**Research Article**

**Plasma Nesfatin Responses Following a Single Session of Interval Exercise in Young Men: Effects of Glucose, Sucrose and Fructose Intake**

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**Abstract**

**Background:** Nesfatin is a key regulator of glucose metabolism. The aim of this study was to identify the effect of glucose, sucrose and fructose intake following acute high intensity interval exercise (HIIE) on plasma levels of nesfatin, insulin and glucose in young males.

**Materials and Methods:** 32 sedentary young males (21.9±2.3 yrs, 77.5±8.9 kg) were assigned into four groups (n= eight per group): glucose, sucrose, fructose and control or water groups. Subjects completed 4×4 min interval running with 90-95% maximal heart rate (HRmax) and 3 min active recovery with 65-70% HRmax between each interval. Blood samples were collected before, immediately after, 30, 60 and 90 minutes after exercise session. Immediately after the second blood sampling, carbohydrate liquids (1.5 g/kg glucose, fructose, sucrose and water) were consumed by the subjects in different groups. The data were analyzed using repeated measures ANOVA test and SPSS-24 software.

**Results:** Results indicated that there was no significant difference between groups for Nesfatin (p=0.519) and glucose (p=0.062) levels; but, there was a significant difference between groups for insulin levels (p<0.001). Bonferroni multiple comparison corrections as a post hoc test showed a significant difference between water and glucose, water and sucrose, glucose and fructose, and sucrose and fructose groups in 30 and 60 minutes after HIIE (P<0.05).

**Conclusion:** with respect to the present study results, acute carbohydrate supplements (glucose, sucrose and fructose) don't affect nesfatin response following exercise. Therefore, it seems that nesfatin doesn't affect acute exercise-induced metabolic status response to different carbohydrate supplements in healthy subjects.

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1. Introduction

Nesfatin-1 was discovered in 2006 by Oh and colleagues as a peptide with 82 amino acids, which derived from NEFA/nucleobindin2 (NUCB2) as its precursor. These researchers identified nesfatin-1 in the hypothalamus and suggested that this peptide can exert an anorexigenic effect (1). However, subsequent studies reported that nesfatin-1 is also expressed by different tissue, including pituitary, adipose tissue and pancreas that all of these tissues play important role in regulating energy homeostasis and metabolism (2). Especially, nesfatin-1 influences glucose metabolism and observed that this peptide regulates glucose homeostasis and insulin secretion in rodent (3) and human (4) subjects. Moreover, Nesfatin-1 cause an increase in glucose-stimulated insulin secretion from b-cells by promoting Ca2+ influx through L-type calcium channels (5) and regulatory effects of nesfatin-1 on insulin resistance is mediated by inhibition of hepatic glucose production which is associated with increased glucose uptake and also increasing insulin receptors (IRs) (6).

It’s suggested that the intravenous injection of nesfatin-1 reduces the blood levels of glucose in hyperglycemic mice (7) and acute subcutaneous infusion of nesfatin-1 reduced food intake in male rats, also a one-day peripheral injection of nesfatin-1 results in increasing physical activity with fat oxidation and simultaneously foot intake was decreased (8), these results represent that nesfatin-1 is a multifunctional peptide. Circulating nesfatin-1 changes in different nutritional and metabolic statuses. it has been shown that there is a correlation between glucose intake and nesfatin-1 changes (4) and suggested that a high dose of glucose consumption is associated with increasing nesfatin-1 gene expression in human islets (9).

