Fasting Ghrelin Levels Are Decreased in Obese Subjects and Are Significantly Related With Insulin Resistance and Body Mass Index

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Abstract

BACKGROUND: Ghrelin is a 28-amino acid peptide that predominantly produced by the stomach. Strong evidence indicates the effects of ghrelin in the regulation of metabolic functions and its potential role in the aetiology of obesity.

AIM: The aim of this study was to investigate the relationship of ghrelin levels with obesity, insulin resistance and glucose in normal and obese subjects.

METHODS: Thirteen normal (n = 13) and seven (n = 7) obese weight subjects aged 20-22 participated in the study. Fasting plasma ghrelin, insulin and glucose levels were measured after overnight fasting. HOMA-IR was calculated to evaluate insulin resistance.

RESULTS: Ghrelin and insulin levels were found to be statistically significantly lower and higher in obese subjects (P < 0.001), respectively. Glucose levels were clinically higher in obese subjects but not statistically significant. Fasting plasma ghrelin was negatively correlated with BMI (r = -0.77, P < 0.001), fasting insulin levels (r = -0.55, P < 0.001) and HOMA-IR (r = -0.66, P < 0.001). There was no correlation between ghrelin and glucose. In multiple regression analysis, insulin levels (Beta: -2.66, 95% CI: -2.49 -2.78, P < 0.001) HOMA-IR (Beta: -2.41, 95% CI: -2.33 -2.55, P < 0.001) and BMI (Beta: -1.77, 95% CI: -1.66 -1.89, P < 0.001) were significant independent determinants of fasting ghrelin.

CONCLUSION: Obese subjects have low fasting ghrelin levels that they are significantly related to insulin resistance and body mass index. More prospective studies are needed to establish the role of ghrelin in the pathogenesis of human obesity.

Introduction

Ghrelin is a 28-amino acid that is produced in the stomach. Other organs such as liver heart and pancreas can also produce ghrelin but in lower levels [1]. Strong evidence indicates the effects of ghrelin in its potential role in the pathogenesis of obesity, insulin resistance (IR) and types 2 diabetes [2].

More recently, research on ghrelin has improved our understanding of the mechanisms involve food intake, fat accumulation, and the development of other metabolic disturbances [3].

The role of ghrelin in the regulation of glucose was initially hypothesized after an observation of a negative correlation of insulin and ghrelin [4].

One year later Schofl et al. [5] supported the involvement of ghrelin in the development of IR and typed two diabetes. Total ghrelin levels have also been found to be negatively correlated with IR in children and adolescents [6].

Food intake is the most important factor that influences ghrelin levels. Usually, total ghrelin levels are rising during the night and decrease after breakfast [7].

A long-term high fat diet has found to reduce plasma total ghrelin levels and stomach content [8]. Also, many studies have reported that ghrelin levels are negatively associated with body mass index (BMI) [9, 10].

We hypothesized that obese individuals would present with elevated fasting ghrelin levels and there is no relationship between ghrelin and insulin levels.
**Methods**

Twenty healthy subjects (13 with normal weight and seven obese) participated in the study aged 20-22 years old. A detailed medical history was taken by the clinic's doctor. Subjects with a medical history of liver, renal, or heart or taking any medications and supplements were excluded from the study. The study received approval from the ethical committees of "Zayed University" and "Doctors Medical Center" while all subjects signed a consent form.

Body weight and height were collected for all subjects using SECA 600 model while obesity was defined as BMI>30 kg/m², according to the criteria of International Obesity Task Force [11].

Plasma ghrelin, glucose, insulin and C-reactive protein (CRP) levels were measured in the morning after fasting overnight. Blood samples were drawn by a certified nurse by venipuncture into 10-ml empty evacuated placed on ice tubes. The tubes were immediately centrifuged at 2000 x g for 10 min. Plasma ghrelin levels were measure with the immunochemiluminometric assay (IDS, SMBH, Germany) with an inter-assay coefficient of 6.2%. Plasma glucose levels were measured using a hexokinase enzymatic reference method (Cobas, Roche USA). Fasting insulin levels were measured using ECLIA method (Cobas 6000, Roche, USA) while CPR was measure with Immunoturbidimetry (Cobas 6000, Roche, USA). HOMA-IR was used to evaluate insulin resistance (fasting serum insulin (pmol/l) × fasting plasma glucose (mmol l⁻¹)/22.5) [12].

**Statistical Analysis**

Fasting levels of ghrelin, glucose and insulin were compared by a t-test between normal and obese groups. Pearson’s correlations were calculated between anthropometric and biochemical variances and ghrelin levels. Moreover, the effect of several variables on ghrelin concentrations was considered with multiple linear regression analysis. In the regression model, we verified ghrelin as a dependent variable and included weight, BMI, glucose, Insulin, glucose and HOMA-IR as independent variables. Values with P < 0.05 were considered statistically significant.

