Original article

Comparatively evaluating the effects of exercising at the anaerobic threshold on oxidative stress and serum levels of leptin, nesfatin-1 and irisin in sedentary male and females

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Abstract. *Study Objectives*: Exercise has a great impact in increases of energy metabolism and imbalance of oxidant-antioxidant status. We aimed to analyse the association between exercise-induced oxidative stress and energy regulatory hormones of leptin, nesfatin-1 and irisin in sedentary male and female subjects. *Methods:* A total of 30 subjects (15 males and 15 females) performed an aerobic exercise approximately 45 min. Blood samples were taken at baseline and the end of the exercise. ELISA method was used to analyse parameters of leptin nesfatin-1, irisin, total oxidant status (TOS) and total antioxidant status (TAS). *Result:* Exercise caused a significant increase in levels of irisin and TOS but TAS level decreased. In addition, leptin and irisin levels increased significantly in females, but they did not change significantly in males. *Conclusion:* However, gender differences have a great impact on energy regulatory hormones which needed to be evaluated in further studies. Energy regulatory hormones did not correlate with the change of TAS and TOS levels.

Key word: Irisin, Nesfatin-1, Leptin, Total Oxidant Status, Total Antioxidant Status, Exercise.

Introduction

It is known that regular exercise training has important protective effects on the body's cardio metabolic system against serious diseases including diabetes obesity and cardiovascular disorders (1, 2). Results of previous studies revealed that skeletal muscle and body fat mass are important hormone secretory organs and play crucial roles in energy metabolic regulation (3-5). Increased physical activity may stimulate the production of several myokine and adipokine, including leptin (6), nesfatin-1 (7, 8). Since their first introduction, the importance of leptin, irisin, nesfatin-1 levels and their role in energy metabolic system regulation has been evaluated in many studies (9-11).

However, exercise has the capacity to transiently stimulate body oxidant oxidant-antioxidant balance and impaired body homeostasis (12, 13). Thus it is important to evaluate the association between exercise induced metabolic stress on oxidant antioxidant balance and metabolic hormones. Oxidant antioxidant balance in response to the running or cycling exercise in trained and untrained subjects have been studied (14-16).

It has been suggested that exercise-induced change in metabolic hormone levels associated with the subject's oxidant status (17). Exercise at the anaerobic threshold is closely associated with moderate intensity and ranged between 40% and 70% of maximal O_2 uptake (1). A study revealed that aerobic exercise at 60% of maximal O_2 uptake has significant potential to provoke the body's metabolic and oxidative stress parameters of the body (18). However, the impact of aerobic exercise on oxidative stress and its association with metabolic hormones of leptin, irisin, nesfatin-1

with regarding genders of the subjects has great interest but has not been well documented.

In the present study, we intended to comparatively evaluate aerobic running exercise induced metabolic stress on leptin, irisin, nesfatin-1, and oxidant-antioxidant parameters in sedentary male and female subjects.

Methods

The study protocol was approved by the Ethics Committee of Bozok University. Prior to the study, all experimental procedures, benefits, and risks of the study were fully explained to all participants. Written informed consent was obtained from untrained who had no more than 1 hour/week regular activity for at least 1 year.

The sedentary subjects' physical characteristics (age, height, weight, body mass index, fat mass, fat-free mass) are 21.1 ±1.5 years, 174±3.1 cm, 64.6±6.7 kg and 21.3±2.1 kg/m^2, 6.77±2.4 kg and 58.26±5.6 kg for male and 21.0 ±1.1 years, 168±2.3 cm, 59.4±3.9 kg and body mass index 21.0±1.1 kg/m^2 11.09±1.4 kg and 48.4±1.7 kg for female, respectively. The body weight and body composition of the subjects were measured using a bioelectrical impedance analyser after an overnight fast. To avoid invalid measurement of body composition analyses using the bioelectrical impedance analyzing method, the hydration status of the subjects was carefully controlled (19).

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. Prior to data collection and during the protocol period, the subjects 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-exercise period.

Exercise protocols:

All subjects were asked to perform running exercise approximately 45 minutes at or close to their anaerobic threshold following overnight fasting at 08:00 am to 09:00. The anaerobic threshold was estimated using 60% to 70% of maximal heart beat levels as proposed by American College of Sports Medicine (20). Subject's heartbeat controlled by polar watch.

Blood collection and biochemical analysis:

Venous blood samples were taken in aprotinin-containing tubes before the test as a baseline and immediately after the running. Serum was separated and immediately centrifuged at 4.000 rpm at 4 °C for 5 min to provide serum samples, and then it was frozen and stored at -80 °C for subsequent analyses performed within 4 weeks. The samples were analysed for leptin, nesfatin-1, irisin in a double-blind condition. The pre and post exercise levels of leptin, nesfatin-1 and irisin were determined using a commercial Enzyme Linked-Immunosorbent Assay (ELISA) kits that specific to each hormone.

