

RESTING PLASMA AgRP LEVELS RESPONSE TO EXERCISE-CONJUGATED DIET AND ONLY DIET IN OVERWEIGHT AND OBESE SEDENTARY FEMALES

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ABSTRACT: Agouti-related peptide (AgRP) is an orexigenic neuropeptide produced mainly in the arcuate nucleus (ARC) of the hypothalamus, which leads to positive energy balance. The purpose of the present study was to investigate the resting plasma AgRP levels' response to exercise-conjugated diet and only diet in overweight and obese sedentary female college students. Thirty subjects were randomly divided into two experimental groups and one control group. Experimental group 1 experienced 12 days of researchers' proposed diet (D), experimental group 2 experienced 12 days of researchers' proposed diet (every day) with 12-day running training (the intensity was 60 to 70% heart rate maximum for 50 minutes) every other day (AED) and the control group remained sedentary with a normal diet in this period. Blood samples were collected 24 hours before and after the enforced protocol. Body fat percentage (BF%) of subjects was measured using the 3-point method. Plasma AgRP levels were measured using the enzyme-linked immunosorbent assay (ELISA) method. The data were analysed by Leven, Kolmogorov-Smirnov, one-way analysis of variance (ANOVA) and Tukey post-hoc test. At the end of the protocols, the resting plasma AgRP in the D group increased significantly ($p < 0.05$) and in the AED group no change was observed ($P > 0.05$). Body weight and BMI were significantly reduced for both experimental groups ($P < 0.001$). Body fat percentage in the AED group was decreased ($p < 0.001$) and no change was observed in the D group. The present results indicate that the AED is better than the D protocol because it leads to stability of AgRP, which may prevent lipogenesis and ultimately reduce body fat percentage.

KEY WORDS: AgRP, aerobic exercise, diet, obesity, overweight

INTRODUCTION

Sedentary life style and low physical activity lead to overweight and obesity [22]. Obesity has long been viewed as an imbalance in the energy equation [31] and represents a state of excess storage of body fat [27]. Rising prevalence of obesity is evident worldwide [26], 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 [6,15,20,22,26]. On the other hand, chronic imbalance between energy intake and energy expenditure leads to obesity [6,12,14,20,31]. 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 [3].

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 behaviour and body weight [17,24,28]. Several different neuronal populations exist in the hypothalamic arcuate nucleus and regulate energy homeostasis [2,17,24,28]. Among them, agouti-related peptide (AgRP) neurons are involved in feeding and weight gain [17]. In the ARC, anorexigenic neurons (producing proopiomelanocortin

List of abbreviations:

AgRP:	Agouti-related peptide
NPY:	neuropeptide Y
BMI:	body mass index
BF%:	body fat percentage
D:	12-day diet protocol
AED:	12-day diet protocol (every day) plus 6 sessions of aerobic exercise (every other day)
ELISA:	enzyme-linked immunosorbent assay
HRmax:	heart rate maximum
ARC:	arcuate nucleus
SE:	mean \pm standard error of mean

and cocaine- and amphetamine-related transcript) and orexigenic neurons (producing neuropeptide Y (NPY) and AgRP) are known to be essential in the food intake and energy expenditure regulation [2]. AgRP is a 132-amino acid peptide [10], released by fasting, undernutrition [2] and caloric restriction [21], 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 [6,20,25]. AgRP induces food intake and positive energy balance via its orexigenic effects [2,17,28]. Caloric restriction increased orexigenic gene expression in the arcuate nucleus (ARC) of the hypothalamus (e.g., AgRP) [21]. On the other hand, it was shown that physical exercise elevates NPY concentration [34]. Exercise and reducing energy intake constitute the first line of treatment for inducing weight loss [27,32]. Energy balance and maintenance of body weight depend on the balance between energy intake and energy expenditure [33].

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 [10]. It has been reported that the effect of fasting resulted in elevation of plasma AgRP levels in obese men [16]. However, few studies have been conducted to investigate the impact of exercise and diet on plasma levels of AgRP [7,9,10,13,15,33]. On humans these studies have been even fewer [11] and most investigations have focused on the effects of hunger on this peptide [3,16,27,33]. Therefore, it has been necessary to evaluate the impact of only diet (D) and aerobic exercise conjugated-diet (AED) on resting plasma concentration of AgRP in overweight and obese sedentary females of college students. The aim of the present study is to compare the AED and the D protocols on the resting plasma concentration of AgRP.

