Original Article

Effects of 8 weeks of aerobic training on Agouti-related peptide, appetite hormones and insulin resistance in overweight sedentary women

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Abstract

Introduction:
The aim of this study was to investigate the effects of 8 weeks of aerobic training on Agouti-related peptide, appetite hormones and insulin resistance in overweight sedentary women.

Materials and Methods:
Thirty overweight female students (25≥BMI≤30) were selected and randomly divided into training and control groups. Before the start of exercise period, and 48 h after their last exercise session, subjects completed the food frequency questionnaire. Then after blood sampling, their anthropometric characteristics were measured. The training group participated in 8 weeks of moderate intensity aerobic training while the control group continued their normal daily activities.

Results:
Agouti-related protein significantly increased in the exercise group ($p<0.05$). Significant changes were also observed between the two groups ($p<0.05$); however, changes in leptin, insulin and insulin resistance were not statistically significant ($P>0.05$). Training significantly reduces energy intake and fat intake ($p<0.05$) and carbohydrate intake ($p<0.05$). The changes between the two groups showed no significant difference ($P>0.05$).

Conclusion:
Although 8 weeks of moderate intensity aerobic training led to a decrease in calorie, fat and carbohydrate intake, these changes were not significantly different from the control group. Agouti-related protein changes may affect factors other than appetite. It appears that nutritional education or program can lead to more effective results if combined with exercise program.

Keywords: Endurance Training, Agouti-related Protein, Leptin, Insulin Resistance, Overweight

Introduction

Due to its high prevalence and direct relationship with other diseases, obesity imposes a huge burden on society (1). Hence, people should carefully control the balance of energy intake and consumption. The energy homeostasis system is regulated by central and peripheral factors (2). Nutritional behavior, Agouti-Related Proteins (AGRP) and leptin are the environmental factors known to play a role in the regulation of food intake and body weight. Human knowledge about the regulation of body weight, appetite, and energy balance dramatically increased upon

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the discovery of neuropeptides, especially AGRP (3) and leptin, which are the most important appetite suppressant and stimulant neuropeptides (2). AGRP gene is a candidate for obesity (3) and a strong appetite stimulating peptide. It affects nutritional behavior, weight regulation, and energy homeostasis, and has higher plasma levels in obese people (4). Studies reported that physical activity under normal circumstances led to overeating through energy intake and stimulation of neuropeptide Y activity and AGRP in the hypothalamus of rats (3, 5). As AGRP and neuropeptide Y are produced by the same neurons, it is assumed that the factors affecting the synthesis and release of neuropeptide Y, will influence AGRP, too. Negative energy balance for appetite stimulation and increased food intake depends on intensity, duration, and type of exercise, initial fuel sources values, and nutritional status (6). Exercise and hunger increase appetite, leading to an increase in negative energy balance and values of appetite stimulating hypothalamic, extrahypothalamic peptides, and peptides such as AGRP and neuropeptide Y (5, 7). It is also believed that AGRP, unlike neuropeptide Y that stimulates eating onset, leads to maintaining the nutritional behavior (8). Levine et al. examined the effect of exercise and calorie restriction on obesity, weight regulation, and expression of hypothalamic neuropeptides in obese rats. Their results indicated that exercise significantly reduced body weight, but had no significant effect on the expression of neuropeptide Y messenger RNA and AGRP (9). Hosseini et al. showed that endurance exercise led to significantly higher plasma levels of AGRP and soleus muscle tissue of rats (10). Ghanbari Niaki et al. suggested that endurance exercise significantly increased the plasma levels of AGRP immediately after exercise (11). There are few studies and conflicting results about the effects of exercise on changes in AGRP (9, 12). Human studies were mainly descriptive and plasma levels of the AGRP in obese women and men were compared with their standard counterparts (13), or they investigated fasting and satiety effects on plasma levels of AGRP (14). Other studies have investigated the AGRP expression in peripheral tissues of animals, AGRP injection, and the behavior due to this intervention (13, 14). AGRP and neuropeptide Y are suppressed under positive energy balance conditions where insulin, leptin, and ghrelin increase (6, 8). Leptin is mainly produced by adipose tissue and is involved in the regulation of metabolic processes (15). Leptin decreases appetite by suppressing synthesis of neuropeptides such as neuropeptide Y (15). Leptin works to reduce weight, but the lack of or resistance to it can cause weight gain (15). In other words, leptin is involved in regulating body weight, fat mass, and energy balance through energy consumption and suppressing appetite (9). It is also the original signal in regulating insulin secretion and glucose homeostasis regulation (15). The results of studies on leptin responses to exercise are controversial, as decrease (6, 16), no changes (17) or increase (18) was reported following exercise. It seems that because of the extent of risk factors associated with obesity and energy intake, many of these factors are still unidentified and the role of factors affecting appetite control and weight control such as AGRP is still unclear. Aerobic exercise along with improved nutritional behavior are important parts of exercise programs for weight control. Due to the contradictory results of the effects of the physical activity type in appetite-related factors, and the confusion in the possible association of aerobics with them, and the impact on the nutritional behavior, the present study aimed to determine the effects of aerobic exercise on plasma levels of AGRP, leptin, insulin resistance, and nutritional behavior in overweight sedentary women.