The oxidant-antioxidant balance response to the aerobic exercise was evaluated with the measurement of total antioxidant (TAS) and total oxidant status of the subjects (TOS). ELISA kits were used to measure the levels of TAS and TOS levels.

Data were expressed as mean (± SD). The Wilcoxon-signed rank test, which is a non-parametric comparison, was used to analyze the significance of within-group comparisons of data. The statistical analyses between the group data were performed using the Mann-Whitney U test. A value of $p < 0.05$ was accepted as statistically significant, $p < 0.0001$ was accepted as highly statistically significant.

Results

The mean (±SD) percent change of TAS, TOS irisin nesfatin-1 and leptin levels in response to the aerobic exercise in male and female groups has been shown in figure 1.

The aerobic exercise-induced increased metabolic activity caused marked alteration in levels of TAS and TOS in both male and female groups. There was significant decreases in TAS levels from 1.01±0.04 mmol/L to 0.88±0.07 mmol/L ($p<0.05$) in male and

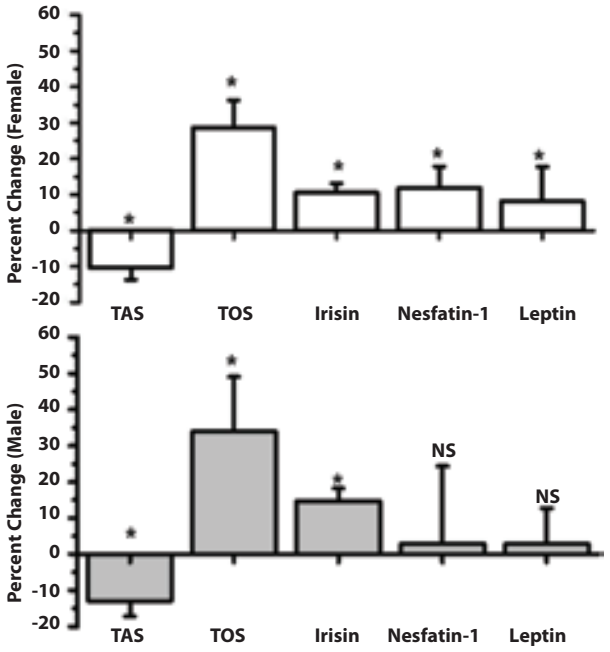

Figure 1. The mean (±SD) percent change of total antioxidant status (TAS), total oxidant status (TOS) irisin, nesfatin-1 and leptin levels during aerobic exercise in female (white column) and male (grey column) groups

1.13±0.05 mmol/L to 1.01±0.05 mmol/L ($p<0.05$) in female. In contrast, significant increases in TOS levels were observed in males from 14.77±2.1 mmol/L to 19.61±2.3 mmol/L ($p<0.05$) and in females from 12.27±1.8 mmol/L to 15.73±2.2 mmol/L ($p<0.05$).

Serum leptin levels in male and female groups showed variation after an acute running, aerobic exercise. Mean (±SD) serum leptin levels in male subjects showed no statistically significant change after aerobic exercise: 43.8±15.6 ng/mL to 44.9±16.1 ng/mL ($p=0.2$). In contrast, an increase in leptin levels after exercise was observed in female subjects: from 109.3±34.2 ng/mL to 117.14±33.1 ng/mL ($p=0.01$).

Aerobic exercise did not cause an increase in nesfatin-1 levels in male group (from 122.1 ±23 pg/mL to 122.4±23 pg/mL, $p=0.9$). However, a statistically significant increase in nesfatin-1 levels was observed in female group from 160.9 ±15 pg/mL to 179.5±16 pg/mL ($p<0.05$). There were statistically significant differences in baseline nesfatin-1 levels between both groups ($p<0.05$).

Irisin levels showed significant increases in all subjects in both groups during aerobic exercise. The increases in irisin levels were found to be from 99.4±11 ng/mL to 113.6±9 ng/mL, ($p<0.0001$) in the male group and from 77.0±5 ng/mL to 85.1±8 ng/mL, ($p<0.0001$) in female group. Mean baseline irisin levels were significantly higher in males than females ($p<0.0001$).

The correlation analysis between TAS, TOS, and energy regulatory hormones during aerobic exercise showed no statistically significant association in male and female groups.

Discussion

In the present study, we have found that serum irisin levels increases with increased metabolic activity without dependent gender of the subjects (9). However, it should be mentioned that male subjects had higher baseline irisin levels compared to females (21). There was a systematic increase in irisin levels in all subjects in both groups (19, 22). The observation of higher baseline irisin levels in male subjects compared to females could be the results of body composition content, especially higher body mass (23) or metabolic profile (24).