MATERIALS AND METHODS

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

Study population

A total of 30 overweight and obese sedentary females of college students participated voluntarily in the present study. All subjects were overweight and obese individuals (mean age 21.9 ± 2.4 years,

mean weight 75.6 ± 5.57 kg, mean height 161 ± 4.79 cm, mean BF% 29.53 ± 3.18 and mean BMI 29.04 ± 2.22 kg m⁻²). Before entering the present study, participants lived in similar college dormitories for at least 7 months. All subjects completed a physical activity and a health history questionnaire. They had no history of regular physical activity or aerobic exercise for several years, were non-smokers, had no history of cardiovascular disease, were not on a weight loss/weight gain diet before the present study, and none used any drug before or during the protocols. Finally, subjects were informed that they could withdraw from the study at any time.

Aerobic exercise and diet protocols

Dietary control

All participants lived in a similar place (college dormitory), and their daily main food intake such as breakfast, lunch and dinner included similar nutritional substances. Participants were asked to complete the dietary questionnaire for details of the energy and macronutrient intakes during 10 days before starting the protocol. Their daily food intakes (dietary habit) were 55-65% carbohydrate, 15-20% fat and 20-25% protein. During the protocols, all subjects were asked to avoid taking any food with high fat.

All subjects were also asked before taking any food, including snacks between main meals, to consult the researcher (exercise physiologist). Thus, participant's diet was controlled

Diet protocol

Participants were asked to use a diet programme, including the researcher's proposed 12-day diet (Table 1). This diet method was extracted from Rashidlamir's diet method [23]. It consisted of three 4-day phases; in each phase in the first three days a decrease in nutrition occurred and on the last day a return of the diet to the previous phase. In the first phase, the subjects decreased their food intake by 10% for three days (lunch and dinner) and then they returned to their usual eating habits on the fourth day (daily dietary habit before the protocol). In the second phase, first they decreased their food intake by 20% for three days then they return to 10% on the fourth day. In the third phase, first they decreased their food intake by 30% for three days then they return to 20% on the fourth day. There was no limitation on drinking water and no decrease in breakfast, but the subjects avoided fat in all meals. One of the most important benefits of this programme was that it was understandable for the subjects because they could follow it easily, which is a positive and important point.

TABLE I. TWELVE-DAYS DIET PROTOCOL

	First phase				Second phase				Third phase			
	1 st day	2 st day	3 st day	4 st day	5 st day	6 st day	7 st day	8 st day	9 st day	10 st day	11 st day	12 st day
Breakfast	No decrease											
The decrease in lunch	10%	10%	10%	Eating like before weight loss phase	20%	20%	20%	10%	30%	30%	30%	20%
The decrease in dinner	10%	10%	10%	Eating like before weight loss phase	20%	20%	20%	10%	30%	30%	30%	20%

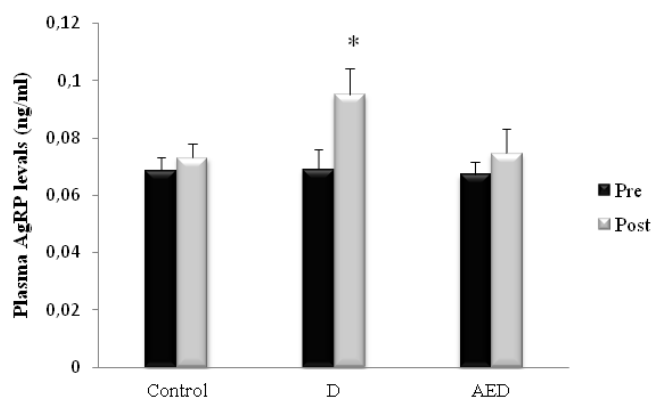


FIG. 1. CHANGES OF PLASMA AGRP LEVELS OF D AND AED GROUPS WHEN COMPARED TO CONTROL GROUP.

* The means difference is at the 0.05 levels.

