The Effect of Exercise on Plasma Ghrelin in Obesity

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Abstract: *Background and Aim of the Work*: Obesity is an important health problem worldwide. Many solutions have been proposed for this problem the most important of which is physical exercise. Exercise affects caloric expenditure signaling to ghrelin-producing cells in the stomach, thereby affecting appetite regulation.

This study examined the effect of weight control programs and exercise on plasma acylated ghrelin concentration in obese subjects.

Subjects and Methods: 120 obese persons were recruited and randomly assigned into two treatment groups: diet group& diet and exercise group. The first group received low caloric diet for 12 weeks, while the second group was treated with same diet and exercise program every other day for the same period. Complete clinical, laboratory investigations and ghrelin concentrations were assessed at the start and end of the study.

Results: In the diet group, the body weight, the BMI, the WHR (Waist hip ratio) and waist circumference were significantly reduced. On the other hand appetite measures, the triglycerides, cholesterol, HDL and LDL serum levels did not change significantly. Acylated ghrelin was significantly increased.

In the diet and exercise group, the body weight, the BMI, the WHR, waist circumference, the feeling of hunger, the desire to eat and prospective food consumption score were significantly decreased. Feeling of fullness score and Serum HDL were significantly increased. Serum triglycerides, serum cholesterol, serum LDL and acylated ghrelin were significantly reduced. Higher reduction of BMI was observed in individuals with lower serum levels of acylated ghrelin.

Conclusion: Exercise training was associated with highly significant reduction of anthropometric measurements, plasma acylated ghrelin (which was associated with significant reduction of appetite measures), whereas diet intervention alone promoted opposite effects. More reduction in BMI was observed in individuals with lower plasma levels of ghrelin at the start of the study.

Keywords: Obesity, Ghrelin, Diet, Exercise, Appetite.

INTRODUCTION

Obesity is considered an important worldwide health issue accompanied with many chronic diseases such as cardiac diseases, hypertension, dyslipidemia and diabetes [1]. Guidelines for management of obesity concentrate basically on food intake restriction in combination with physical exercise [2]. Exercise affects caloric expenditure signaling to ghrelin- producing cells in the stomach, thereby affecting appetite regulation [3].

Ghrelin is the only known appetite stimulant (orexigenic) hormone, by sending signals to the hypothalamus. It exists in circulation in two forms: des-acylated (90%) and acylated (10%) ghrelin [4]. Acylated ghrelin is more involved in appetite regulation because it is the only form that can cross blood brain barrier [5].

The effect of exercise on plasma total ghrelin concentrations has been studied but the findings are equivocal. Some studies show total ghrelin does not change after exercise [6, 7], whereas others show increases [8, 9], and yet others show decreases [10, 11]. These studies are limited by the fact that total ghrelin was measured. Other studies have examined the impact of exercise on acylated ghrelin in normal-weight individuals [12, 13]. These studies were done with normal-weight individuals, but there is evidence that adiposity may influence acylated ghrelin concentrations [14].

Considering the conflicting results of these studies, therefore the objective of this study was to investigate the effect of weight control programs and exercise on plasma acylated ghrelin concentrations in obese subjects.

SUBJECTS AND METHOD

A. Subjects

One hundred and twenty obese persons were recruited from the obesity clinic at Physical Medicine,

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Rehabilitation and Rheumatology department in Mansoura University Hospital. They were 69 females and 51 males. Their ages ranged from 22-49 years, weight ranged from 84-130 kg and height ranged from 159-177 cm.

This study was approved by the Ethical Committee of Faculty of Medicine, Mansoura University.

All the benefits and discomfort involved in this study were explained to the participants and all subjects provided written informed consent for participation in the study.

Inclusion Criteria

Obese subjects not taking any medication which affect body weight or appetite, lifelong non-smokers with a sedentary to moderately active life style without any regular exercise or diet program for the last 6 months before the study.

Exclusion Criteria

Uncooperative and unmotivated volunteers, Patients suffering from DM, Ischemic heart disease, autoimmune, gastrointestinal, endocrinal, kidney, liver diseases, recent systemic infections, fracture or surgery, pregnant and lactating, Patients with any contraindication to exercise therapy e.g. acute pain or marked musculoskeletal disorders.

B. Methods

All Participants in this study were subjected to detailed history taking: personal history, present history, past history, family history.