In addition to nutrition, blood levels of nesfatin-1 are affected by acute and chronic exercise in human subjects. Exercise is a powerful physiological stimulant that affects the metabolism, oxidation of substances, secretion of hormones and brain neurotransmitters. The range of acute exercise (depending on the type, severity and duration of exercise) can cause short-term suppression of appetite and lower insulin levels (10). Some researchers have examined the effect of acute exercise on nesfatin-1 response. Ganbari Niaki et al. (2010) investigated the effects of acute high intensity and circuit resistance exercise on nesfatin and glucose regulating hormones in male kickboxers but observed no significant changes in nesfatin-1 after two exercise protocols. However, there was a significant change in glucose regulating hormones including, glucagon and cortisol (11). In another study, Mohebbi et al (2015) investigate the effect of exercise in the anaerobic threshold or maximum fatty oxidation intensity on plasma levels of nsafatin-1 and leptin in overweight men. These results indicated that higher intensity exercise resulted in a more significant decrease in nesfatin-1 and leptin compared to exercise with maximum fat oxidation (12). Generally, the effect of acute exercise on nesfatin-1 is controversial. Because of the interaction of glucose metabolism, nesfatin-1 and the effect of acute exercise on nesfatin-1 levels. On the other hand, the aim of the present study was to investigate the acute effect of exercise along with sugars (glucose, fructose and sucrose) supplemnetations on nesfatin-1 levels in young males.
According to our knowledge is the first study that investigates the acute effect of interval exercise in combination with sugars especially fructose and sucrose supplementation on nesfatin-1 levels. We hypothesized that sugars supplement can affect nesfatin-1 response to acute exercise.

2. Materials and Methods

To perform this study, 32 sedentary healthy young males (the age, weight, height and BMI were 21.9±2.3 yrs, 77.5±8.9 kg, 176.8±1.1 cm, 24.3±2.1 kg/m2 respectively) were recruited for participation in the present study protocol. None of the subjects participated in the regular exercise training program in the last year. One week before performing the exercise protocol, the methodology for conducting the study was explained to the subjects and finally, all of them reviewed and signed Informed consent, and also completed the medical questionnaire. All of the study stages were conducted according to ethical guidelines of the Helsinki Declaration and Ethical approval was obtained from Islamic Azad University, Science and Research Branch, Tehran, Iran. Inclusion criteria for to present study included: non-drug and alcohol addiction, lack of regular exercise activity for at least one year, no history of kidney, liver, cardiovascular diseases, diabetes and any type of injury or physical problem. Since the participants were residents of the university of Mazandaran (Mazandaran, Iran) dormitory, they used the same food as the university self-service. However, the participants were emphasized to consume the same and certain food two days before blood sampling.

The following conditions were required for blood sampling: 1) no use of drugs or supplementation during the course of the study; 2) no change in diet at least two days before the test; 3) avoid heavy physical activity at least one week before the test; 4) no consumption of coffee, banana, cereals and fatty food at least 24 hours before exercise protocol. Finally, participants were randomly assigned into four groups each group consists of eight subjects, including glucose, sucrose, fructose and control groups, who all participated in an acute exercise session. Exercise sessions were performed between 8-11:48 a.m. exercise intervention started with 10 minutes warm-up including running at 70 per cent of maximum heart rate (HRmax) followed by a main acute exercise protocol which conducted as a high-intensity interval exercise (HIIE). HIIE protocol consists of 4×4 minutes’ interval running that each interval performed in 90-95% HRmax that conducted on the treadmill and immediately after each intensive four minutes interval, three min active recovery with 65-70% HRmax was performed (13). Immediately after completing the HIIE protocol, Blood samples were collected (second blood sampling after pre-test sampling) and as soon as possible after the second blood sampling, sugary liquids, including 1.5 per kilogram of body weight (g/kg) of glucose, fructose and sucrose consumed by glucose, fructose and sucrose groups respectively. Moreover, subjects in the control group consumed 3.5 ml water per g/kg immediately after the HIIE and blood samples were again collected 30, 60 and 90 minutes after sugar (or water) supplementation.
Briefly, blood samples were collected five times: before, immediately after, 30, 60 and 90 min after the completion exercise protocol. Plasma nesfatin-1 and insulin levels were measured by the ELISA method and glucose levels were determined by a commercial Pars Azmon kit, Tehran, Iran. Data analyzed by SPSS version 24. To determine the normality of data distribution, Kolmogorov-Smirnov (KS) test and for homogeneity of variances, Levene tests were used. Differences between and within groups calculate by repeated-measures ANOVA that was followed up with Bonferroni post-hoc test.

