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Research Article

Effect of Arginine Supplementation and High Intensity Training on Appetite Hormones and Body Composition of Obese Boys

Tohid Mabhut Moghadam[1], Mehrdad Fathi[2*], Sayed Reza Attarzadeh Hosseini[2], Mohammad Mosaferi Ziaaldini[2], Amir Rashidlamir[2]

[1]Department of Sport Sciences, Khavaran Institute of Higher Education, Mashhad, Iran
[2]Department of Sport Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

***Correspondence to**
Mehrdad Fathi,
Email: mfathei@um.ac.ir

Abstract

Background: There is little information about the effects of high intensity aerobic exercise training (HIT) and L-arginine supplementation on appetite-regulating hormones among obese male adolescents. We aimed to determine the effect of eight weeks of HIT and L-arginine supplementation on appetite-regulating hormones and body composition indices in obese adolescent boys.

Methods: Twenty obese adolescents were randomly divided into two groups of HIT and placebo (P-HIT, n = 10) and HIT with supplementation of L-arginine (A-HIT, n = 10). The HIT protocol was treadmill running with ventilation threshold (VT) intensity and training sessions were isoenergetic and energy consumption were set to 350 kcal per session for each participant, which were evaluated indirectly by calorimetry. The A-HIT group received 3 g of L-arginine per day for 8 weeks. Before the interventions and 48 hours after the last exercise session, anthropometric indices and levels of appetite-regulating hormones were measured.

Results: There was no significant changes between the groups with respect to leptin, agouti, and PYY3-36 peptide levels. There were significant changes in weight reduction between the groups ($P \leq 0.05$). However, body max index (BMI) and percent body fat (PBF) changes were not significant in between groups ($P \geq 0.05$).

Conclusion: Our findings suggest that co-supplementation of L-arginine with HIT training had no further effects on appetite regulatory hormones and body composition of obese male adolescents.

Keywords: L-arginine, Leptin, Agouti, PYY3-36, High intensity training, Obesity

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Background

Obesity is one of the main health problems of people in many countries (1). Child obesity, as one of the most severe public challenges in the 21st century, affects global population growth (2). Several peptides released by peripheral tissues interact with specific regions of the brain and release neurons secreting anti-appetite and appetite neuropeptides that are involved in controlling nutritional behavior and energy patterns (3). Recent studies have focused on the effect of physical activity on appetite and energy intake (4). In this regard, many studies have suggested that physical activity is a robust non-pharmaceutical strategy for the prevention and management of obesity and the beneficial effects of exercise may not be merely through influencing energy consumption, but by regulating appetite, which can in turn affect the energy equilibrium equation (5). Appetite-reducing and stimulating hormones, such as peptide PYY3-36, leptin, and Agouti-related protein (AgRP) play an important role in appetite regulation (6-8). Studies have shown that among the appetite-regulating hormones, PYY3-36 peptide has attracted the most attention and plays a more important role in suppressing appetite and hunger (9). Moreover, the contradictory results of some studies concerning leptin as an anti-appetite neuropeptide show that decrease the levels of leptin in response to physical activity in children and adolescents with (10-12) or without (13) has been weight loss. Some results also indicate the insignificant effects of exercise on leptin levels (14-16). Even in some cases that changes in levels of leptin may not be tangible in the blood, a regular physical activity can induce the effects of anorexia and thermogenesis by increase in the binding of leptin receptors and the expression of the leptin gene (17).

The effects of physical activity on the AgRP as an appetite peptide that can affect nutritional behavior, weight adjustment, and energy homeostasis are contradictory to some extent but most of the results indicate an increase in agouti in both the plasma and gene expression (18, 19). Several studies have shown that aerobic exercise has not led to an acute and recognizable increase in energy in children (15) and adolescents (20) with obesity. On the other hand, increasing evidence from studies shows the special effects of L-arginine on obesity, while the clinical study on the effects of L-arginine on obesity