The following items from the general examination were recorded: anthropometric measurements; weight [15], height [15], ideal body weight [16], body mass index [17], waist circumference [18], hip circumference [18], Waist/Hip ratio.

Subjective Measures of Appetite

Participants were asked to rate their baseline appetite on 10-cm visual analogue scales (VAS) after whole night fast at least 48 h after the last exercise session to avoid acute effects of exercise [19]. They were assessed using 10-cm self-rated VAS, before and after breakfast.

Investigations: (done at the start and at the end of the study)

Fasting blood sugar [20], total lipids: serum cholesterol, serum triglycerides, serum low density lipoprotein (LDL), serum high density lipoprotein (HDL) [21], complete blood count, liver function tests (AST, ALT, Albumin, Billirubin) [22], kidney function tests **(**serum creatinine).

Determination of Acylated Ghrelin in the Serum by ELISA

Blood samples were taken at the obesity clinic after whole night fast. Samples were taken at the start and at the end of the study (48 hour at least after finishing exercise protocol to exclude acute effect of exercise).

Experimental Design

The Subjects were Randomized to One of the Two Treatment Groups

Diet Group

Comprised 60 individuals (33 females and 27 males). The mean age ranged from 25-49 years. The mean body weight ranged from 84-129 kg. They received low caloric diet for 12 weeks.

Diet & Exercise Group

Comprised 60 individuals (36 females and 24 males). The mean age ranged from 22-49 years. The mean body weight ranged from 83-130 kg. They were treated with exercise program in combination with low caloric diet for 12 weeks. At the end of the study, anthropometric measures, subjective measures of appetite and blood samples were taken for each subject.

Diet Protocol

Subjects of this study were instructed to receive balanced low caloric diet. They were encouraged to consume approximately 15% of calories from proteins, 30% from fat and the rest from carbohydrates. The patients were allowed to change items of the prescribed diet according to the exchange list quoted from the Egyptian National Institute of Nutrition within their daily caloric requirements [23].

The Harris-Benedict Equation for Basal Energy Expenditure (BEE) is used to figure energy requirements based on sex, height, weight and age [24].

Men: BEE = 88.362 + (13.397 x weight in kg) + (4.799 x height in cm) - (5.677 x age in years)

Women: BEE = 447.593 + (9.247 x weight in kg) + (3.098 x height in cm) - (4.330 x age in years).

Exercise Program

Subjects in diet & exercise group were instructed to perform exercise program. Exercise training program performed every other day for 12 weeks. Each session lasted for 30-40 min of aerobic exercise. Aerobic exercises in each session included walking on electrical treadmill. Subjects were instructed to wear heart rate monitor during each session to control heart rate. Intensity of exercise was at 60 to 80 percent of maximum heart rate [25].

Maximal heart rate (HR max): is the highest heart rate a person can attain during heavy exercise. It can be calculated using a formula:

Maximal HR = 220 – age [26].

Statistical Analysis

The results of this study processed using SPSS version 10.0®under windows. The descriptive analyses were demonstrated as means ± standard deviation (SD) for continuous data variables. Intergroup differences of continuous data variables were analyzed by *t*-tests .Chi-square test was used to evaluate categorical variables. Correlation coefficient was used for detection of correlation between quantitative variables. The P- values ≤0.05 was considered statistically significant.

RESULTS

The differences between diet & diet and exercise group as regards the age and gender, BP, BMI, WHR, waist circumference, feeling of hunger, desire to eat, prospective food consumption, feeling of fullness and hematological data were insignificant.

In the diet group there was significant decrease of body weight (p=0.016), BMI (p=0.005), WHR (p=0.039) and waist circumference (p=0.042) after intervention (Table **1**).

On the other hand desire to eat, feeling of hunger score, prospective food consumption, feeling of fullness and lipid profile did not change significantly in the obese subjects in the diet group after the intervention compared to the baseline values. However, acylated ghrelin was significantly increased in the diet group after the diet regimen (p<0.001) (Table **1**).

In diet and exercise group there was significant decrease of body weight (p<0.001), BMI (p<0.001), WHR (p<0.001) and waist circumference (p<0.001) after intervention. There was significant decrease of feeling of hunger (p<0.001), desire to eat was (p<0.001) and prospective food consumption (p<0.001). While feeling of fullness score was significantly increased (p<0.001) (Table **2**).

