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The Effect of Intermittent Fasting on the Growth Hormone and Ghrelin in Rats Feeding on A Standard Diet

Standart Diyet ile Beslenen Ratlarda Aralıklı Beslenmenin Büyüme Hormonu ve Ghrelin Üzerine Etkisi

Nizamettin Günbatar, Bahattin Bulduk

Health Sciences Faculty, VanYuzuncuYıl University, Van, Turkey

Abstract

Aim: In this study, the effect of intermittent fasting on growth hormone (GH) and ghrelin in rats that fed on a standard diet was investigated.

Material and Method: Twelve Wistar albino rats were randomly divided into two groups (n=6 per group). Control group (C): a standard nutrition programme was applied. Intermittent fasting group (IF): a 24-hour break from the non-consecutive diet for 2 days a week (all food restricted except water) was applied together with a standard diet.

Results: As a result of the analysis, it was found that the GH in the intermittent fasting together with the standard diet group tended to increase compared to the control group, and while this value difference was not statistically significant, the ghrelin level was found to be statistically lower than the control group.

Conclusion: As a result, it was found that intermittent nutrition tends to increase the level of GH, and it has a statistically significant lowering effect on ghrelin.

Keywords: Growth Hormone, ghrelin, intermittent fasting, standard diet

Öz

Amaç: Bu çalışmada aralıklı beslenmenin standart diyet ile beslenen ratlarda büyüme hormonu (GH) ve ghrelin üzerine olan etkisi incelendi.

Gereç ve Yöntem: On iki Wistar albino sıçan rastgele kontrol ve aralıklı beslenme olmak üzere iki gruba ayrıldı (n = 6). Kontrol grubu (C): Bu gruba standart beslenme programı uygulandı. Aralıklı beslenme grubu: bu gruba haftada sadece 2 gün (ardışık olmayan) diyet verilmesine 24 saat ara verildi (su hariç tüm besin kısıtlaması)

Bulgular: Yapılan analizler sonucunda standart diyet ile birlikte aralıklı beslenme uygulanan gruptaki büyüme hormonu kontrol grubuna göre artma eğiliminde olup bu değer farkı istatistiki önemde bulunmazken, ghrelin seviyesinin ise kontrol grubuna göre istatistiki önemde düşük olduğu bulundu.

Sonuç: Aralıklı beslenme uygulamasının büyüme hormonu seviyesini arttırma eğiliminde, ghrelin seviyesi üzerinde ise düşürücü yönde etkisinin olduğu görülmüş olup, obezitenin tedavisinde ve oluşumunu önlemede kullanılacak yöntemler arasında değerlendirilebileceği kanısına varılmıştır.

Anahtar Kelimeler: Büyüme hormonu, ghrelin, aralıklı beslenme, standart diyet

Corresponding *(İletişim)*: Nizamettin Günbatar, Health Sciences Faculty, VanYuzuncuYıl University, 65090, Van, Turkey
E-mail *(E-posta)*: nizam_gun2011@hotmail.com
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INTRODUCTION

Intermittent fasting has a significant effect on reducing the rate of occurrence of complications that may be associated with weight loss and obesity. In addition to weight loss, intermittent fasting prolongs life with regards to blood sugar levels, heart and brain functions by reducing the incidence of chronic non-communicable diseases associated with aging, such as cancer, kidney disease and diabetes mellitus.[1]

Obesity has become a major social health problem worldwide as a result of the consumption of many unhealthy nutrients and limited physical exercise. By restricting daily food intake with intermittent fasting, triglycerides, total cholesterol, low-density lipoprotein cholesterol, blood pressure, glucose, insulin and C-reactive protein levels are ensured to be within normal ranges due to weight loss.[2]

Intermittent fasting (IF) or time-restricted eating is a form of diet that is practiced by restricting food and energy intake for a certain period of time. By restricting the total calorie intake with this diet, circadian rhythms of nutrition are supported and metabolic homeostasis is created.[3,4]