There was no fundamental change in the subjects' eating habits. This point is very important for applied dietary programmes for most people, because most people who follow a diet programme, due to changes in diet, food habits and the difficulty of implementing it, withdraw from the programme. Implementation of a programme depends on simplifying it.

Another positive point is that this programme is designed on the basis of daily food intake. On the other hand, daily food intake (i.e., type of macronutrient) is not designed according to this programme. This shows that the present diet programme can be implement worldwide with each diet.

Aerobic exercise protocol

The aerobic exercise programme (6 sessions) consisted of four times ten-minute running along with three times two-minute walking between each running time plus a four-minute time for participants' active recovery per session for twelve days (every other day) [4×10(min) running+3×2(min) walking between each running time+4min active recovery]. The total time of the aerobic exercise was 50 minutes in each session. Training intensity was 60-70% heart rate maximum (HR max) for participants. Subjects were running in an outdoor environment. Furthermore, they were supervised by an exercise physiologist.

Groups division

All the subjects were randomly divided into two experimental and one control groups. Experimental group I (N=10) followed researchers'

recommended 12-day diet protocol (D group); experimental group II (N=10) underwent 12-day aerobic exercise every other day and diet recommended by researchers every day (AED group); the control group (N=10) remained sedentary and participants were asked to avoid any physical activity or exercise during the experiment and to maintain their usual dietary habits.

Body weight, height and body mass index measurement

The weight of all fasting subjects was measured before and after the enforced protocol by a digital scale; 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. All measurements were performed twice during the study, once before and once after the study.

Body fat percentage measurements

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

Laboratory measurements

Blood samples (5 cc from brachial vein) were collected between 8:00 and 9:00 AM (in the case of fasting), 24 hours before the first and after the last day of the protocol. Blood samples were then drawn into a pre cooled tube containing EDTA. After sampling, it was immediately centrifuged at 3000 g for 10 min. The plasma was stored at -80°C until analysis. Then plasma AgRP levels were measured by a special kit, which was performed with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Phoenix Pharmaceuticals, Belmont, USA, Sensitivity 0.07 ng·mL⁻¹).

Statistical analysis

All calculations were made using SPSS/PC version 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.

TABLE 2. BASELINE PHYSICAL AND METABOLIC CHARACTERISTICS IN 30 IRANIAN OVERWEIGHT AND OBESE SEDENTARY FEMALE COLLEGE STUDENTS BEFORE THE PROTOCOLS (MEAN±SE)

Group	Age (years)	Height (cm)	Weight (kg)	BMI (kg·m ⁻²)	BF (%)	Plasma AgRP levels (ng·mL ⁻¹)
Control	21.8 ± 0.81	1.60 ± 2.00	76.59 ± 1.85	29.81 ± 0.64	29.42 ± 0.67	0.0687 ± 0.0045
Only diet	22.2 ± 0.71	1.63 ± 1.58	75.06 ± 2.37	28.23 ± 0.87	31.05 ± 2.17	0.0691 ± 0.0071
Aerobic exercise conjugated	21.8 ± 0.71	1.61 ± 1.08	75.05 ± 1.61	28.80 ± 0.68	28.86 ± 0.72	0.0673 ± 0.0045

TABLE 3. SOMATIC CHARACTERISTIC AND THE RESTING PLASMA AgRP CONCENTRATION BEFORE AND AFTER PROTOCOLS

Dependent variables	Control		Only diet (D)		P-Value	Aerobic exercise conjugated diet (AED)		P-Value
	Before protocol	After protocol	Before protocol	After protocol		Before protocol	After protocol	
Body weight (kg)	76.59 ± 1.85	76.45 ± 1.76	75.06 ± 2.37	73.38 ± 2.13*	<0.001	75.05 ± 1.61*	72.90 ± 1.62*	<0.001
Body fat (%)	29.42 ± 0.67	29.44 ± 0.69	31.05 ± 2.17	30.55 ± 2.00	0.458	28.86 ± 0.72	28.04 ± 0.74*	<0.001
Body mass index (BMI) (kg·m ⁻²)	29.81 ± 0.64	29.44 ± 0.60	28.23 ± 0.87	27.60 ± 0.79*	<0.001	28.80 ± 0.68*	27.97 ± 0.67*	<0.001
Plasma AgRP levels (ng·mL ⁻¹)	0.0687 ± 0.0045	0.0732 ± 0.0048	0.0691 ± 0.0071	0.0953 ± 0.0089*	<0.05	0.0673 ± 0.0045	0.0747 ± 0.0084	0.924