There was significant decrease serum triglycerides (p<0.001), serum cholesterol (p<0.001) and serum LDL (p<0.001). While serum HDL was significantly increased (p<0.001) (Table **2**).

Acylated ghrelin was significantly decreased in the diet and exercise group (p<0.001) (Table **2**).

Table 1: Pre-Post-Intervention Obesity Related Measures, Appetite Measures, Lipid Profile and Acylated Ghrelin in the Diet Group

	Pre-intervention	Post-intervention	Student's t test	
	Mean ±SD	Mean ±SD	T	p
Body weight (kg)	102.6 ±11.3	97.5 ±11.6	2.493	0.016
BMI (kg/m^2)	38 ±3.6	36.1 ±3.6	2.891	0.005
WHR	1.28 ±0.10	1.24 ±0.11	2.084	0.039
Waist circumference (m)	0.98 ±0.08	0.95 ±0.08	2.054	0.042
Feeling of hunger	6.9 ±2.2	7.1 ±1.9	0.119	0.905
Desire to eat	6.8 ± 1.8	6.4 ± 2.2	1.090	0.278
Prospective food consumption	7 ± 2.1	7.4 ± 2.1	1.043	0.229
Feeling of fullness	3.1 ± 2.2	2.9 ± 1.9	0.119	0.905
Triglycerides (mg/dl)	267.9 ±6.9	265.7 ±6.8	1.7591	0.081
Cholesterol (mg/dl)	229.9 ±12.3	227.8 ±12.4	0.9313	0.354
HDL (mg/dl)	32.8 ±3.3	33.2 ±3.4	0.6539	0.514
LDL (mg/dl)	146.8 ±5.4	145.9 ±5.6	0.8961	0.372
Acylated Ghrelin (pg/ml)	45.3 ±5.5	47.7 ±6.7	6.398	<0.001

Table 2: Pre-Post-Intervention Obesity Related Measures, Appetite Measures, Lipid Profile and Acylated Ghrelin in the Diet + Exercise Group

	Pre-intervention	Post-intervention	Student's t test	
	Mean ±SD	Mean ±SD	T	P
Body weight (kg)	101.7 ±13.1	94.7 ±13.7	20.132	<0.001
BMI (kg/m^2)	38.3 ±4.1	35.7 ±4.4	19.601	<0.001
WHR	1.30 ±0.12	1.15 ±0.14	31.093	<0.001
Waist circumference (m)	1 ±0.09	0.88 ±0.11	6.540	<0.001
Feeling of hunger	7 ±2	5 ±2.2	10.883	<0.001
Desire to eat	6.9 ±2.1	5.9 ±2.3	6.724	<0.001
Prospective food consumption	7.4 ±2	5.8 ±2.2	7.705	<0.001
Feeling of fullness	3 ±2	5 ±2.2	10.883	<0.001
Triglycerides (mg/dl)	268.1 ±7.3	261.9 ±6.9	4.7810	<0.001
Cholesterol (mg/dl)	233.4 ±12.1	221.4 ±11.5	5.5683	<0.001
HDL (mg/dl)	32.9 ±4	36.3 ±4.2	4.5407	<0.001
LDL (mg/dl)	148.5 ±4.9	143.1 ±4.7	6.6169	<0.001
Acylated Ghrelin (pg/ml)	43.9 ±3.9	42.3 ±5.2	3.980	<0.001

Table 3: The Acylated Ghrelin Serum Level in the Upper Quartile Versus Lower Three Quartiles of BMI Reduction in the Diet Group at Baseline and Post Intervention

	Upper quartile with highest BMI reduction (n=15)	Lower three quartiles with lowest BMI reduction (n=45)	Student's t test	
	Mean ±SD	Mean ±SD	t	p
Acylated Ghrelin (pg/ml)				
At baseline	44.8 ±5.3	45.4 ±5.7	0.399	0.602
Post-intervention	43.5 ±5.3	49.1 ±6.6	3.012	0.004

Table 4: The Acylated Ghrelin Serum Level in the Upper Quartile Versus Lower Three Quartiles of BMI Reduction in the Diet + Exercise Group at Baseline and Post Intervention

	Upper quartile with highest BMI reduction (n=15)	Lower three quartiles with lowest BMI reduction (n=45)	Student's t test	
	Mean ±SD	Mean ±SD	t	p
Acylated Ghrelin (pg/ml)				
At baseline	43.6 ±3.5	44.1 ±4.2	0.464	0.644
Post-intervention	37.9 ±3.8	43.8 ±4.8	4.317	<0.001

More reduction of BMI was observed in individuals with lower serum levels of acylated ghrelin (Tables **3**, **4**).