Materials and Methods

The study population consisted of overweight female students of Damavand

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University, 30 of whom were selected through a call and then randomly divided into two groups: control group (n = 15) and exercise group (n = 15). Later, 4 subjects in the control group, who had no interest in continuing cooperation with the research, were excluded according to the ethical principles. The number of subjects in this group reduced to 11 people. Inclusion criteria were BMI between 25 and 30, mental health, and not following a special diet for weight loss. After describing all the research processes by the researchers, participants were asked to complete and sign a participation consent form if they decided to participate. Descriptive characteristics of the subjects are presented in Table 1.

This was a quasi-experimental study. The subjects were divided into two groups of exercise and control in order to match them for weight. The subjects in the exercise group had aerobic exercise, including jogging on a treadmill, three days a week for eight weeks. Exercise intensity was determined on the basis of maximum heart rate and using the formula of [HR_{max} = 220 - age] (19). The exercise intensity in the first two weeks was 50-60 HR_{max} for 30 minutes. (20). Then, it increased for 5% every two weeks in order to respect the principle of overload, and finally reached 65-75% HR_{max} at the end of the last two weeks (21). The exercise period also increased every two weeks for 5 minutes, such that it reached 45 minutes at the end of the last two weeks. Moreover, a ten-minute warm-up at the beginning of each exercise session, including walking and jogging, and a five-minute cool-down at the end of the exercise program was considered. Exercises intensity was controlled with a pulse meter (Esaote Biomedica, Hungary). The intensity was regulated by increasing or decreasing the running speed such that the heart rate falls in the determined limits for each person. After reaching the desired intensity, time was measured by the treadmill watch. During the test, the control group did not participate in any exercise programs and followed their normal life.

Prior to the start of exercise period and 48 hours after the last exercise session, anthropometric characteristics of the participants were measured including height (Seca Wall Mount Stadiometer, Germany), weight (Seca digital scale, Germany) and body composition (*In body*, South Korea). The subjects were also asked to complete a food frequency questionnaire (FFQ). FFQ was designed based on Willett questionnaire (22) and adjusted for Iranian food items. FFQ consists of questions about the consumption frequency of food items according to the standard serving size or the amount that is usually familiar for people. Finally, the energy intake was calculated by N4 software adjusted for Iranian diet (23).

Prior to the start of exercise period and 48 hours after the last exercise session, 5 ml blood was drawn from the brachial vein of the subjects under fasting conditions. After clotting, the samples were centrifuged to separate serum. Serum samples were kept frozen at -80 °C until the time of measuring the variables. Glucose was measured by the photometric method (Pars Azmoon, Iran), and leptin and AGRP by ELISA (Cusabio, China). Insulin resistance index was calculated by Homeostasis model (HOMA-IR) using the following formula (24).

Homeostasis model = [Fasting glucose (mmol/l) × Fasting insulin (μIU/ml)/22.5]

Collected data was analyzed using SPSS software Version 16 at a significance level of $P \leq 0.05$. The Excel software version 2010 was used for drawing diagrams. The normal distribution of data was verified by Kolmogorov-Smirnov test. After ensuring the normal distribution of data, the dependent and independent t-tests were used to compare group means.

Results

Data on subjects' characteristics and variables (mean ± SD) are presented in Tables 1 and 2.

Statistical analysis of the calorie intake before and after the exercise period using t-

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test showed that energy intake in the control group did not change significantly over time (p>0.05), while the exercise group showed a significant reduction in energy intake after exercise (p<0.05). It was while the comparison of the changes in the two groups was not significant (p>0.05). In addition, changes in the consumed nutrients did not show any significant change in the control group. (p>0.05). However, the comparison of changes in the exercise group showed a significant decrease in the consumption of fat (p=0.05) and carbohydrates (p>0.05) compared to the time before exercise. A comparison of changes in the two groups among the three nutrients (fat, carbohydrate, and protein) using an independent t-test showed no significant difference between the two groups (p>0.05). Intragroup changes assessment showed that AGRP levels had no significant changes compared to pre-exercise time in the control group (p>0.05) while they significantly increased in the exercise group after completing the exercises (p<0.05). There was a significant difference in AGRP changes between the two groups (p<0.05). The results showed that exercise did not result in the change of serum leptin levels in the control group (24.84±12.48 vs. 27.88±12.45) and the exercise group (25.17±11.36 vs. 28.13±10.53) compared to the pre-test time (p>0.05). The comparison of changes between the two groups also showed no significant difference between changes in the two groups (p>0.05). The glucose level in the control group showed no significant changes compared to pre-exercise time (p>0.05), while it decreased significantly after exercise in the exercise group (p<0.05). However, this comparison indicated no significant difference between the two groups (p>0.05). Statistical analysis showed that insulin resistance index in the control group had no significant changes compared to the pre-exercise time (p>0.05), whereas the index decreased in the exercise group (p<0.05). The comparison of changes between the two groups also showed no significant difference between changes in the two groups (p>0.05).