is insufficient (21). The mechanism of action of the physiological levels of L-arginine stimulates the oxidation of glucose and long-chain fatty acids while reducing the repeated synthesis of glucose and triacylglycerols (22). Besides, the use of L-arginine supplements increases lipolysis and inhibits lipogenesis by regulating the expression and function of the key enzymes involved in the antioxidant response and lipid metabolism in insulin-sensitive tissues (23). In recent years, various studies have shown that the use of L-arginine supplements has been beneficial in reducing obesity and improving insulin sensitivity in multiple animal models and some human samples (12). However, the results of some studies suggest that high-intensity physical activity significantly reduces the amount of energy consumed within 24 hours after activity in adolescent boys (24). The apparent point of this study is the isoenergetic nature of the training sessions so that the subjects burn a certain number of calories during each session of the exercise. This may prevent the impact of different energy consumption between the groups. This technique is commonly used in studies that attempt to evaluate the effect of exercise intensity (12). The synergetic effect of L-arginine consumption and high intensity training (HIT) is not clearly understood. In this study we investigated the effect of eight weeks of L-arginine supplementation along with HIT training on appetited regulatory hormones and body composition of obese adolescent boys.

Materials and Methods

Subjects

In this quasi-experimental study, 20 adolescent boys were selected and assigned into two groups randomly as high-intensity aerobic exercise and placebo (P-HIT, 10 subjects) and high-intensity aerobic exercise group with supplementation of l-arginine (A-HIT, 10 subjects) for eight weeks. The inclusion criteria were homogeneity of subjects' weight, not having a history of specific diseases such as cardiovascular disease, high blood pressure, diabetes mellitus, and lack of perceptual impairment, or any disorder that affects one's ability to perform the protocol. all parents and students were introduced to the purpose and manner of conducting the research and related coordination regarding the study was made. Then, the consent form was received from the student and the parents and then the necessary permissions were obtained from the Ethics Committee of the Ferdowsi University of Mashhad. The characteristics of the participants are shown in Table 1.

Blood Sampling and Measurement of Biochemical Variables

Before and 24 hours after the eight-week intervention, 10 cc blood samples were collected from the antecubital vein under fasting conditions (> 12 hours). The blood samples were centrifuged for 20 minutes at 3600 rpm and stored at −80°C until analysis. Levels of leptin, AgRP and PYY3-36 (Hangzhou East Biopharm, code No CK-E10738, CK-E11648, CK-E92030 respectively) were measures using enzyme-linked immunosorbent assay (ELISA).

Exercise Protocol

The design of the HIT protocol primarily required the assessment of peak oxygen consumption (VO2 peak) and ventilation threshold (VT) of the participants. To do this, participants performed an incremental test to volitional exhaustion on a treadmill (Italy-K4b2 model). Oxygen uptake was measured (Cosmed) throughout the test and the greatest 15s average was defined as VO2 peak. VT was determined as the point where a systematic increase in the ratio of ventilation to consumed oxygen (VE/VO2) occurred without an increase in the ventilation to carbon dioxide ratio (VE/VCO2).

After evaluating VO2 peak and VT of each participant, the HIT protocol was designed on the treadmill for three times per week during the eight weeks of study. The running speed in the training session was based on the speed at which the participants achieved their VT. The training sessions were isoenergetic and energy consumption was set to 350 kcal per session for each participant, which was evaluated indirectly by calorimetry (12). To apply progressive lode of exercise, every two-week the VT of each subject were evaluated and the new speed was applied for treadmill running. To determine the approximate duration of the training session, the following equation were used:

Table 1. Body Composition Characteristics of the Participants Before and After HIT Training and L-Arginine Intervention

Variable	Groups	Mean ± SD		Intragroup Changes		Intergroup Changes	
		Before Intervention	After Intervention	T Value	*P* Value	T Value	*P* Value
Weight (kg)	P-HIT	71.85 ± 11.79	69.12 ± 10.74	3.17	0.011*	5.19	<0.01*
	A-HIT	76.80 ± 5.93	73.72 ± 6.47	8.50	0.01*		
BMI (kg/m²)	P-HIT	28.60 ± 2.95	27.25 ± 2.04	2.98	0.017*	0.279	0.783
	A-HIT	30.02 ± 1.77	28.56 ± 1.2	9.64	<0.01*		
PBF (%)	P-HIT	43.37 ± 4.89	40.29 ± 3.75	2.25	0.050*	0.577	0.047*
	A-HIT	41.61 ± 5.59	36.80 ± 2.06	2.45	0.036*		

BMI: body mass index; PBF: percent body fat; P-HIT: placebo and high intensity training; A-HIT: L-arginine and high intensity training.

