



## Studying the serum ghrelin levels of the elite athletes and sedentary controls fasting at the time of Ramadan

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### Abstract

The Ghrelin is produced primarily by enteroendocrine cells in the gastric mucosa, and increases gastric emptying in normal subjects. The purpose of this study is to determine the differences in the Ghrelin level during different times along the day, and to assess the differences between the athletes and sedentary at the end of Ramadan fasting period of one month. The results indicated that there were no significant difference among the Ghrelin levels of athletes and sedentary during different times through the day ( $p>0.05$ ). Significant differences were observed between pre- and post-tests mean Ghrelin scores of subjects ( $p<0.05$ ) without making a separation as an athlete and sedentary. Also, the results indicated no significant difference between repeated Ghrelin measurements of athletes and sedentary ( $p>0.05$ ). A significant and positive relationship was found in the middle level at the percentages of BMI and body fat of both groups. Moreover, there were significant differences among BMI scores ( $p<0.01$ ) and body fat % ( $p<0.05$ ) of the athletes and sedentary. Consequently, fasting during Ramadan did not cause any differentiation on the serum Ghrelin level of athletes and sedentary, but without group differentiation there was significant increase from the initial measurement to the last measurement of the serum Ghrelin level. There was a significant and positive relationship at middle level between BMI and body fat % of both groups of athletes and sedentary. There was a significant relationship, on the other hand, between BMI and repeated Ghrelin measurements of subjects.

**Keywords:** Athletes, Ramadan, fasting, ghrelin.

### INTRODUCTION

Each year during the lunar month of Ramadan, adult Muslims refrain from eating, drinking, smoking or having sexual relationships during the daytime. This fasting is complete, intermittent, and does not require caloric restriction. Free eating is allowed from sunset to dawn. Since Ramadan is a lunar month, its occurrence changes with time. Each year the Ramadan month occurs 11 days earlier. Consequently, each 9 years, Ramadan happens in a different season; the length and the temperature of the fasting days also change (1). During the last 20 years, several studies were undertaken to elucidate the effects of Ramadan fasting in healthy subjects.

Ghrelin, a peptide secreted by endocrine cells in the gastrointestinal tract, is thought to play a significant role in the regulation of energy balance due to its effects on the stimulation of food intake

(27,28) and weight gain (5,24,27,28) in rodents. It has been suggested that ghrelin may also play a role in meal initiation in humans, since the concentration of ghrelin increases immediately prior to a meal (5) and decreases after eating (5,7,25). In addition to having a powerful effect on the secretion of growth hormone, ghrelin stimulates food intake and transduces signals to hypothalamic regulatory nuclei that control energy homeostasis. Thus, it is interesting to note that the stomach may play an important role in digestion and central feeding regulation (11).

Furthermore, ghrelin infusions are associated with feelings of hunger and increased energy intake during a buffet-style lunch (26). Despite the evidence indicating a role in acute food intake, little is known about the factors regulating ghrelin and its effects on long-term energy balance in humans. One hypothesis is that ghrelin secretion is up-regulated in periods of

negative energy balance and down-regulated in periods of positive energy balance (20). Since energy balance is a function of both energy intake and energy consumption, Ghrelin concentrations should increase or decrease with fluctuations in food intake (macronutrient composition and/or energy intake) and/or energy expenditure. In turn, decreased ghrelin concentrations should be associated with lower food intake. However, the effects of monthly change in food intake and energy expenditure on ghrelin have not been investigated in humans.