3. Results

The Kolmogorov-Smirnov and the Levene test indicated that the present study data have a normal and appropriate distribution (P > 0.05). Inter-group results for insulin levels showed a significant difference between groups (p < 0.001). Bonferroni post-hoc test for insulin levels indicated that there was a significant difference between the glucose group with water group in 30 min (p = 0.002) and 60 min after HIIE (p = 0.008); also significant differences between the glucose group with the fructose group in 30 min (p = 0.01) and 60 min after HIIE (p = 0.04) were observed. In addition, Bonferroni post-hoc test indicated that there is a significant difference between the sucrose group and with water group in 30 min (p = 0.001) and 60 min after HIIE (p = 0.001). Moreover, there was shown a significant difference between the sucrose group with the fructose group in 30 min (p = 0.003) and 60 min after HIIE (p = 0.02). In summarizing, insulin levels significantly increased in glucose and sucrose groups in comparison to water and fructose groups 30 minutes and 60 minutes after HIIE completion. Intra-group results of insulin for different groups showed that there was a significant difference only in the glucose and sucrose group. According to the present study results, insulin levels of glucose and sucrose groups significantly increased in 30 minutes and 60 minutes after HIIE in comparison to before and immediately after HIIE stages (p < 0.05) (Fig 1).
Inter-group results of nesfatin levels showed that there was no significant difference between different groups (p = 0.519). Regarding intra-group differences, didn’t observe a significant difference between any stages of blood sampling (p > 0.05, Fig.2).

Figure 1: Changes in insulin levels. * Indicated a significant increase in comparison to water and fructose groups. # Indicated a significant difference in blood sampling time in glucose and sucrose groups: significant insulin increases in glucose and sucrose groups in 30 min and 60 min after HIIE in comparison to before and immediately after HIIE. Stage1: before HITE, stage2: immediately after HIIE, stage3: 30 min after HIIE, stage4: 60 min after HIIE, and stage5: 90 minutes after HIIE.
Inter-group analysis of glucose levels showed no significant difference between different groups (p= 0.062). Within groups, results indicated that glucose levels significantly increased 30 minutes after HIIE in comparison to immediately after HIIE in the glucose group. There are no significant changes between other stages in a different group (p> 0.05, Fig. 3).
4. Discussion

The aim of the present study was to investigate the effect of carbohydrate supplementation (glucose, sucrose, fructose or water) after acute high intensity interval exercise on plasma levels of nesfatin, glucose and insulin in sedentary healthy males. The main finding in the present study is that carbohydrate supplementations don’t affect nesfatin response to HIIE protocol. Nesfatin-1 has a widely distribution and different organ, including stomach, pancreas, pituitary and adipose tissue remarkably express and secreted nesfatin-1 which indicates this peptide can influence feeding or another metabolic status (14, 15). In a hunger state, a selective reduction in nucleosidein 2/nsafatin-1 mRNA expression has been reported in the ventricular lateral nuclei of the mice but observed that re-feeding cause returns nesfatin-1 expression to the initial levels (1). It was shown that intravaginal injection of nucleobudinine-2/nsafatin-1 to male Wistar rats reduced food consumption for 6 hours after the injection in a dose-dependent manner (16).

