Effect of a Single Session of Aerobic and Circuit-Resistance Exercise on Plasma Ghrelin and Agouti-Related Peptide (AgRP) Levels in Well-Trained Females

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Introduction

Sedentary life style and low physical activity lead to overweight and obesity [1]. Obesity has long been viewed as an imbalance in the energy equation [2] and represents a state of excess storage of body fat [3]. Rising prevalence of obesity is evident worldwide [4], and it is known to be a main cause of many diseases such as hypertension, atherosclerosis, type II diabetes, certain types of cancer and respiratory and gastrointestinal disorders; a strong relationship between obesity and these diseases has been reported [5, 6]. Body weight is regulated by a balance between food intake and energy expenditure, and it is reported that exercise is an effective method of increasing the latter [7]. The arcuate nucleus (ARC) is a key hypothalamic nucleus in appetite regulation and is an important integration site linked to the control of food intake and energy homeostasis. The hypothalamus and its neural circuits play a critical role in the regulation of feeding behavior and body weight [8-10]. Several different neuronal populations exist in the hypothalamic arcuate nucleus and regulate energy homeostasis [8-11]. Among them, agouti-related peptide (AgRP) neurons are involved in feeding and weight gain [8]. AgRP is a 132-amino acid peptide [12], released by fasting, undernutrition [11] and caloric restriction [13], and is a neuropeptide expressed mainly by the arcuate nucleus of the hypothalamus. Probably, the most important role of AgRP is its role in energy balance and homeostasis. Therefore, these researchers have suggested that AgRP neuropeptide may contribute to the control of food intake and obesity [12-14]. AgRP induces food intake and positive energy balance via its orexigenic effects [8-10]. Ghrelin is a 28-amino acid appetite-stimulating peptide that is released primarily by the stomach and the gastrointestinal tract [14]. Ghrelin levels tend to rise before meals and decrease after meals, suggesting that ghrelin prompts meal initiation [15-17]. Furthermore, ghrelin modulates peripheral metabolism, weight gain, and adiposity [18, 19]. The orexigenic effects of ghrelin are exerted through the growth hormone secretagogue receptor 1a (GHS-R1a) [20], which is expressed in agoutirelated peptide/neuropeptide Y (AgRP/NPY) neurons in the arcuate nucleus of the hypothalamus (ARC) [21]. Exercise and reducing energy intake constitute the first line of treatment for inducing weight loss [22, 23]. Energy balance and maintenance of body weight depend on the balance between energy intake and energy expenditure [24]. Food restriction increased AgRP and NPY mRNAs in both running and sedentary rats, which reflected a stronger negative energy balance and also indicated that AgRP and NPY expression was dependent on the availability of energy stores [25]. Single circuit resistance exercise (at 35% 1RM) increased plasma AgRP in male college students [26]. It has been reported that the effect of fasting resulted in elevation of plasma AgRP levels in obese men [27]. Researches on ghrelin responses to a single session acute exercise have
Materials and Methods

Before administrating this study, the researchers obtained approval from the ethical committee of Ferdowsi University of Mashhad, Iran.

Participants: Twenty-four well-trained females were randomly assigned into three groups of eight participants: (1) Control (no exercise); (2) Circuit resistance group [resistance training at 60% of a one-repetition maximum (1-RM)]; (3) Aerobic group (1.5 miles run at 70% of VO₂max). Subjects were asked to complete a medical examination and a medical questionnaire to ascertain that they did not take any medication, were free of cardiac, respiratory, renal, metabolic diseases. According to research findings on the impact of estrogen on ghrelin [34], all participants were all in luteal phase of their menstrual cycle. All subjects were accustomed to the training protocol.

Dietary control: Participants were asked to complete the dietary questionnaire for details of the energy and macronutrient intakes during 3 days before the protocol. Their daily food intakes (dietary habit) were 55-65% carbohydrate, 15-20% fat and 20-25% protein. Then, the diet for the night before the blood sampling proposed to all participants.

Training protocol: Before the main trial, participants were taken to the weight training room three times. On the first two visits, all of the participants performed strength tests to determine their 1-RM for each of the nine free-weight resistance exercises (arm curl, triceps extension, back extension, squat, leg curl, bench press, overhead press, dead lift, seated row). On the third visit, the subjects completed a practice session to make sure that each participant was able to complete the entire exercise session. Subjects were asked to perform 3 sets of 9 non-stop circuits (9 exercises with 60% of one-repetition maximum for each exercise at their maximum speed) with a 5-minute rest between the sets. The aerobic group was asked to run for 1.5 miles at 70% of their VO₂max. The employed procedure was the same as the previously reported one [28-36]. The training session for both the aerobic and anaerobic groups consisted of a 10-minute warm-up, followed by the main exercises, and a 10-minute cool-down period in the end. The duration of the entire session was 35-45 minutes, and the control group remained sedentary during this time.