No significant correlation was found between the baseline acylated ghrelin level and age, sex, BP, blood picture, lipid profile, fasting blood sugar, serum creatinine, ALT and AST . On the other hand, baseline acylated ghrelin level was inversely correlated with the body weight, BMI and WHR (p=0.010, p=0.023 and p=0.022 respectively), also was significantly correlated with feeling of hunger (r=0.192, p=0.036), desire to eat (r=0.207, p=0.023) and prospective food consumption score (r=0.205, p=0.025) while inversely correlated with feeling of fullness (r=-0.192, p=0.036).

There is no significant correlation between the baseline serum level of acylated ghrelin and the laboratory findings of the patients.

Table 5: Correlation of Acylated Ghrelin Serum Level with Age and Clinical Data among the Patients (n=120)

	r	P
Age	0.111	0.228
DBP (mmHg)	0.004	0.964
SBP (mmHg)	0.100	0.275
Body weight (kg)	-0.235	0.010
BMI (kg/m^2)	-0.207	0.023
WHR	-0.210	0.022
Feeling of hunger	0.192	<0.036
Desire to eat	0.207	<0.023
Prospective food consumption	0.205	<0.025
Feeling of fullness	-0.192	<0.036

Table 6: Correlation of Acylated Ghrelin Serum Level with Laboratory Data among the Patients (n=120)

	r	P
Hb concentration (gm/dl)	0.101	0.271
RBCs count (x10^6/mm^3)	0.180	0.049
WBCs count (x10^3/mm^3)	0.012	0.893
Platelets count (x10^3/mm^3)	0.170	0.064
Triglycerides (mg/dl)	0.115	0.065
Cholesterol (mg/dl)	0.094	0.307
HDL (mg/dl)	0.192	0.036
LDL (mg/dl)	0.036	0.695
Fasting blood sugar	0.009	0.924
Serum creatinine	0.102	0.267
AST	0.015	0.871
ALT	0.056	0.543

DISCUSSION

Current evidence suggests that ghrelin stimulates appetite and regulates energy balance, and thus is one of the candidate genes for obesity and some chronic diseases [27]. After the discovery of ghrelin in 1999, its role in stimulating hunger and appetite has been repeatedly reported [28-30], it effectively stimulates food intake in animals and increase in body weight and fat tissue.

A specific effect of ghrelin on lipid metabolism was suggested by the observation that rodents treated with ghrelin showed enhanced fat content independently of feeding behavior by enhancing carbohydrates utilization and decreasing fat utilization leading to increase in the amount of omental and retroperitoneal fat pads [28, 31, 32]. In visceral adiposity, ghrelin increases fat accumulation by increasing expression of adipogenic genes as acetyl-coA carboxylase [33], inhibiting lipolysis enzymes [34], regulating adipogenesis [35, 36] and suppressing the release of noradrenaline in adipose tissue [37].

The interventions with diet or diet and exercise in this study produced different effects on the extent of reduction of obesity parameters, on the feeding and appetite parameters and on acylated ghrelin serum level in obese subjects enrolled in this study. This finding is in agreement with the findings of two studies [19, 38].

The body weight in the diet and exercise group ($p<0.001$) is significantly better than the diet group ($p<0.016$) after intervention indicating that exercise and diet produced significantly greater weight reduction than diet alone. Our results were in agreement with that of Lopes *et al*. who found that diet and exercise group showed a significant decrease in the obesity parameters [39].

While the serum level of acylated ghrelin was significantly elevated in the diet group, the serum ghrelin of the diet and exercise group was significantly reduced. Our results were in agreement with that of Lopes *et al*. who found that although the both diet and diet plus exercise groups showed a significant decrease in the obesity parameters (measured by a decrease in body fat percentage and fat mass in that study) the diet plus exercise group showed a significant decrease while the diet group showed a significant increase in the acylated ghrelin serum levels [39].