Ghrelin stimulates secretion of GH, food intake, and body weight gain when administered peripherally or centrally (2,24). Ghrelin activates neuropeptide Y (NPY) and agouti-related protein (AGRP)- producing neurons localized in the arcuate nucleus of the hypothalamus (3,13,19,21), which is one of the brain regions of primary importance in the regulation of feeding. The secretion of ghrelin increases under conditions of negative energy balance, such as starvation, cachexia, and anorexia nervosa, whereas its expression decreases under conditions of positive energy balance, such as feeding, hyperglycemia, and obesity (18,24). Obesity and related disorders are among the leading causes of illness and mortality in the developed world (17). The problem of obesity has reached epidemic proportions in the United States. More than 50% of adults are overweight or obese, and 5% are severely obese (body mass index [BMI]>35) (8). In Turkey in 2000, the prevalence of obesity was 22.3% (BMI>30) (4). In 2004, this prevalence was 25.2% (36.7% in women, and 21.6% in men) (9). To better understand the pathophysiological mechanisms that underlie metabolic disorders, increasing attention has been paid to central regulatory elements in energy homeostasis, including food intake and energy expenditure. The purpose of this study was to determine Ghrelin level differences during different times through the day and to assess differences among the athletes and sedentaries at the end of Ramadan fasting period that was one month.

In this study, a critical analysis of these results was undertaken in order to elucidate the Ramadan effects on ghrelin parameters of changes.

## MATERIALS & METHODS

### Subjects

The study exclusion criteria were totally 30 male subjects who were 15 elite physically active athletes and 15 sedentary university students. The volunteers who fast during Ramadan have been taken into consideration in the study. The mean age, height and body weight of subjects were as follows:  $21.33 \pm 2.58$  yrs.,  $173.0 \pm 8.08$  cm,  $75.80 \pm 15.15$  kg for athletes, and  $19.86 \pm 1.72$  yrs,  $172.53 \pm 7.72$  cm and  $65.33 \pm 7.67$  kg for sedentaries. The body mass index (BMI) for the athletes was  $25.09 \pm 2.81$  kg/m<sup>2</sup>, and it was  $21.93 \pm 1.75$  kg/m<sup>2</sup> for the sedentaries. All subjects were healthy ones according to their medical history, clinical examination, and routine laboratory findings.

### Blood Sampling and Laboratory Analyses

Blood sampling was performed at 04:00 A.M. after an overnight fast for at least 8 hours. Blood samples were taken at 13:00 after 8-hour hunger at 18:00 after 13-hour hunger and at 19:00 when subjects were full. Blood samples were put immediately on melting ice and centrifuged within 1 hour from sampling. Plasma was stored at -80 °C until analysis. Active serum Ghrelin (pg/mL-LINKO) was determined by the Enzyme-Linked Immunosorbent Assay (ELISA) method.

### All Anthropometrics, Fat Distribution

All anthropometric measurements were performed with the subjects wearing underwears. Body weight was rounded to the nearest 0.1 kg, and body height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. The body mass index (BMI) was calculated as weight divided by height in square meters (kg/m<sup>2</sup>).

Body fat was determined using triplicate skinfold measurements by one investigator (test-retest) at various areas including the chest and subscapula with a constant-pressure skinfold caliper. The body fat percentage was calculated by Sloan and Weir method. (Holtain Ltd, Crymych, UK) (13).

### Statistical Analyses

The results are expressed as mean values  $\pm$  SD with  $P < 0.01$  and  $P < 0.05$  indicating significance. Two-way ANOVA for Mixed Measures was used to compare the serum ghrelin levels at midnight, lunch time, pre- and post-dinner time, and Mann-Whitney

U-test or unpaired Student's t-test comparisons were conducted for percentage of Body Fat and BMI (Body Mass Index) changes between athletes and sedentaries respectively. Correlations with BMI were sought after Pearson's squares method. The statistical analyses were performed using SPSS software (version 10.0; SPSS Inc., Chicago, IL).

## RESULTS

No significant difference was found between sedentary and athletes on Ghrelin values when measured at four different times of the day [ $F_{(3,49)} = 0.87, p > 0.46$ ].

As a shown in table 3, no significant difference was found between the general averages of sedentary

and athletes for Ghrelin values measured at different times [ $F_{(3,49)} = 0.126, p > 0.944$ ].

It can be claimed that there is a significant difference between average values of Ghrelin from the initial measurement to the last one without group separation [ $F_{(3,49)} = 5.010, p < 0.030$ ].