RESULTS

The baseline physical and metabolic characteristics of all subjects are described in Table 2. Subjects' body mass index (BMI) ranged from 25.03 to 33.04 kg·m⁻². According to the result of Levene's test and the Kolmogorov-Smirnov test, all dependent variables were normally distributed and homogeneous. At the end of the protocols, one-way ANOVA indicated a statistically significant difference between the groups in the resting plasma AgRP levels ($F=4.035$; $P=0.029$), body weight (kg) ($F=42.226$; $P<0.001$), BMI (kg·m⁻²) ($F=40.138$; $P<0.001$) and body fat percentage ($F=13.158$; $P<0.001$). Therefore, there is sufficient evidence to confirm the effect of the interventions.

At the end of the protocols, the results of the Tukey post hoc test showed that resting plasma AgRP levels (ng·mL⁻¹) in D group were significantly higher when compared to the control group ($P=0.036$). Significant increases were noted in circulating AgRP of D protocol subjects (Fig. 1, Table 3) (0.0691 ng·mL⁻¹ to 0.0953 ng·mL⁻¹). There was also no significant change in the resting plasma AgRP levels ($P=0.924$) after the AED protocol when compared to the control group (Fig. 1, Table 3).

Body weight (kg) after the protocols was significantly reduced for both AED and D groups ($P<0.001$) in comparison with the control group, which was less in the D group. BMI (kg·m⁻²) in both D ($P<0.001$) and AED ($P<0.001$) groups decreased when compared to the control group (Table 3). A decrease in body fat percentage of the AED group was also observed ($p<0.001$) when compared to the control group. We observed no significant change in body fat percentage of the D group ($p=0.458$) in comparison with the control group (see table 3). Physical and metabolic characteristics before and after aerobic exercise in all subjects are also shown in Table 3.

DISCUSSION

This is the first study demonstrating the effect of AED and D on the resting plasma concentration of AgRP in overweight and obese sedentary females of college students. Many studies had changed macronutrients such as fat, protein or carbohydrate percentages in the diet (change in dietary habit) [1,4,5,11,18], which is difficult for individuals and causes them to abandon their diet programme

(discontinue their diet plans). In our study, in addition to aerobic exercise, the percentage of daily food intake was reduced. The present diet protocol is easy for individuals to implement (no change in dietary habit), which motivates individuals to continue their diet programme. The main findings of this research are as follows: the resting plasma AgRP levels were significantly increased in the D group, whereas there was no significant change in the resting plasma AgRP levels in the AED group. At the end of the protocols, body weight was significantly reduced for both groups. Furthermore, in the AED group, body fat percentage was significantly reduced.

It has been shown that AgRP had both an acute and long-term effect on food intake [12]. 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 [2,17,21,24,28]. In such circumstances, small changes in cellular charge lead to a response of AgRP neurons and change its neurotransmission.

This change in the resting plasma AgRP levels may be due to decreased energy stores and/or cellular charge during the only diet protocol. A changed ATP/ADP ratio stimulates or suppresses several mechanisms, which increase/decrease orexigenic gene expression such as AgRP [2]. These positive changes in energy balance could reintegrate homeostasis of energy in the body. Moreover, previous studies strongly support our suggestion that AgRP secretion is the cellular target by which diet-induced changes in cellular charge.

Fat mass is a major source of leptin production. Leptin has inverse functions (e.g., restrains food intake promotion and lipogenesis) of AgRP [2]. On the other hand, NPY and AgRP have co-localization and co-expression in the ARC [2,24,28]. Furthermore, NPY increases white fat lipid storage, and reduces brown fat thermogenesis [24]. Likewise, it has been reported that AgRP increases body fat storage, and reduces brown fat thermogenesis [24], which may be a role of AgRP in the pathogenesis of obesity [16]. Activation of AgRP neurons causes increased food intake and weight gain, decreased energy expenditure and reduced fat oxidation. Body fat percentage reduction might be attributed to stabilisation of AgRP by the AED and stability of body fat percentage in the D group might be attributed to increased

AgRP levels. The present findings are consistent with the above studies.