P < 0.05 was considered significant.

Exercise session time (min) $_{=}$ *350/(vo*$_2$*×496)*

The speed and duration of running were proportional to the VT and VO2 peak gained by the subjects themselves. Nevertheless, to apply the additional load because of a possible improvement in performance, VT and VO2 peak of all subjects were re-assessed at the end of the fourth week and became the criteria for determining the duration of the training session and treadmill speed in the next four weeks.

Arginine Supplementation Protocol

A-HIT received 3 g of L-arginine a day for 8 weeks (tablet, 99.9% purity, Karen, Iran) by dissolving the tablet and mixing with drinking water. P-HIT group received dextrin in similar manner with A-HIT group (25).

Statistical Analysis

SPSS, version 22.0 (SPSS Inc., USA) was used to determine the effect of 8-week HIT training and L-arginine supplementation. An independent *t* test between the groups or a paired *t* test between times were applied. All values are expressed as mean ± standard deviation (SD). $P<0.05$ were considered significant.

Results

Body Composition

The results show a significant reduction in weight in both P-HIT and A-HIT groups after 8 weeks ($P=0.011$ and $P=0.000$, respectively). Also, body weight reduction in the A-HIT group was significantly higher than P-HIT group after eight weeks of intervention ($P=0.000$). We found a significant change in BMI indicis in both groups ($P=0.017$, $P=0.015$, P-HIT and A-HIT respectively), However, no significant difference were shown between groups. There was a significant reduction in PBF after eight weeks of study in both groups of P-HIT and A-HIT ($P=0.050$, $P=0.036$ respectively). However, the A-HIT group experimented higher reduction then P-HIT group ($P=0.047$; Table 1).

The Regulatory Appetite Hormones

The results showed that after 8 weeks of training and taking arginine supplementation, the changes of leptin, Agouti and PYY3-36 levels did not change significantly compared with the pre-test ($P>0.05$). Also, no significant difference was observed in any of the hormones between the two groups ($P=0.681$, 0.742, and 0.858 respectively, Table 2).

Discussion

The purpose of this study was to investigate the effect of 8 weeks of HIT training and L-arginine supplement under isoenergetic conditions on appetite-regulating hormones and anthropometric indices in obese adolescent boys. We found that the levels of leptin, AgRP, and peptide PYY3-36 after the protocol were not significantly different from the pre-protocol values in both groups of P-HIT and A-HIT. In this regard, the intergroup analysis also showed a non-significant difference in changes in levels of leptin, AgRP, and peptide PYY3-36 between the P-HIT and A-HIT groups (Table 2).

Saghebjoo and colleagues recently investigated effect of six weeks of high-intensity interval training and L-arginine supplementation on serum levels of adiponectin and lipid profile in overweight and obese young men. They found no significant changes in serum adiponectin levels, lipid profile, body mass index, and fat percentage after high-intensity interval training and/or L-arginine intake (26).

The physiological stress resulting from exercise is a potential leptin secretion regulator. Changes associated with the flow of fuel, circulating hormonal concentrations and energy costs may affect leptin concentrations (27). Despite the expectations that regular physical activity can induce effects of anorexia and thermogenesis of leptin by increasing the binding of receptors of leptin and the expression of the leptin gene, in some cases, these effects do not change leptin levels in the blood (17). In the present study, after eight weeks of HIT under isoenergetic conditions in the P-HIT and A-HIT groups, the comparison of the values between the two groups was not significant. In line with this study, Letizia and colleagues examined the effect of exercise and caloric intake on obesity, weight adjustment, and hypothalamic neuropeptides' and agouti presentation in obese mice. Their results showed that exercise significantly reduced

Table 2. Appetite Hormonal Response to 8 Weeks of Intervention in P-HIT and A-HIT Groups

Variable	Groups	Mean ± SD		Intragroup Changes		Intergroup Changes	
		Before Intervention	After Intervention	T Value	*P* Value	T Value	*P* Value
Leptin (ng/mL)	P-HIT	529.76 ± 156.64	529.32 ± 134.96	0.01	0.989	-0.42	0.681
	A-HIT	437.50 ± 54.04	450.03 ± 55.14	1.18	0.269		
Agouti (ng/mL)	P-HIT	147.52 ± 52.05	146.86 ± 52.54	0.13	0.900	-0.33	0.742
	A-HIT	122.70 ± 9.34	132.92 ± 12.99	-0.40	0.693		
PYY_{3-36} (pg/mL)	P-HIT	36.49 ± 13.09	37.09 ± 11.18	-0.25	0.805	0.18	0.858
	A-HIT	32.66 ± 3.45	32.84 ± 4.60	-0.19	0.850		

P-HIT: placebo and high intensity training; A-HIT: L-arginine and high intensity training.

body weight, but did not significantly affect the AgRP (18).

