Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
C M B Association Publisher

Original Research

Nesfatin-1 and irisin levels in response to the soccer matches performed in morning, afternoon and at night in young trained male subjects

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Received January 14, 2018; **Accepted** June 30, 2018; **Published** July 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.10.20

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Abstract: This study aimed to investigate the potential effects of acute soccer matches performed in morning, afternoon and at night on both nesfatin-1 and irisin levels in trained subjects. Total of 20 male subjects performed in soccer matches at three different times of day: morning, afternoon, and night. Pre- and post-match venous blood samples were taken, and levels of both nesfatin-1 and irisin were analysed using the enzyme-linked immunosorbent assay (ELISA). Following all matches, the subjects' irisin levels increased significantly in all subjects ($p < 0.0001$). Nesfatin-1 levels were also increased after the matches; however, the increase was statistically significant for morning ($P=0.01$) and night-time ($p=0.009$). The subjects' nesfatin-1 levels did not increase in all subjects and decrease of nesfatin-1 levels observed in some subjects after matches. This study finds that soccer matches performed different workout times have strong stimulatory effects on irisin levels in all subjects but nesfatin-1 response varied among the subjects and it did not change significantly in afternoon match.

Key words: Energy; Irisin; Metabolism; Nesfatin-1; Soccer match.

Introduction

Cardiopulmonary exercise testing has widely used procedure in clinical medicine and sports science because of its beneficial role on decision making process in subjects metabolic fitness status. Body metabolic system is critically depended on the balance between energy intakes to consumption, called as metabolic homeostasis. The important preventative effects of regular physical activity on impairments of body metabolic system have been well established. Skeletal muscle and adipose tissue have been identified as important secretory organs of irisin and nesfatin-1 (1). The increased physical activity may have an important effect on release of miyokines and adipokine hormones (1).

It has been shown that nesfatin-1 is an anorectic peptide derived from the posttranslational processing of the nucleobindin 2 genes, and it is involved in energy homeostasis, metabolic regulation and feeding behaviour (2-4). Irisin, an important hormone secreted by myocytes and adipocytes, has also been proposed to regulate several physiological and metabolic processes, including metabolic control (5, 6).

In literature, there is considerable confusion among previously published data about the magnitude of the contribution of exercise to nesfatin-1 levels. During acute exercise, a significant decrease in nesfatin-1 levels (7), an increase in nesfatin-1 levels (8) and unchanged nesfatin-1 levels have all been reported (9). Aerobic running exercise may cause significant variations in nesfatin-1 levels of each individual subjects with regarding exercise time performed at morning and night (10). Notably, an increase of irisin levels in response to acute and chronic exercise was a major finding for some researchers (5, 11, 12).

Application of various type and duration of exercise protocols in laboratory condition provides valuable measurements on the alteration of nesfatin-1 and irisin levels. However, effects of various intensity mixed exercise performed outside the laboratory condition on nesfatin-1 and irisin levels has not been studied yet. It is known that soccer games contains various type of exercise, including vigorous exercise intercepted with moderate to lower exercise intensity (13, 14) and has very effective influence on energy consumption rate. It is not known that soccer game had differential effects on energy regulation hormones of irisin and nesfatin-1 depending on the time of day.

In the present study, effects of soccer game played in the morning, afternoon and late night on the energy-regulating hormones nesfatin-1 and irisin were examined in trained young male subjects.

Materials and Methods

This study's protocol was approved by the Ethics Committee at Firat University. Prior to the study, all experimental procedures, benefits and risks were fully explained to all participants. Written informed consent in accordance with the Declaration of Helsinki was obtained from trained subjects who had participated in physical activity three to five times per week for at least five years via soccer competitions in the province. The physical characteristics of the subjects are given in

Table 1. Physical characteristics of trained subjects.

	Subjects
Age (year)	18.4 ± 0.1
Weight (kg)	61.5 ± 1.7
Height (cm)	174 ± 1.9
BMI (kg/m^2)	20.3 ± 0.6
FM (kg)	6.26 ± 0.5
FFM (kg)	55.25 ±1.3

Values are mean ± S.E. BMI, body mass index; FM, fat mass; FFM, fat free mass.

Table I.

All subjects completed a medical questionnaire and a medical examination to ensure that they were not taking any medication; were free of cardiac, respiratory, renal and metabolic diseases; were not using steroids; and were in good health. Body weight and body composition of the subjects were measured using a bioelectrical impedance analyser after an overnight fast (Tanita Bioelectrical Impedance; TBF 300M, Tanita, Tokyo, Japan). Prior to data collection and during the protocol period, soccer players were instructed not to change their normal eating habits and to refrain from additional vitamin or antioxidant dietary supplementation. Subjects were also instructed to abstain from exhaustive exercise during the 72-hour pre- and post-match period, except for functional tests.