Ghrelin is a 28-amino acid hunger-stimulating peptide hormone that is mainly formed by P/D1 in the fundus of the stomach and epsilon cells in the pancreas. Ghrelin levels, which increase before meals, decrease after meals. It has an opposite task to the leptin hormone which is released from the adipose tissue and creates a feeling of satiety when it is in sufficient amount.[5] Ghrelin has an important role in the body's energy homeostasis and is an important gastrointestinal hormone that stimulates food intake and adiposity.[6] The ghrelin hormone, whose receptors are located in the hypothalamus, pituitary and vagal afferent nerve endings and trunks located throughout the gastrointestinal tract, strongly stimulates the release of GH from the anterior pituitary.[5] It has been reported that circulating ghrelin levels decrease after food intake.[7]

GH, also known as somatotropin, is produced by somatotropic cells in the anterior pituitary and has a single-chain polypeptide structure with 191 amino acids.[8] GH production is regulated through stress, exercise, nutrition, sleep, and feedback mechanisms. Among the factors regulating the release of GH are growth hormone releasing hormone (GHRH) produced in the hypothalamus, somatostatin produced in various tissues, and ghrelin hormone produced in the gastrointestinal tract. GH levels increase in childhood, reach their highest levels in adolescence, and decrease with advancing age.[9] GH is extremely important in regulating growth during adolescence. For this reason, irregularities in the levels of GH in the organism cause significant health problems. GH deficiency and hypopituitarism in adults are closely related to diseases such as vascular endothelial dysfunction, dyslipidemia and insulin resistance, which are important risk factors for cardiovascular diseases.[10]

MATERIAL AND METHOD

The study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Dater: 25.06.2015, Decision No: 192616). This study was conducted according to the Declaration of Helsinki, as revised in 2000. The study was performed on 12 Wistar albino male rats aged 3-4 months and weighing 200-250 g. All other conditions, except for the experimental diet, are provided for in such a way that within the standards of laboratory animal care there are 6 rats in each cage. Rats were classified as the 1st group as the control group (CG) with 6 rats in each group, and a standard diet program was applied to this group (2.8% crude fat, 23.1% crude protein, 5% crude fiber, 7.1% crude ash, and 12.8% moisture). Our 2nd group, on the other hand, was determined as the intermittent fasting together with the standard diet group (with a 24-hour break from the non-consecutive diet for 2 days a week and all food restricted except water). The study was continued for a total of 8 weeks. at the end of the study, blood samples were taken from the hearts of rats euthanized with intraperitoneally administered ketamine (50mg/kg) and serum growth hormone and ghrelin levels were measured by ELISA method.[11]

Taking Blood Samples

At the end of the experiment period, the abdominal region of the rats (control and experimental groups) to which general anesthesia was performed with ketamine (50 mg/kg) was excised in the form of an inverted V letter from the anal (pubis) area to the chest cavity, the abdominal cavity was opened, and the required amount of blood was taken by entering the heart with an injector. The blood taken was transferred to yellow-capped biochemistry tubes and centrifuged at 4000 RPM (RCF=1240xg) for 15 minutes and serums were removed. Serums removed were placed in Eppendorf tubes and stored at -80°C until the study time.

Determination of Ghrelin and Growth Hormone

Serum ghrelin and growth hormone levels were determined using the commercial enzyme-linked immunosorbent analysis (ELISA) rat ghrelin (Cusabio in Biology Research, Houston, USA) and growth hormone (MyBioSource, Inc, San Diego, USA) kits with the method of Quantitative Sandwich.[12]

Statistical Analysis

Descriptive statistics of the groups were given as mean and standard deviation. The Shapiro-Wilk test was used to determine whether the data were distributed normally or not. For the same parameter, whether the differences were significant between the groups or not was evaluated by Kruskal-Wallis Test. In order to determine which group the differences were caused by, post hoc analysis (Tukey HSD) was performed and results with a p value of ($p<0.05$) or less were considered significant. For calculations, SPSS (ver.22) statistical package program was used.

RESULTS

While the serum GH value tended to increase in the group on which intermittent fasting was applied compared to the control group, this difference in value was not found to be statistically significant. The ghrelin value was found to be low in statistical significance compared to the control group (**Table 1**).

Table 1. Mean and standard deviation values of growth hormone and ghrelin in serum samples.

Parameter	Control	Intermittant Fasting	P value
Growth hormone (ng/mL)	10.00±0.585[c]	10.83±1.022[c]	0.584
Ghrelin (pg/mL)	233.64±6.16[a]	201.86±4.95[b]	<0.001

"a,b,c: Shows the difference between groups (Tukey HSD)" bp: It is significant compared to the control group (p<0.05)

While the increase in serum growth hormone was insignificant in the group on which intermittent fasting was applied compared to the control group, the decrease in the ghrelin value was significant (p<0.05) (**Figure 1**).