The heart rate of the subjects was constantly checked (70-85% of the maximum heart rate) using the polar device (Finland). The exercise sessions were held in the morning between 08:30 and 11:00, to avoid the effects of circadian rhythms. Subjects were instructed to follow normal lifestyle habits, avoid medications, refrain from exercise three days before the study, 12 hours of fasting and visit the lab after a 12-hour fast (only allowed to drink pure water) for the blood sampling. All the subjects were randomly divided into two experimental and one control groups.

Experimental group I (N=8) followed researchers’ recommended running 1.5 miles with a fixed heart rate (VO₂max 70%) (AE group); experimental group II (N=8) was asked to completed a circuit-resistance exercise protocol (RE group). The participants were instructed to follow a normal lifestyle, maintain daily habits, avoid all medications, and refrain from exercising 3 days before the experiment session.

Blood samples: Blood samples (10 ml) were taken before and immediately after the protocol, while the subjects were overnight fast (at least 12 h). Plasma was obtained for AGRP and ghrelin determination. Plasma AgRP was measured by using a special kit, which was performed with commercially available enzyme-linked immuno sorbent assay (ELISA) kits (Pharmaceuticals, Belmont, USA, Sensitivity 0.07 ng/ml).

The total level of plasma ghrelin was measured by using a special kit, which was performed with commercially available enzyme-linked immuno sorbent assay (ELISA) kits (Pharmaceuticals, Belmont, USA, Sensitivity 6.25 pg/ml).

Body fat percentage measurements: Percentage of body fat from skin fold thickness of subjects was measured by using the 3-point skin fold thickness method [30]. The skin fold thickness at three sites was obtained using a caliper. The skin fold sites were triceps, subscapular and abdomen.

Anthropometric measurement: The subjects were weighted by a digital scale of 0.01 kg accuracy; all subjects were without any clothes and shoes. Subjects’ height was measured using a meter on a wall; all subjects were without clothes and shoes. Body mass index (BMI) was also calculated as weight (kg) divided by height (m) squared. Fat percentage of the subjects was measured using caliper device by the method of three skin fold thickness (subscapular, abdominal, and triceps) [37].

Statistical analysis: All calculations were made using SPSS-16.0. All data are presented as mean±standard error of mean (SE). We used Levene’s test to assess homogeneity of variances and used Kolmogorov-Smirnov test to examine normal distribution of data. One-way analysis of variance (one-way ANOVA) and Tukey post hoc test were used to examine the difference between the groups. Statistical significance was accepted at the 5% level.
Results

Individual characteristics of the subjects determined by are highlighted in Table 1. According to the result of Levene’s test and the Kolmogorov-Smirnov test, all dependent variables were normally distributed and homogeneous. Significant increases were noted in circulating AgRP of control group ($t=-8.775; p=0.001$), circuit resistance ($t=-24.77; p=0.001$) and aerobic group ($t=-36.940; p=0.001$). Also, there was significant increases in circulating Ghrelin of control ($t=-12.320; p=0.0001$), circuit resistance ($t=-92.884; p=0.001$) and aerobic subjects ($t=-135.227; p=0.001$).

At the end of the protocols, one-way ANOVA indicated a statistically significant difference between the groups in plasma AgRP levels ($F=385.715; p=0.001$), plasma Ghrelin levels ($F=4011.9; p=0.001$). At the end of the protocols, the results of the Tukey post hoc test showed that plasma AgRP levels (ng/ml) in circuit resistance group were significantly higher when compared to the aerobic group ($p=0.011$) (0.0575 ng/mL to 1.235 ng/ml). According to the results of the Tukey post hoc test, there was also an increase in the plasma Ghrelin levels of circuit resistance subjects when compared to the aerobic subjects, but the increase was not significant ($p=0.545$).

Discussion

As the results show, a single bout of circuit resistance exercise and aerobic training can significantly increase AgRP and ghrelin concentration in plasma. Andersson et al. [38] reported that a single session of treadmill running (22 m/min, 10% grade) resulted in a significant increase in plasma total ghrelin levels. In a study, regardless of the increase in GH, ghrelin was significantly depressed following the single bout of circuit resistance exercise [32]. Currently two studies exist concerning plasma ghrelin response to an aerobic exercise [39, 40].