Our study showed that 4% reduction in serum ghrelin (from 43.9 to 42.3 pg/ml) in diet and exercise group is associated with significant reduction in the desire to eat, feeling of hunger and the prospective food consumption and significant elevation of feeling of fullness among the obese subjects in the diet and exercise group together with decreased body weight and obesity indices in these subjects. In this regard, A study concluded that reduced ghrelin levels due to long-term exercise training leads to decreased appetite and body weight [40].

Another study showed that caloric restriction induced a significant increase in acylated ghrelin concentrations compared with baseline values,

whereas exercise promoted opposite effects. Our results demonstrated that a diet plus exercise-induced 5% weight loss was able to significantly reduce acylated ghrelin concentrations in obese individuals. The other study revealed a 15.4% increase in acylated ghrelin concentrations in subjects assigned to diet alone and a 39% decrease in acylated ghrelin concentrations in those assigned to the diet and exercise group [41]. Likewise, another study found similar results after a 3-month aerobic training in eutrophic subjects who lost weight [42]. Similar to our results, other researchers found increased total ghrelin concentrations following weight reduction in obese women attending a diet-induced weight-loss program [43].

However, our findings contrast with those reported by other authors who found increased total ghrelin concentrations after an exercise program [44]. We can speculate that acylated ghrelin concentrations may respond differently than total ghrelin.

Although it has already been shown that exercise-induced weight loss induces a compensatory increase in total ghrelin plasma levels [44-46] no significant changes have been reported on acylated ghrelin [46]. Acylated ghrelin is the molecular form that is able to cross the blood brain barrier, able to bind to the hypothalamic receptor and therefore exert its orexigenic effects at the hypothalamic level.

Increased acylated ghrelin concentrations was also associated with increased hunger during treatment and difficulty in reducing food intake and losing weight while aerobic exercise was able to reduce acylated ghrelin concentrations and feeling of hunger in the obese patients compared to baseline values [47].

Diet plus exercise increase resting metabolic rate, which may be related to the maintenance of muscle mass in group of subjects who performed exercise. In turn, participants assigned to diet alone had either reduced or no differences in resting metabolic rate after treatment [48, 49].

Interestingly, our study revealed that in the subjects in diet plus exercise where serum ghrelin was significantly reduced after intervention, serum cholesterol, LDL and triglycerides were also significantly reduced while serum HDL was significantly elevated. In contrary, the serum level of lipids did not change significantly after intervention in the diet group. These findings were in agreement with other researchers who reported that a significant reduction in serum ghrelin was associated with significant reduction in triglyceride, LDL and cholesterol concentrations as well as the triglyceride to high-density lipoprotein ratio [38]. This come in agreement with another study which reported that is, 'favorable' levels of HDL, TG, TC/HDL, and LDL/HDL have been showed in trained volunteers [50]. These findings point out that the decrease or increase in serum ghrelin is associated with similar changes in circulation lipids.

Another important finding of the current study is that, the extent of weight reduction after diet and exercise is produced in individuals with more reduction of the acylated ghrelin. In obese subjects in the upper quartile with highest reduction in BMI (i.e. those who lose more weight) serum acylated ghrelin was reduced by 13%. On the other hand, obese subject in the lower quartiles with lowest reduction in BMI (i.e. those with slight weight loss), the serum acylated ghrelin was reduced by 0.7%.

Our results had shown that baseline acylated ghrelin serum level was inversely correlated with the body weight, BMI and WHR. These findings were in agreement with the findings of Stepien *et al*., 2011. Interestingly Stepein and co-workers reported that WHR better correlated with serum acylated ghrelin than BMI and they attributed this finding to fact that WHR better reflect the visceral fat deposit than BMI [51]. This opinion is confirms the findings of another study [52]. Our results also showed that baseline acylated ghrelin serum level was significantly correlated with feeling of hunger, with desire to eat and with prospective food consumption score while inversely correlated with feeling of fullness. These findings are in agreement with the findings of another study [41].

CONCLUSION

Reviewing our study, we concluded that exercise training- that reduced plasma acylated ghrelin significantly- has a beneficial effect on BMI, waist circumference, WHR and lipid profile in obese subjects, while diet alone promoted the opposite effect. This effect was associated with significant reduction of feeling of hunger, desire to eat and prospective food consumption. More reduction in BMI was observed in individuals with lower levels of ghrelin at the start of the treatment.

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