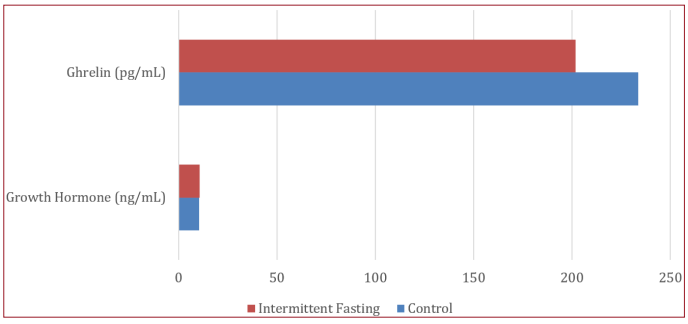

Figure 1. The mean values of serum Growth hormone and Ghrelin between the groups (ng/ml ,mg/dl).

DISCUSSION

It has been reported that decreased circulating ghrelin levels in obesity[13] is caused by disturbances in the ghrelin system, such as, central ghrelin resistance,[14] and ghrelin's inability to induce cessation of food intake.[15] In a study, it was reported that there was a significant decrease in ghrelin levels in six healthy volunteers who fasted for 33 hours.[16] Also, in a study conducted during Ramadan, it was reported that there was a significant decrease in circulating ghrelin levels at the end of Ramadan.[17] In another study, Alzoghaibi et al (2014) indicated that there was no change in ghrelin levels due to fasting during Ramadan.[18] In our current study, it was observed that the level of ghrelin in the group on which intermittent fasting was applied decreased significantly compared to the control group, which is considered compatible with the above studies. It has been reported that gastric nutrient chemosensors have significant contributions to the regulation of ghrelin secretion, which is important for the regulation of food intake and energy homeostasis.[19] In our study, it is thought that the low ghrelin level in the group on which intermittent fasting was applied is caused by the inhibitory effect of intermittent fasting on gastric nutrient chemosensors.

In a study, it was reported that there was an increase in GH levels after fasting for 24 hours.[20] GH has an important role in metabolic adaptations during fasting,[21,22] but there are discrepancies regarding the absolute and relative increase in GH levels during the fasting period. In a study conducted by Alken et al (2008) they again showed that GH levels increased 7 times after a 24-hour fasting.[23] In another study Beer et al (1989) showed that there was an approximately 10 times increase in GH levels after 24-hour fasting, in 6 healthy young men, but this increase was not observed in the group of 8 healthy young women.[24] An increase in GH levels was noted in a 24-hour fasting study in 14 healthy adults.[25] GH levels have been shown to rise after 24 hours in healthy adults who fast for 12 to 36 hours.[26] In another study, it was reported that fasting for less than 3 days significantly increased GH secretion compared to long-term fasting.[27] In another study, conducted by Bouhlel et al. they reported that there was no change in GH levels at the end of fasting held during the month of Ramadan.[28] While the above-mentioned studies of intermittent feeding for a few days showed that the level of GH hormone increased, Bouhlel et al. showed that there was no change in GH levels at the end of Ramadan. In our study, it was observed that GH levels tended to increase in the group on which intermittent fasting was applied compared to the control group, but this increase was not at a significant level. It is reported that ghrelin hormone strongly stimulates the release of GH from the anterior pituitary.[5] It is thought that the GH increase that is not significant in our study is caused the duration of intermittent feeding with the significantly low (**Table 1**) ghrelin level in our intermittent fasting group.

CONCLUSION

In this study we carried out, we are of the opinion that 8-week intermittent fasting practice, 2 days a week, which we applied to the group that fed on a standard diet will have an important contribution to preventing the development of obesity by preventing excessive food consumption by providing a significant decrease in the level of ghrelin hormone, which provides the feeling of hunger, and also in the level of GH hormone, which has many metabolic effects, it will have an important contribution to the regulation of metabolic activities, since it has an effect that tends to increase, although not at a significant level.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee (Dater: 25.06.2015, Decision No: 192616).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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