Markofski and colleagues reported a 4.3% significant increase in AgRP compared with the control group following a long-term aerobic protocol (28). AgRP was introduced as an appetite-boosting signal in the classification of environmental signals (9), and negative energy balance was a major contributor to AgRP which can be affected by physical activity (28). The negative energy balance to stimulate appetite and increase food intake can depend on intensity, duration, exercise, the initial amount of fuel sources and nutritional status (19, 28) which could lead to negative energy balance and AgRP peptide values (29, 30). Therefore, in the present study it seems that because of the isoenergetic nature of the nutritional diet of the subjects, the opportunity to supply excess energy resulting from an increase in appetite after exercise is limited. In a similar study using the HIT protocol for 12 weeks on obese adolescents, PYY3-36 levels increased. Prado and colleagues compared the effect of low and high-intensity aerobic exercise training program on obese adolescent girls and found that 12 weeks of high-intensity training led to improved levels of PYY3-36 and may have reduced eating in obese adolescents. Changes in PYY3-36 levels were associated with the HIT group (31). The mechanism of PYY increase was vague, but one of the causes seems to be changing in the nervous system that increases with the intensity of exercise (32). Other possible causes of the absence of differences in PYY3-36 values in this study were that in other studies, most of the exercise protocols were long-term or aerobic and the measurements included total PYY values of human samples (16, 33) and perhaps the contradiction in PYY values in studies is a reflection of the type of total PYY measurement and change rather than the serum blood levels. One of the reasons for the increase in PYY3-36 levels is the decreased appetite and nutrition following high-intensity aerobic exercises (31).

Along with the present study, in most studies with or without supplementation in which long-term aerobic exercise protocols were used, the changes in physical characteristics of the subjects were tangible (12, 34). For example, Prado et al showed that following an intervention of 12 weeks of high and low intensity training in 36 adolescent boys and girls, the physical features such as weight, BMI and PBF after the protocol significantly reduced compared to pre-protocol conditions so that this decrease was more significant in the HIT group (12). In another randomized clinical study on the specific effects of arginine on obesity over 21 days, 8.3 g/d arginine (approximately 80 mg/kg body weight per day) and placebo were given to 33 obese people (mean BMI = 39.1 kg /m^2) with a low calorie diet (1000 calories per day), a regular exercise program (45 minutes, twice daily, 5 days a week). The participants were randomly divided into two HIT + arginine and HIT + placebo groups, the body indices such as weight, BMI, and PBF significantly improved with the completion of the research compared with baseline values. It should be noted that these changes in the arginine group were greater than the placebo group (34). The factors contributing to the supplemental role of L-arginine as a factor in reducing obesity are probably a complex mechanism at cellular-molecular levels and the whole body so that the mitochondrial biogenesis stimulation and regulating gene expression and metabolic pathways via L-arginine are expected. Therefore, the use of L-arginine supplementation for obese people induces fat loss and prevents the reduction of body mass during weight loss (34).

Conclusion

Our findings suggest that co-supplementation of L-arginine with HIT training had no further effects on appetite regulatory hormones and body composition of obese male adolescents. However, in future studies, nutritional habits of the participants should be monitored

Authors' Contribution

Study concept and design: TMM, MF and MMZ; analysis and interpretation of data: TMM, SRAH, MMZ, and AR; drafting of the manuscript: TMM, MF, SRAH and MMZ; critical revision of the manuscript for important intellectual content: TMM, MF, AR, SRAH and MMZ; statistical analysis: MMZ and SRAH.

Conflict of Interests

Authors have no financial interests related to the content of the manuscript.

Ethical Approval

Ethics Committee of the Ferdowsi University of Mashhad approved the study.

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