Correlations between fasting serum ghrelin and BMI levels in athlete and sedentary subjects ( $r = 0.674, p < 0.01$ ).

It is observed that there is a moderate, positive and meaningful correlation between BMI and Body Fat Percentage (BFP) for both sedentary and athlete subjects ( $r = 0.590, p < 0.05$ ).

Table 1. The characteristics of subject at the time of enrollment (Mean  $\pm$  SEM).

	Athletes		Sedentary		Sig.
	n	X1	n	X2	P $\leq$
Age (years)	15	21.33 $\pm$ 2.58	15	19.86 $\pm$ 1.72	NS
Height (cm)	15	173.0 $\pm$ 8.08	15	172.53 $\pm$ 7.72	NS
Weight (kg)	15	75.80 $\pm$ 15.15	15	65.33 $\pm$ 7.67	0.05
Body mass index (kg/m <sup>2</sup> )	15	25.09 $\pm$ 2.81	15	21.93 $\pm$ 1.75	0.001
Percentage body fat (%)	15	9.57 $\pm$ 4.37	15	11.58 $\pm$ 4.34	0.05

Table 2. Effect of hunger and satiety on Ghrelin level differences during different times through the day and differences among the athletes and sedentary at the end of the Ramadan fasting period of one month. Peripheral serum active ghrelin (pg/ml  $\times$  0.302 = pmol/liter) levels in 30 healthy subjects (mean  $\pm$  SEM).

	Ghr. Nocturnal (pg/mL)	Ghr. Lunch time (pg/mL)	Ghr. Before Dinner (pg/mL)	Ghr. After Dinner (pg/mL)
Time	04:00 A.M	13:00 P.M	18:00 P.M	19:00 P.M
Athletes	11.14 $\pm$ 2.65	12.33 $\pm$ 4.61	10.24 $\pm$ 1.73	10.36 $\pm$ 1.62
Sedentary	12.06 $\pm$ 3.74	12.27 $\pm$ 2.56	13.62 $\pm$ 5.95	14.33 $\pm$ 7.31

P  $\leq$  0.05 vs. baseline period.

Table 3. ANOVA results for ghrelin measurement values.

Source of Variance	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	802.11	52			
Between Groups (Individual/group)	6.16	3	2.054	0.126	0.944
Error	795.94	49	16.244		
Within Groups	1213.79	53			
Measurement	107.37	1	107.376	5.010	0.030
Group* Measurement	56.17	3	18.725	0.874	0.461
Error	1050.23	49	21.43		
Total	2015.90	105			

Meaningful difference was found in terms of between sedentary and athlete subject groups involving in the study (BFP) ( $U = 65.00$ ,  $p < 0.05$ ). When averages of BFP are taken into consideration, it is understood that the sedentary' values ( $x=11.58$ ) are higher than the values of athletes ( $x=9.57$ ).

Meaningful difference was found, in terms of BMI, between the groups of sedentary and athlete subjects involving in the study [ $t_{(23.72)} = 3.67$ ,  $p < 0.01$ ]. It was determined that BMI values of athletes ( $X = 25.09$ ) were higher than the BMI values of sedentary ( $X = 21.93$ ).

## DISCUSSION

This study has been accomplished to make clear the role of exercise and fasting on Ghrelin levels after fasting during one-month Ramadan period, and to determine if those have any effect on protecting from obesity.

Our study illuminates the difference of Ghrelin measurement and has been performed on the athletes and sedentaries at the end of fasting which lasts a long time (29 days). That is why our study is the first among the relevant studies. While other studies in the literature concentrate on the short term hunger results of Ghrelin, we have taken, with this study, the results at the end of fasting for 30 days.

Zoladz et al. determined, therefore, by their study that the decrease in heart beating number during exercises due to fasting has no significant effect on Leptin and Ghrelin concentrations. If the body weight is reduced by making exercises without reducing the consumption of eating, the value of Ghrelin increases by the weight balancing role of Ghrelin (29). Heather determined with his study that rested metabolism's higher values were related with the high values of Ghrelin. The individuals having balanced body weight consume more energy and take more energy as a result of higher values of rested metabolism (10).