Increased irisin levels in response to the acute exercise support the findings of previous studies (11, 25, 26) but are contrary to some findings (27, 28). In addition, decreased blood irisin levels following 12 weeks of exercise training have also been reported (29,) while it increase after acute exercise.

It has been observed that nesfatin-1 and leptin showed complicated outcomes regarding the gender of the subjects during exercise (Figure 1). Nesfatin-1 and leptin increased in female subjects, while they both did not change significantly in male subjects. In literature, conflicting findings have been reported with regarding acute or chronic exercise and nesfatin-1 levels. A decrease in plasma levels of nesfatin-1 after acute aerobic exercise has been reported (30). In another study, an increase in nesfatin-1 levels following high intensity interval training has been reported (31). Unchanged nesfatin-1 levels has been reported following acute aerobic running exercise (32) performed in morning but increased in exercise performed in male subjects (26). Nesfatin-1 levels have been shown varied responses

during soccer match performed in different times of the day (26). It should be emphasized that nesfatin-1 levels could be significantly affected by some conditions. For example, it increases during mental stress in patient with major depressive disorders (33) but decreases in the case of metabolic stress in patient with diabetes mellitus (34).

Baseline leptin levels were be higher in females than males, with related differences in fat mass (11, 35). Leptin levels increased in female while it did not change in male subjects (Figure 1).

On the other hand, in a study, acute exercise performed in obese females has revealed unchanged leptin levels (36). However, training caused decrease in basal leptin levels with closely related decrease in body fat tissues (36). However, there is a decrease in leptin levels after exercise in patient with metabolic syndrome (37).

Despite the absent of statistically significant correlation, exercise induced changes of oxidative stress markers may have an impact on leptin, nesfatin-1 and irisin levels. The response of energy regulatory hormones of leptin and nesfatin-1 during aerobic exercise may vary with the gender of the subjects. However, irisin is clearly influenced exercise induced hormones. Performing further studies in a large number of subject groups and with different fitness statuses or body mass indexes may provide better results to understand the associations between exercise-induced metabolic stress and energy regulatory hormones and their role in oxidant-antioxidant status.

Conflicts of interest: The authors declare that there is no conflict of interest in this manuscript.

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References

1. Wasserman K, Hansen JE, Sue DY, et al. Principles of exercise testing and interpretation: including pathophysiology and clinical applications. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins 2012; 19103.
2. Ozcelik O, Ozkan Y, Algul S, Colak R. Beneficial effects of training at the anaerobic threshold in addition to pharmacotherapy on weight loss, body composition, and exercise performance in women with obesity. Patient Prefer Adherence 2015; 9, 999-1004.
3. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. Nat Rev Endocrinol 2012; 8(8):457-65.
4. Görgens SW, Eckardt K, Jensen J, Drevon CA, Eckel J. Exercise and regulation of adipokine and myokine production. Prog Mol Biol Transl Sci 2015; 135:313-36.
5. Leal LG, Lopes MA, Batista ML JR. physical exercise-induced myokines and muscle-adipose tissue crosstalk: a review of current knowledge and the implications for health and metabolic diseases. Front Physiol 2018; 9: 1307.
6. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372(6505): 425-32.
7. Oh-I S, Shimizu H, Satoh T, et al. Identification of nesfatin-1 as a satiety molecule in the hypothalamus. Nature 2006; 443(7112): 709-12.
8. Boström P, Wu J, Jedrychowski MP, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 2012; 481(7382): 463-8.
9. Kraemer RR, Shockett P, Webb ND, Shah U, Castracane VD. A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. Horm Metab Res 2014; 46(2): 150-4.
10. Kim HJ, Lee HJ, So B, Son JS, Yonn D, Song W. Effect of aerobic training and resistance training on circulating irisin level and their association with change of body composition in overweight/obese adults: a pilot study. Physiol Res 2016; 65(2): 271-9.
11. Algul S, Ozcelik O, Ozdenk C. Variations in leptin, nesfatin-1 and irisin levels induced by aerobic exercise in young trained and untrained male subjects Biol Sport 2017; 4: 339-44.
12. Steinberg JG, Ba A, Brégeon F, Delliaux S, Jammes Y. Cytokine and oxidative responses to maximal cycling exercise in sedentary subjects. Med Sci Sports Exerc 2007; 39(6): 964-8.
13. Berzosa C, Cebrián I, Fuentes-Broto L, et al. Acute exercise increases plasma total antioxidant status and antioxidant enzyme activities in untrained men. J Biomed Biotechnol 2011; 2011: 540458.
14. Ozcelik O, Ozkan Y, Karatas F, Kelestimur H. Exercise training as an adjunct to orlistat therapy reduces oxidative stress in obese subjects. Tohoku J Exp Med 2005; 206(4): 313-8.
15. Powers SK, Radak Z, Ji LL. Exercise-induced oxidative stress: past, present and future. J Physiol 2016; 594(18): 5081-92.
16. Wiecek M, Maciejczyk M, Szymura J, Wiecha S, Kantorowicz M, Szygula Z. Effect of body composition, aerobic performance and physical activity on exercise-induced oxidative stress in healthy subjects. J Sports Med Phys Fitness 2017; 57(7-8): 942-52.
17. Samy DM, Ismail CA, Nassra RA. Circulating irisin concentrations in rat models of thyroid dysfunction -- effect of exercise. Metabolism 2015; 64(7): 804-13.