Table 1: Descriptive characteristics of the subjects (mean ± SD)

Characteristics	Control group (N=11)		Exercise group (N=15)	
	Before	After	Before	After
Weight (kg)	75.14±8.15	74.83±8.15	75.49±9.92	74.48±10.03
BMI (kg/m^2)	28.20±2.75	28.02±2.72	27.50±2.47	27.00±2.36
WHR	0.92±0.04	0.92±0.03	0.91±0.03	0.88±0.11
Body Fat Percentage	40.97±5.09	42.61±3.23	39.44±4.00	38.12±4.07

Table 2: Information on variables (mean ± SD)

Group and Time / Variable	Control group (N=11)		Exercise group (N=15)	
	Before	After	Before	After
Energy intake (kcal)	2056.50±192.31	1804.84±277.81	2133.42±277.81	1763.67±199.20*
Consumed Fat (g)	75.80±37.07	61.99±19.06	75.09±38.38	56.09±28.11
Consumed Carbohydrates (g)	253.16±103.43	237.45±67.26	270.95±85.20	223.85±68.74
Consumed Protein (g)	86.52±53.42	59.06±11.85	75.48±47.28	72.06±32.71
AGRP (pg/ml)	24.82±8.34	25.71±9.73	16.81±7.17	38.69±24.88*#
Leptin (ng/ml)	24.84±12.48	27.88±12.88	86.52±53.42	86.52±53.42
Glucose (mg/dL)	95.91±7.48	88.80±8.41	99.57±6.96	92.00±6.74*
Insulin resistance	2.05±0.48	1.62±0.60	2.36±1.20	1.20±0.83*

[*] Indicates intra-group changes compared to the time before exercise
[#] Indicates a significant difference between the two groups.

Discussion

The present study aimed to investigate the effect of aerobic exercise on plasma levels of AGRP, leptin, insulin resistance and nutritional behavior in overweight

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sedentary women. The amount of food consumption and the overall size of the main meals and snacks in both exercise and control groups generally showed a reduction after eight weeks of exercise. The notable point here is the changes in the meals consumption pattern between the two groups. In other words, the exercise group, despite the lack of a specific nutrition education, experienced a reduction in the overall size of consumed food in lunch (29%) and snacks after dinner, while these values increased in the control group. Considering enhanced nutrients intake at breakfast as one of the key meals in a healthy diet, one can conclude that physical activity and an active lifestyle can lead to positive changes in people's nutritional behavior.

One of the most important factors measured in this study was the energy intake which plays an important role in the nutritional behavior. The general trend of energy intake changes in the present study showed a reduction after eight weeks of exercise. However, this trend was not significantly different between the two groups despite the 14% reduction in the exercise group. In line with the results of this study, several studies of King et al. during 1996 and 1997, (25, 26) and Lluch et al. (27) also suggested an insignificant decrease in energy intake, while they emphasized the role of physical activity, in addition to a low-calorie diet and control of nutritional behavior in order to control appetite. Ebrahimi et al. examined a low-intensity activity (55% of the maximum heart rate reserve for 45 minutes) and a high intensity activity (75% of the maximum heart rate reserve for 30 minutes) in 5 consecutive days and found no significant changes in energy intake or energy balance (28). Similar studies on low-intensity and high-intensity exercises for 7 days and 14 days (29) also reported no significant difference. According to studies and the findings of the present study on this factor, it is concluded that although the period of investigation is an effective factor on the energy intake, the intensity of activity and energy consumption in one session are also probably important elements. One of other possible reasons for the lack of a significant difference in decreased energy intake between the two groups could be the type of exercise program in this study. The present study focused more on exercises to strengthen the cardiorespiratory system than the muscular system in the experimental group. Since muscular system accounts for a great share of energy intake and consumption in the body, a slight change in this tissue can affect the results. Quality and volume of the consumed nutrients are the main determinants of energy intake, too. The present study measured the main nutrients (carbohydrates, fat, and protein) in the diet of subjects through FFQ adjusted for Iranian food items before and after eight weeks of exercise. Fat and carbohydrate intake reduced in the experimental group, while protein did not change significantly. Generally, the changes in all three nutrients were insignificant between the control and experiment groups. Nutritional knowledge and attitude are two main determinant factors of volume and amount of nutrients in people (30). The present study did not provide the subjects with any specific nutritional education before or during the exercise period. The reported amount only represents the knowledge and attitudes of the subjects toward the consumption of nutrients. Since overweight women in the present study may have targeted losing weight as their main goal, they may have particularly tried to avoid fat and carbohydrates, hence changes observed in the consumption of fat and carbohydrates, especially in the exercise group can be attributed to this regimen and not the improvement of their nutritional knowledge and attitude, because the observed changes were not significantly different compared to the control group. On the other hand, lack of a significant correlation between reduction of total energy intake and observed changes in nutrient consumption can also prove this claim.