Our study shows that the D protocol enhances plasma AgRP levels. In addition, the fasting induced an increase in AgRP release in response to decreased energy stores and/or decreased cellular charge. These data suggest that AgRP neurons increase the release of AgRP neuropeptide by negative energy balance. Thus, in the presence of AgRP neuropeptide, energy balance returns to homeostasis. This prolonged effect of the D protocol on plasma AgRP levels (24 h after the end of the protocol) creates overfeeding and ultimately increases weight gain. Finally, negative energy balance due to fasting induces AgRP gene expression, increases plasma AgRP levels and ultimately increases food intake and leads to positive energy balance via their orexigenic effects [2].

On the other hand, stability of plasma AgRP levels during the AED protocol shows no elevated AgRP levels by the ARC of the hypothalamus for 24 h after the end of the protocol. This status is a sign of energy homeostasis. The AED protocol per se played an inhibitory role on activation of AgRP neurons in the long term. There may be an effect of cellular charge and energy store adaptations on AgRP neurons due to the AED protocol.

Exercise had a greater effect than diet in inducing negative energy balance in the short and medium term [19]. Other studies have shown that regular exercise, regardless of the effect on energy status [29,32], changes acylated ghrelin concentrations, and it stimulates the energy intake in women but not in men directly. Moreover, women may need to increase energy expenditure and decrease energy intake to achieve body fat loss [29]. Our findings demonstrated that the AED group decreased body fat percentage and lost weight without change of resting plasma AgRP. It has been reported that administration of AgRP leads to elevated food intake, body weight, and body fat and to reduced energy expenditure and brown fat thermogenesis [24], which are important roles of the arcuate nucleus of the hypothalamus in body weight and body fat management. These documents have supported our findings. It has been shown that when women were performed to walk/to jog (in comparison to the control group) [8] and were also performed to follow exercise and low energy intake [32], their body weight decreased and their body fat had a significant reduction. These findings support our finding that a significant change in body weight and body fat percentage was due to AED, while the D group's body weight decreased without a significant reduction in their body fat

percentage. Weight loss of the D group may be due to other physiological factors such as proteolysis, which demands future studies.

Exercise may lead to a better coupling between energy intake and energy expenditure in the long term that ensures that body weight is maintained, which may be due to appetite regulation. Furthermore, it may cause an increase of energy intake with increased levels of physical activity [3]. It has been indicated that physical exercise or training can decrease adiposity [3,32] and plays an important role in energy expenditure and influences hormonal concentrations [3]. More research is necessary to explain the mechanisms behind the post-exercise adjustments in short-term appetite control, and their long-term consequences [3], while there have been few studies on the effect of both acute and chronic exercise combined with diet or either one alone, on circulating levels of AgRP peptide in humans involved in appetite control. We demonstrated one of those mechanisms. It explained an optimum influence of the AED in neuropeptide involved in food intake behaviour and appetite. According to our finding, stability of the resting plasma AgRP may prevent the increasing appetite (appetite control) and ultimately lead to prevention of positive energy balance in overweight and obese individuals. On the other hand, the AED leads to reduced weight and fat percentage.

CONCLUSIONS

In conclusion, the results of the present study demonstrated that the AED leads to stability of the resting plasma AgRP levels, reduced body weight and significantly reduced body fat in the overweight and the obese individuals, while the D protocol leads to plasma AgRP level elevation, reduced body weight and no significant change in body fat percentage. According to previous studies, negative energy balance leads to excitation of AgRP neurons in the ARC of the hypothalamus, elevated AgRP expression and ultimately increased plasma AgRP levels. Furthermore, increasing AgRP leads to reduced energy expenditure, glycogen and fat oxidation, increases food intake, and induces lipogenesis and ultimately weight gain, which displays anabolic effects of AgRP neuropeptide. The main role of the AED in preventing overweight and obesity is stability of the resting plasma concentration of AgRP, which prevents increase in appetite and body fat and ultimately prevents overweight and obesity. Finally, the AED is better than the D protocol.

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