18. Brown M, McClean CM, Davison GW, Brown JCW, Murphy MH. The acute effects of walking exercise intensity on systemic cytokines and oxidative stress. Eur J Appl Physiol 2018; 118(10): 2111-20.
19. Ugras S. Evaluating of altered hydration status on effectiveness of body composition analysis using bioelectric impedance analysis. Libyan J Med 2020; 15(1): 1741904.
20. American College of Sports Medicine (ACSM). Guidelines for Exercise Testing and Prescription (9th ed.). Baltimore, MD: Lippincott, Williams and Wilkins, 2014.
21. Löffler D, Müller U, Scheuermann K, Friebe D, Gesing J, Bielitz J, et al. Serum irisin levels are regulated by acute strenuous exercise. J Clin Endocrinol Metab 2015; 100(4): 1289-99.
22. Fox J, Rioux BV, Goulet EDB, et al. Effect of an acute exercise bout on immediate post-exercise irisin concentration in adults: A meta-analysis. Scand J Med Sci Sports 2018; 28(1): 16-8.
23. Huh JY, Panagiotou G, Mougios V, et al. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism 2012; 61(12): 1725-38.
24. Benedini S, Dozio E, Invernizzi PL, et al. Irisin: a potential link between physical exercise and metabolism-an observational study in differently trained subjects, from elite athletes to sedentary people. J Diabetes Res 2017; 2017: 1039161.
25. Daskalopoulou SS, Cooke AB, Gomez YH, et al. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. Eur J Endocrinol 2014; 171(3): 343-52.
26. Ozcelik O, Algul S, Yilmaz B. Nesfatin-1 and irisin levels in response to the soccer matches performed in morning, afternoon and at night in young trained male subjects. Cell Mol Biol (Noisy-le-grand) 2018; 64(10): 130-33.
27. Kurdiova T, Balaz M, Vician M, et al. Effects of obesity, diabetes and exercise on Fndc5 gene expression and irisin release in human skeletal muscle and adipose tissue: in vivo and in vitro studies. J Physiol 2014; 592(5): 1091-107.
28. Timmons JA, Baar K, Davidsen PK, Atherton PJ. Is irisin a human exercise gene? Nature 2012; 488(7413): E9-10.
29. Norheim F, Langleite TM, Hjorth M, et al. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. Febs J 2014;281(3):739-49.
30. Mohebbi H, Nourshahi M, Ghasemikaram M, Safarimosavi S. Effects of exercise at individual anaerobic threshold and maximal fat oxidation intensities on plasma levels of nesfatin-1 and metabolic health biomarkers. J Physiol Biochem 2015; 71(1): 79-8.
31. Amanat S, Sinaei E, Panji M, et al. A randomized controlled trial on the effects of 12 weeks of aerobic, resistance, and combined exercises training on the serum levels of nesfatin-1, irisin-1 and HOMA-IR (homa-ir). Front Physiol 2020; 11: 562895.
32. Ghanbari-Niaki A, Kraemer RR, Soltani R. Plasma nesfatin-1 and glucoregulatory hormone responses to two different anaerobic exercise sessions. Eur J Appl Physiol 2010; 110(4): 863-8.
33. Algul S, Ozcelik O. Evaluating the levels of nesfatin-1 and ghrelin hormones in patients with moderate and severe major depressive disorders. Psychiatry Investig 2018; 15(2): 214-8.
34. Algul S, Ozkan Y, Ozcelik O. Serum nesfatin-1 levels in patients with different glucose tolerance levels. Physiol Res 2016; 65(6): 979-85.
35. Friedman J. 20 years of leptin: leptin at 20: an overview. J Endocrinol 2014; 223(1): T1-8.
36. Ozcelik O, Celik H, Ayar A, Serhatlıoglu S, Kelestimur H. Investigation of the influence of training status on the relationship between the acute exercise and serum leptin levels in obese females. Neuro Endocrinol Lett 2004; 25(5): 381-5.
37. Pop D, Dădârlat A, Bodizs G, Stanca L, Zdrenghea D. The response of circulating leptin levels to exercise stress testing in subjects diagnosed with metabolic syndrome. ISRN Endocrinol 2014; 2014: 689260.

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