It has been observed, meanwhile, that there was no significant difference in aspect of statistical importance between the averages of athletes and sedentaries although sedentaries have higher averages for the Ghrelin levels taken at four different times of the day when compared to the values of athletes.

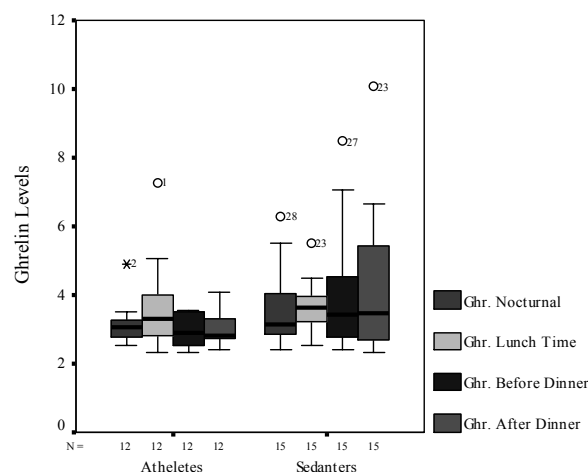


Figure 1. Box plot showing a significant difference ( $P \leq 0.05$ ) in satiated and basal fasting serum ghrelin levels between athlete and sedentary subjects.

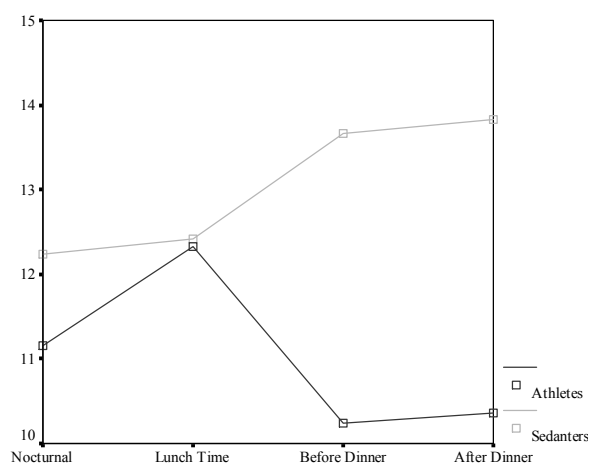


Figure 2. Graphics showing the changes those dependent on Ghrelin values which were taken at different times of the day.

Souza et al. determined in their study that amongst menstrual disorders of sportswomen have no correlation with their Ghrelin levels (23). Kramer et al. examined whether Ghrelin has a role in GH's response to the exercise, but they found out that whereas running exercises have boosted GH it has played no effect on the Ghrelin levels. Ghrelin levels of human plasma shows a sharp increase before each meal, and a slow decrease after each meal (14). Finding the values of Ghrelin levels which were taken just after the dinner higher than expected in both athlete and sedentary subjects participated in this study has reminded us that the slow decrease was due to the measurements taken just after eating. There is a relationship, therefore, between high

plasma levels of Ghrelin during fasting and excretion of stomach.

Some studies support the hypothesis claiming that the changes in plasma Ghrelin levels do not organize the central appetite mechanism using the circulation feature of stomach. According to a research, for instance, accomplished on some rodents, the Ghrelin mRNA in stomach and the Ghrelin level in plasma increase irrelevant of the changes in the volume of stomach during fasting, and it declines when a person eats. The fasting mentioned hereby makes reference to some 12 hours night hunger in contradiction to the research carried out as specified (22).

We could not find, meanwhile, any meaningful difference between measurement values of Ghrelin in this study; that is, it has been observed that common effects of the values of athlete and sedentary subjects were insignificant on Ghrelin ( $F(3,49)=0.87, p<0.46$ ). Ghrelin releasing increases during conditions of negative energy balance such as starvation and anorexia nervosa, and it decreases under positive energy conditions such as nutrition, hyperglycemia and obesity. It has been determined, on the other hand, in this study that the Ghrelin values for both sedentaries and athletes do not differ statistically from each other in case both starvation and satiety.

