjects in both groups after the morning exercise (Fig. 2). The observation of variations in exercise-induced responses of nesfatin-1 among individuals are in accordance with the results of some previous studies [12,13,14].

The mechanism underlying the exercise-induced nesfatin-1 response is not well known. The present results showed that night-time exercise amplified nesfatin-1 levels. Furthermore, baseline nesfatin-1

levels (normalised to body weight) of the untrained group were significantly higher than those of the trained group. It is difficult to draw any definitive conclusions about the causes of the differences in nesfatin-1 levels among the subjects or about the effects of night-time exercise. Possibly, they may be explained by differences in the subjects' nutritional status [21, 22], diet composition [23] or thermoregulation [24].

Irisin response

When irisin was first discovered, it was described as a muscle-derived hormone (myokine), which was secreted in response to exercise [7]. The results of the present study confirmed this finding (Fig. 3). Elevated irisin levels following acute exercise could be related to exercise-induced stimulation of sympathetic activity [25]. In the present study, the higher baseline irisin levels observed in the trained subjects compared to those of the untrained subjects, despite their similar body weights, pointed to a possible role of increased fitness status. A previous study suggested that the muscles cells in trained subjects stimulated irisin secretion, which might provide protection against carbohydrate and lipid metabolism disorders [26]. Studies also suggested that higher baseline irisin levels in trained subjects might be attributed to the content or type of muscle cell rather than total muscle mass [27, 28]. The results of the present study suggest that serum irisin levels may be upregulated in accordance with the training status of the individual. Thus, at a cellular level, exercise training may improve bioenergetic functions of skeletal muscle, including the release of irisin.

Exercise-induced anorexia has attracted the interest of many investigators. Theoretically, using exercise to amplify levels of leptin, nesfatin-1 and irisin hormones may represent a non-pharmacological treatment to improve energy balance. Previous studies demonstrated the effectiveness of leptin [5] and nesfatin-1 [6] in reducing food intake and increasing energy expenditure [29, 30]. Studies also showed that irisin significantly increased energy consumption and oxidative metabolism [28, 31].

However, there are conflicting results in the literature on exercise and energy balance, with some studies reporting that acute exercise reduced or increased energy intake but others finding that it had no effect on energy intake [32, 33]. To shed light on this issue, it needs to be determined whether the use of mean data or individual-based data is the most effective for analysing the effect of acute exercise on hormone levels. In the present study, we did not measure energy consumption during the exercises or post-exercise energy intake. The variation in the hormone levels of the subjects may explain the different observations on exercise-induced energy consumption versus post-exercise energy intake.

The timing of exercise seems to be important with regard to increasing nesfatin-1 levels. However, it is not known whether night-time exercise-induced increases in nesfatin-1 levels can suppress nocturnal food intake. A previous study reported that exogenous administration of central nesfatin-1 reduced food intake during spontaneous nocturnal feeding [34]. Future studies should determine the optimal change in levels of energy-regulated hormones during exercise to obtain clinically acceptable benefits for suppressing food intake and increasing energy consumption.

CONCLUSIONS

Regarding group differences, the present study revealed no significant changes in serum leptin levels in response to acute exercise bouts in the morning compared to at night in either trained or untrained male individuals. In contrast, nesfatin-1 levels increased in both groups after the night-time exercise, and irisin levels increased significantly in both groups and at both time points. Both the timing of the exercise (morning vs. night) and individual responses to the exercise influenced levels of leptin and nesfatin-1, which regulated energy intake. Investigators should focus on intra- and inter-individual variations in circulating levels of leptin, nesfatin-1 and irisin and analyse their relationships with body fat mass.

Conflict of interest statement

The authors declared no conflict of interests regarding the publication of this manuscript.

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