Exercise protocols

Trained subjects were asked to perform exercise workouts by way of an indoor recreational soccer match. All soccer matches were performed on the same field (30 m x 50 m), included the same subjects (one goalkeeper and seven players) and occurred on three different days between either 8:00 and 10:00 am (morning, M), 2:00 and 4:00 pm (afternoon, A), or 8:00 and 10:00 pm (night, N). The sequence of exercise times was randomly chosen. The matches lasted 60 min. each (30 min. in the first half and 30 min. in the second half, without rest), and there were 3 days between each soccer match.

Blood collection and biochemical analysis

Venous blood samples were taken in aprotinin-containing tubes both before each match as a baseline and immediately after the match. The serum was separated and immediately centrifuged at 4.000 rpm at 4 °C for 5 min. and then frozen and stored at -80 °C for subsequent analyses to be performed within 4 weeks. The samples were analysed for nesfatin-1 and irisin via the double-blind method.

Serum nesfatin-1 levels were measured using a commercial enzyme linked-immunosorbent assay (ELISA) kit with a measurement range of 31.2 pg/mL to 2,000 pg/mL (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 15%, respectively.

Plasma irisin levels were measured using an ELISA kit (Phoenix Pharmaceuticals Inc, Burlingame, California, USA). A number of validation studies have been performed using the ELISA kit. The detection range of the kit was 0.1–1000 ng/ml, with intra- and inter-assay coefficients of variation and sensitivity of 5.61% and 14.56%, respectively.

Statistical Analyses

Data are expressed as mean ± S.E.. The Wilcoxon signed-rank test, which is for non-parametric comparisons, was used to analyse the significance of within-group comparisons of data. A value of $p < 0.05$ was accepted as statistically significant, and $p < 0.0001$ was accepted as highly statistically significant.

Results

The percentage of (mean ± SE) altered nesfatin-1 levels in response to the soccer matches performed in the morning, in the afternoon, and at night are presented in Figure 1.

We observed significant increases in nesfatin-1 levels after the soccer match performed in morning (from 95.7 ± 7 pg/ml to 117.3 ± 8 pg/ml) (P=0.01) and at night (from 92.0 ± 5 pg/ml to 116.7 ± 9 pg/ml) (p=0.009). However, nesfatin-1 levels did not increased significantly (from 107.2 ± 7 pg/ml to 113.5 ± 6 pg/ml) after the soccer matches performed in afternoon (p=0.4).The analysis of the data obtained from all soccer matches showed that the baseline nesfatin-1 levels did not significantly differ in three games (Figure 1).

In the present study, the all players showed consistent increases in irisin levels after soccer matches performed in the morning, afternoon and at night (Figure 2). There were significant increases in irisin levels in morning (from 240 ± 4 ng/ml to 279 ± 3 ng/ml, p<0.001), afternoon (from 251 ± 3 ng/ml to 301 ± 2 ng/ml, p<0.001) and at night (from 263 ± 3 ng/ml to 309 ± 3 ng/ml, p<0.001) soccer matches. The baseline irisin levels increased significantly ($p < 0.001$) in both the afternoon and night matches compared to the morning match.

Discussion

The present study was the first to examine both nesfatin-1 and irisin levels in trained subjects during three acute soccer matches, which were performed in the morning, in the afternoon and at night. Since the first

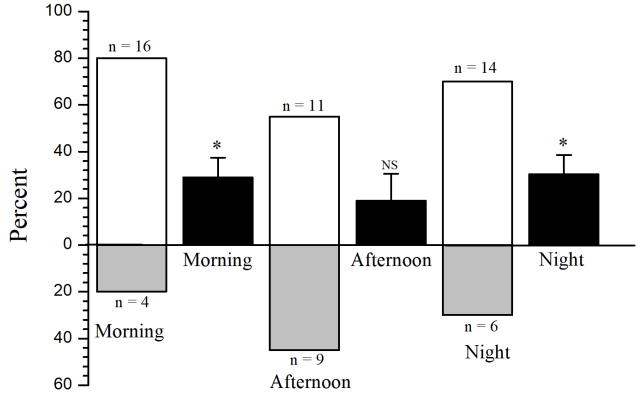

Figure 1. The percentage and number of subject have an increased (white column) or decreased (grey column) in nesfatin-1 levels after the soccer matches in morning, afternoon and at night. The (Mean ± S.E.) percentage increase of nesfatin-1 levels (black column) in morning, afternoon and at night soccer matches. The symbol * represent statistically significant differences between basal and end-match values ($p < 0.05$). NS: not significant.