Acute effects of a single bout of exercise on appetite and energy intake have been investigated by several researchers [35, 41]. According to previous studies findings, negative energy balance to stimulate appetite and increase food intake depends on intensity, duration, mode of exercise, initial levels of fuel sources, and nutritional status [41, 42].

Table 1. Anthropometric characteristics of participants

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (N=8)</th>
<th>Circuit resistance group (N=8)</th>
<th>Aerobic group (N=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.37±1.76</td>
<td>21.87±3.04</td>
<td>21.12±2.35</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161.69±1.85</td>
<td>160.5±3.99</td>
<td>163.12±5.20</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>59.68±7.65</td>
<td>56.35±9.31</td>
<td>58.25±7.80</td>
</tr>
<tr>
<td>BMI(kg/m²)</td>
<td>22.88±3.49</td>
<td>21.82±3.01</td>
<td>21.82±1.92</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>19.67±1.65</td>
<td>18.29±3.14</td>
<td>19.67±5.51</td>
</tr>
</tbody>
</table>

Values are means±SE

Table 2. Pre and post training values of plasma AgRP and ghrelin concentrations in the three groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group (N=8)</th>
<th>Circuit resistance group (N=8)</th>
<th>Aerobic group (N=8)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (ng/ml)</td>
<td>0.712 ± 0.0324</td>
<td>1.300 ± 0.150</td>
<td>8.302 ± 0.2014</td>
<td>8.155 ± 0.177</td>
</tr>
<tr>
<td>AgRP (ng/ml)</td>
<td>0.0375 ± 0.0158</td>
<td>0.0650 ± 0.0220</td>
<td>1.235 ± 0.1421</td>
<td>1.090 ± 0.0767</td>
</tr>
</tbody>
</table>

Values are means±SD, *: $p<0.05$

![Figure 1. Plasma ghrelin (A) and AgRP (B) levels in the three groups before and after training protocol](image-url)
The main findings of this research are as follows: plasma AgRP and ghrelin levels were significantly increased in the three groups, whereas the increase in experimental groups was significant higher when compared to the control group. There are few researches about the effect of exercising on plasma AgRP, especially in humans. In these few studies, the effects of fasting and satiety have been investigated on plasma AgRP [39]. It has been shown that AgRP had both an acute and long-term effect on food intake [43].

During positive energy balance, reduced AgRP gene expression inhibits feeding and suppresses appetite. In contrast, during fasting, when energy balance is negative and also energy stores are low, increased AgRP gene expression stimulates feeding or promotes appetite [8, 11, 13, 25]. This change in the resting plasma AgRP and ghrelin levels may be due to decreased energy stores and/or cellular charge during the protocol. A changed ATP/ADP ratio stimulates or suppresses several mechanisms, which increase/decrease orexigenic gene expression such as AgRP and ghrelin [11]. These positive changes in energy balance could reintegrate homeostasis of energy in the body. In study by Ghanbari-Niaki et al. [26]. Plasma AgRP levels increased immediately after exercising and returned to the pre-exercise level in the recovery period. It has been determined that food restricting would significantly increase plasma AgRP levels both on rats in humans [25, 44]. On the other hand, exercising and physical activity disorders energy balance and homeostasis between muscle cells and increases the demands of energy for cells. This probable decrease of energy resources of cells with overnight fasting could be an important factor in increasing plasma AgRP and ghrelin concentration in the subjects. It could be concluded from the findings that hypothalamus increases plasma AgRP after exercising. Findings of this study are in compliance with this concept that: inner and outer signal channels of the cells are responsible for increasing appetite due to increase of energy expenditure after exercising and AgRP appetite increasing signal channel could be an important channel toward response to exercising [45].

The present study has investigated the effect of two types of different exercising in young athlete females. The results obtained from this study are in accordance with these few researches. Since ghrelin and AgRP collaborate in increasing appetite, and in previous studies increasing of these two peptides have been reported to be concurrent [45], has agreed with the present study. Nonetheless, it seems that further investigation is necessary to understand possible mechanisms in this field. It seems that the increase in plasma AgRP can be in line with the increase of this peptide in hypothalamus and in response to the negative energy balance due to the exercising which increases the appetite in order to recover energy resources of the subjects’ body. In summary, the data indicate that a single circuitresistance exercise (at 60% 1RM) and aerobic exercise increased plasma AgRP and ghrelin. A post-exercise elevated plasma AGRP and ghrelin level might thus be considered as a possible stimulus for a post-exercise induced hyperphagia state.

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Authors’ Contributions
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Conflict of Interest
The authors declare no conflict of interest.

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