Tschop et al. observed in their study that feeding rodents through intracerebroventricular and peripheral way in physiological doses of Ghrelin increases their appetite, leading to eating too much consequently, and decreases fat needs of them. It was also observed that when they were fed by Ghrelin antagonists, the effects of Ghrelin were suppressed, causing some decreases in appetite. As a result, it was determined that applying Ghrelin on animals caused putting on weight and fattening due to taking too much nutrients (24). Leidy et al. examined by their study the Ghrelin replacement on young women with normal body weight and on those took exercise and diet programs, and they determined that Ghrelin responded reactively to the behavioral changes in energy level, and it was also sensitive to the changes in body weight (16).

It makes, therefore, us think that sensitiveness to Ghrelin might have decreased when we observe no decrease in appetite although the plasma Ghrelin levels are low in obese, and furthermore recurring

hunger during Ramadan fasting might have reduced and influenced the sensitiveness in Ghrelin levels.

A meaningful difference has been observed in this study in the levels of Ghrelin from initial measurement to the last measurement without any group privilege.

Cummings et al. (6) and Krarup et al. (15) determined, in their study, that Ghrelin levels have been affected from weight-losing. Lindeman et al. found, contrarily to aforespecified findings, in their study that the plasma Ghrelin levels haven't been influenced by weight-losing. However, same study claimed that the more the BMI level was increased, the more the Ghrelin level was decreased. Tschop et al. (24) determined in their study, similarly with the study of Lindeman, that when BMI level was increased, the Ghrelin level was decreased accordingly. The results of former surveys indicated that exposing to long time lack of energy due to diet and exercise programs boosted the circulating Ghrelin levels significantly. It was observed, meanwhile, that there was a negative correlation between BMI and % Body Fat in cases of anorexia nervosa and other eating disorders. All of former findings remind us that there is a direct correlation between Ghrelin and energy depots.

Although there is no significant relationship between measured BMI and Ghrelin levels of athletes and sedentaries, the sedentaries have much more Ghrelin levels than athletes as shown in the Graphics those drawn depending on the findings. It reminds us that making sports during fasting (in hunger) does not increase the Ghrelin level on contrary to expected; that is, it does not raise or exsogenic effects, but it tends to reduce it. We need more researches to be applied on vast population so that we can obtain more objective results on relevant subject.

Whereas that the athletes have higher BMI values when compared to the sedentaries though their % Body Fat is less than of the sedentaries shows a reverse correlation, this situation can be explained by an excess of muscle tissue. Taking the Ghrelin secretion under control with diet and exercise programs can be useful in maintaining normal body weight and in the inhibition of weight-gaining. Effects of Ghrelin on insulin and glucose are still being

argued. Some long-term studies on this subject, on the other hand, may be useful somehow.

All of these results indicate that the serum Ghrelin values create no difference for sedentary and athletes by fasting in the month of Ramadan, but they suggest some statistically significant increases on between the values from initial measurement (meal before dawn) to the last measurement (after dinner hours) without making any differentiation among groups.

A medium level, positive and meaningful correlation has been determined between BMI and Body Fat Percentages of both athlete and sedentary subjects. No meaningful correlation has been observed between BMI and Ghrelin levels of the athletes and sedentaries by means of recurring measurements on the other hand.

### Recommendations

Taking the Ghrelin secretion under control with diet and exercise programs can be useful in maintaining normal body weight and in the inhibition of weight-gaining.

The Ghrelin can be used as a valid indicator in evaluating the Obesity and Anorexia Nervosa conditions.

The effect of Ghrelin on insulin and glucose metabolism is being discussed. Long-term studies in this direction will be useful.

Much more enlightening information can be accessed, unlike these studies, between the measurements of Ghrelin to be made prior to the month of Ramadan, during and after the Ramadan and Ramadan fasting.

It would be useful with similar studies, therefore, to examine the changes in Ghrelin in the obese and in ones with different BMI values to be carried out through Ramadan.

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