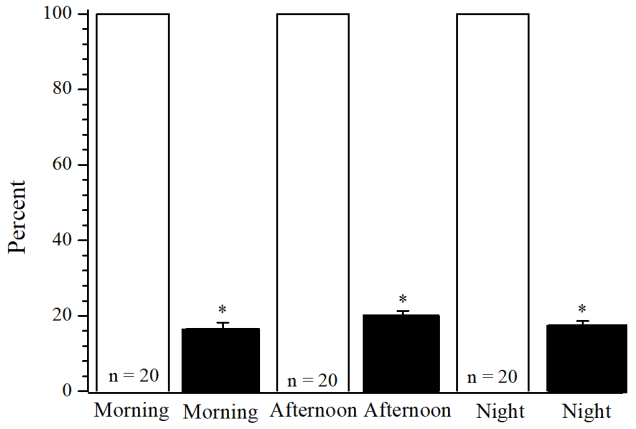

Figure 2. The percentage and number of subject have an increased (white column) in irisin levels after the soccer matches in morning, afternoon and at night. The (Mean ± S.E.) percentage increase of irisin levels (black column) in morning, afternoon and at night soccer matches. The symbol * represent statistically significant differences between basal and end-match values ($p < 0.0001$).

identification of nesfatin-1 and irisin, the results of related studies have revealed its possible role in the treatment of and/or preventative actions for serious metabolic disorders, including diabetes and obesity (15-18).

Soccer game is an intermittent team sport and characterised by a combination of some factors including physical, psychological, technical and tactical (13). This game is one of the most popular sports in most countries in the world. During soccer match, player efforts could be varies from low to high intensity, contains jump and sprint (19). However, the game intensity is mostly performed aerobically (14).

The results of previous studies clearly showed that exercise may have beneficial effects on many orexigenic and anorexigenic hormones that lead to a negative energy balance and body weight improvement (20, 21). To the best of our knowledge, there have been no studies concerning the effects of acute soccer matches performed at different times of day in terms of both nesfatin-1 and irisin hormones.

In the present study, the players showed neither consistent increases nor consistent decreases in nesfatin-1 levels after soccer matches performed in the morning, afternoon and at night (Figure 1). Statistically significant increases in nesfatin-1 levels were observed after morning and night-time soccer matches (Figure 1). Signifcantly increased nesfatin-1 levels following training at high intensity of exercise in sedantery overweight men (8) and during boxing and taekwondo exercises (energy supplement based on anaerobic metabolism) have been reported (22). In contrast, no significant effects of anaerobic sprint exercise and kickboxing on plasma nesfatin-1 levels has been reported (9).

Despite wide documentation of the noticeable effects of nesfatin-1 on negative regulation of energy balance and food intake, its response during exercise-induced energy consumption is still poorly understood. Increased nesfatin-1 is known as an indicator of anorectic effects (2) and it is logical to expect decreases following exercise due to the increased energy deficit. The exact mechanisms responsible for the increased nesfatin-1 levels after morning and night-time soccer matches remain unclear. Several factors have been shown to influence nesfatin-1 levels, including nutritional status (23), metabolic impairments (15), diet composition (24), thermoregulation (25) and circadian rhythm (26).

It has been shown that a single bout of moderate and high intensity exercise performed in the afternoon cause modest increases in circulating irisin concentrations in obese females (27). In our study, irisin levels increased after all soccer matches (Figure 2). The increase in circulating irisin levels is consistent with the results of other studies (5, 11, 28). However, one report found that acute exercise does not cause an increase in irisin levels (29). It has been shown that endurance and resistance training causes an increase in irisin levels in both healthy and older subjects (5, 10, 30).

The increased irisin levels we found may reflect an alteration of the metabolic status of subjects' muscle cells during exercise-induced stress. Surprisingly, the baseline irisin levels increased significantly in afternoon and at the night-time soccer match. Previous observations have suggested that the obtained differences in pre- and post-match irisin levels are not a function of diurnal rhythm (31).

This study has some limitations that should be considered. First, a possible limitation of this study is the relatively small sample size of only young and healthy male subjects. Future studies should be performed in large subject groups with both male and female older or obese subjects to understand the relationships between exercise and levels of nesfatin-1 and irisin. Second, both the nesfatin-1 and irisin levels were evaluated immediately after soccer matches. It would be valuable to instead measure these hormone levels many hours post-exercise to provide a better interpretation of how exercise affects levels of both hormones as well as their subsequent influence on energy balance.

Acute exercise may affect energy balance via alteration of nesfatin-1 and irisin levels in addition to its well-known mechanical-activity-induced increase in energy consumption.

Acute soccer matches performed in morning, afternoon and at night may influence both nesfatin-1 and irisin levels, which could be considered by exercise scientists and clinicians. Especially, morning and night-time soccer matches seems to be the preferable exercise times for the improvement of metabolic system regulation via increased nesfatin-1 and irisin hormones, which may have important implications for both weight management and impaired energy metabolism.

Disclosure of conflict of interest

All authors have no any potential conflicts of interest associated with this research.

Funding/support

This work was funded and supported by the by Firat University Scientific Support Unit (FUBAP) (TF. 14. 68.).

Authors' contributions

Each author have participated sufficiently in the work to take public responsibility for appropriate portions of the content. All authors have read and approved the final version of the manuscript and agree with the order of presentation of the authors.

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