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The assessment of AGRP as an appetite-stimulating factor showed that eight weeks of aerobic exercise changed this factor significantly (a 2.5-fold increase compared to before the exercise period). The results of various studies on this factor, although often focused on its gene expression in rats, were inconsistent (9, 12). However, the overall outcome of the results showed an increase in AGRP both in plasma level and gene expression (12). The significant increase in AGRP was observed by other researchers, too. For example, Rejeck et al. reported a 4.8-fold increase in AGRP compared to the control group. Negative energy balance is the main factor affecting the AGRP (6) which can be affected by the main components of physical activity (i.e. the overall intensity, duration and volume of the activity). With regard to plasma levels of AGRP, it can be argued that activities lead to its production stimulation or release by creating a negative energy balance. Since AGRP has a mediating role along with its regulatory role in appetite and energy balance, significant changes in plasma levels of this protein in the present study have probably influenced factors other than appetite and other relevant factors. The mediating role of AGRP is substantial in neuroendocrine response to inflammation, which in turn can increase obesity and type 2 diabetes. Evidence also suggests that AGRP has an inhibitory effect on hypothalamic–pituitary–thyroid axis, that is, the induction of AGRP suppresses the TSH-secreting hormone, (i.e. TRH), and reduces the amount of circulating thyroid hormones (6). Cytokine leptin is another appetite-affecting factor measured in this study. The results showed very small and insignificant increase in leptin. Leptin suppresses appetite by inhibiting neuropeptide Y in people with no insulin or leptin resistance. The results of the effect of physical activity on levels of leptin are very inconsistent. In line with the results of this study, ZilaeiBouri et al. did not report changes in leptin plasma levels after eight weeks of moderate-intensity aerobic exercise (31), while Parastesh et al. reported a reduction after twelve weeks of aerobic exercise. It seems that at least 12 weeks of activity is required to observe possible changes in leptin levels (32). On the other hand, leptin is highly dependent on energy consumption and the overall volume of performed work in one exercise session. Therefore, the present study did not meet the needed exercise session volume and energy consumption amount (at least 800 kcal) (33) for observing changes in leptin and consequently in appetite after an exercise course. Plasma glucose levels were also measured in this study. The results suggested that changes in glucose between the control and exercise groups had no significant difference after eight weeks. Although the changes in the experimental group were significant and leptin showed a reduction after eight weeks of moderate-intensity aerobic exercise. Glucose is a variable with relatively high and quick variability and is affected by intensity, duration and the amount of stress. The amount of glucose in both groups was in the normal range of less than 100 mg/dL that somehow reflects the insulin sensitivity of the cells and the ability of insulin to maintain glucose in the normal range despite the overweight in the subjects. The values obtained from insulin resistance index can also emphasize this point. The values obtained for insulin resistance index in this study suggest that changes of this factor were not significantly different in the two groups. However, the exercise group values significantly reduced (31%) which is in line with the observed changes in glucose. Reduced insulin resistance was reported in many studies on aerobic physical activity (28). It was supposed that the responses of leptin, insulin resistance, and glucose in this study were associated together because there was a significant difference between the two groups in all the three cases. However, there was no significant relationship between changes in these variables. Accordingly, changes in the insulin resistance index were possibly

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induced by temporary changes in insulin and glucose as the key components of the index at the time of blood sampling, but not the cellular adaptations in the body. On the other hand, as the subjects did not follow a special diet in this study, and nutrient intake changes were not significant, we can conclude that stomach volume did not change significantly, either. It appears that changes in these factors as a result of a need for structural changes and fundamental functions in the relevant tissues require stronger nutritional and exercise stimuli.

Conclusion

The results showed that, although the eight-week moderate-intensity aerobic activity led to significant changes in energy intake and the amount of carbohydrates and fat consumption in the exercise group, the changes were not enough to make a significant difference with the control group. Probably significant changes in plasma levels of AGRP have affected factors other than appetite and some related factors. It seems that providing a diet or nutritional education combined with exercise can lead to more effective results.

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Conflict of interest

The Authors declare that there is no conflict of interest in this paper.

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