In the present study, it was observed that nesfatin levels don’t change immediately after exercise and also, nesfatin levels remain unchanged after the consumption of glucose, sucrose and fructose supplementation. To explain these findings, we should note the intensity and duration of exercise. In agreement with our findings, Ghanbari-Niaki et al (2010) indicated that acute anaerobic interval exercise or circuit resistance exercise session (20 min) doesn’t affect plasma levels of nesfatin-1 in males’ boxers (11). These results indicated that acute high-intensity exercise can’t change circulating levels of nesfatin after exercise. Ghanbari-Niaki et al. (2010) suggested that probably long-term exercise is needed for observing significant changes in nesfatin-1 levels. Present study HIIE protocol duration (25 min) is similar to Ghanbari-Niaki et al conducted exercise and we also don’t observe a significant change in plasma nesfatin. It has been shown that intense and prolonged exercise can result in the depletion of most body’s energy resources such as glycogen. Depletion energy sources of the body cause an increase in appetite-related peptides such as ghrelin and Agrp. On the other hand, it can decrease anti-appetite peptides such as nesfatin, leptin and insulin (11, 12, 17, and 18). In this study, a relative decline in plasma levels of nesfatin was observed immediately after exercise that statically wasn’t significant. These results can be attributed to the different intensity and duration of the HIIE protocol, conducted in the present study in comparison to other researchers. To support the importance of exercise duration effectiveness on nesfatin-1 response to acute exercise, Mohebbi et al (2012) reported that one session cycling on a computer-controlled Ergometer until reaching higher than 800 kcal significantly decreased plasma levels of nesfatin-1 in overweight males, but an exercise in fat max intensity doesn’t affect nesfatin-1 (12). These researchers concluded that the controversial result between different intensities is the consequence of differing substrate utilization during the two-exercise trial. In support of this idea, observed that glucose homeostasis results in nesfatin-1 secretion into the blood circulation (19). Oral administration of glucose is associated with a significant increase in basal nesfatin-1 levels than the saline-treated control group (4).
In contrast, throughout the day fasting decrease nesfatin-1 levels in plasma. Our result indicated that glucose administration cause increases nesfatin levels in comparison to other groups, although this increase wasn’t significant that can attribute to low doses of glucose. In this way, Riva et al, 2011 have proven that a high dose of glucose consumption can elevate nesfatin-1 expression in human islets, but a lower dose doesn’t exert a significant effect (9). Increasing the dose of consuming sugars after exercise, quickly restores the lost resources such as glycogen and results in further increases of the anti-appetite hormones in the after-exercise hours (20, 21). However, to our knowledge is the first research that investigates the effect of carbohydrate supplementation on nesfatin changes after acute high-intensity exercise. Regarding about long-term effect of high-intensity interval training (HIIT), Ahmadizad et al (2015) reported that six weeks of HIIT significantly increased nesfatin levels in sedentary overweight men. However, these researchers indicated that a 6-week moderate intensity interval exercise training doesn’t change nesfatin-1 levels (22). These findings supported the impact of exercise on nesfatin changes after the exercise training period. Unfortunately, in the present study, we don’t examine the chronic effect of high intensity exercise alone or with carbohydrate supplementations on nesfatin changes.

In addition to nesfatin-1 result, the present study has shown that sucrose and glucose increase the insulin levels in 30 and 60 minutes after acute exercise sessions compared to water and fructose groups. These differences between carbohydrate supplements are related to the nature of the supplement, as well as the absorption pathway of glucose and sucrose. Since the glucose is absorbed directly through its transporters (GLUTs), and because glucose transporters increase after exercise and in fact, exercise has an insulin-like effect, the increased insulin in the glucose group is lower in comparison to the sucrose group immediately after the HIIE protocol. On the other hand, it has been shown that sucrose and fructose first should be glucose-digested to be absorbed, or fructose should go to the liver to be absorbed (23, 24).


Conclusion

In conclusion, it seems that different carbohydrate supplementations (sucrose, glucose, and fructose) immediately after high-intensity exercise can’t affect nesfatin levels. These results can be related to the dose of glucose consumption and duration and intensity of exercise. With respect to a relative increase in nesfatin levels after glucose ingestion in the glucose group, an increase in the dose of glucose or probably duration of exercise can be associated with further changes in nesfatin levels.

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Author contributions

Conceptualization: M.GH.; Methodology: M.GH.; Software: M.GH.; Validation: M.GH.; Formal analysis: GH.T.; Investigation: M.GH.; Resources: M.GH.; Data curation: M.GH.; Writing - original draft: M.GH.; Writing - review & editing: M.GH.; Visualization: M.GH.; Supervision: M.GH.; Project administration: M.GH.; Funding acquisition: M.GH.
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