**Results**

Fasting plasma levels were significantly lower in an obese subject compared to normal ones (Table 1, Figure 1). Also, BMI, plasma insulin and HOMA-IR were significantly higher in the obese group (Table1).

**Table 1: Baseline characteristics of Normal and Obese subjects**

| Variables          | Normal (n = 13) | Ob (n = 7) | p-value |
|--------------------|----------------|------------|---------|
| Age, y             | 21±0.8         | 21±0.07    | 0.298   |
| Height, cm         | 159±8.2        | 161±3.7    | 0.183   |
| Weight, Kg         | 53±8.7         | 79±11      | 0.001*  |
| BMI, kg/m²         | 20±6.3         | 31.3±2.1   | 0.001*  |
| CRP                | 1.2±1.1        | 2.0±1.0    | 0.065   |
| Glucose, mmol/l    | 5.1±6.5        | 5.5±6.7    | 0.072   |
| Insulin, pmol/l    | 8.2±2.7        | 14.5±2.3   | 0.041*  |
| HOMA-IR            | 1.85±0.5       | 3.54±0.8   | 0.004*  |
| Ghrelin, pg/ml     | 541±202        | 440±140    | 0.002*  |

Data is presented as mean ± SD; *P < 0.05 = Statistically significantly difference.

Fasting plasma ghrelin was negatively correlated with BMI (r=-0.77, P<0.001), fasting insulin levels (r=-0.55, P<0.001) and HOMA-IR (r=-0.66, P<0.001). There was no correlation between ghrelin and glucose (Table 2).

**Discussion**

In our study contrary to our hypothesis, obese subjects have lower concentrations of ghrelin and...
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higher insulin levels that the age-matched lean control subjects. This is an indication that ghrelin is downregulated in human obesity. This result may be because of elevated insulin levels since ghrelin levels were independently associated with insulin and HOMA-IR. These findings are in agreement with other research studies [13, 14]. Ghrelin was shown to inhibit insulin secretion from pancreatic islets in rodents [15].

Table 3: Multiple regression analysis of Ghrelin with anthropometric and biochemical variables

| Variable   | Beta  | 95% CI       | P   |
|------------|-------|--------------|-----|
| Weight     | -0.44 | -0.32, -0.55 | 0.505 |
| BMI        | -1.77 | -1.65, -1.89 | 0.001* |
| Glucose    | -0.32 | -0.37, -0.49 | 0.668 |
| Insulin    | 2.66  | -2.49, -2.78 | 0.001* |
| HOMA-IR    | -2.41 | -2.33, -2.55 | 0.001* |

*p < 0.05: Statistically significant.

Ghrelin secretion may be affected by adiposity through insulin and glucose metabolism [16]. Studies performed in humans demonstrated that i.v. Administration of insulin induces a fall in ghrelin levels [17]. Thus, reduced ghrelin levels in obesity may be the consequence of increased insulin levels in these subjects.

In our study, ghrelin levels were negatively correlated with body weight and independently associated with BMI. Research evidence shows that plasma ghrelin levels are negatively correlated with body mass index and body fat percentage [18]. Also, reduced ghrelin secretion in obese patients was found to be an adaptive mechanism to a long-term positive energy balance [19].

The present study has some limitations that should be addressed. The small sample of the study has an important influence on the results. Also, we did not take in consideration any dietary habits, genetic information and socioeconomic factors that could also play an important role in obesity. Moreover, this was a one-time measure and may not represent the reality of the indices. Nevertheless, the results will provide some evidence of the relation of fasting ghrelin levels with insulin resistance and give some light on the pathogenesis of human obesity.

In conclusion, Ghrelin is deregulated in obesity and associated with insulin and insulin resistance and thus may be considered a suitable target for the management of insulin resistance. More studies are needed to elucidate the effects of ghrelin in the pathogenesis of human obesity.

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Authors’ Contributions

DP contributed to the conception and design of the study, interpretation of data writing of the manuscript and final approval of the version to be published. CK contributed to the design of the study, interpretation of the results writing a part of the manuscript and final approval of the paper. FA contributed to the collection of data, draft a part of the manuscript and final approval of the paper to be published. EK contributed to the collection and analysis of data, draft a part of the manuscript and final approval of the manuscript to be published. FS contributed in the data collection, draft a part of the manuscript and final approval of the manuscript to be published.

References

1. Ghezalordi S, Carnicelli V, Frascarelli S, et al. Ghrelin tissue distribution: comparison between gene and protein expression. J Endocrinol Invest. 2006; 29:115–121. https://doi.org/10.1007/BF03344083 PMid:16610236
2. Ukkola O. Ghrelin in Type 2 diabetes mellitus and metabolic syndrome. Mol Cell Endocrinol. 2011; 340:26–28. https://doi.org/10.1016/j.mce.2011.02.009 PMid:21419192
3. Boguszewski CL, Paz-Filho G, Velloso LA. Neuroendocrine body weight regulation: integration between fat tissue, gastrointestinal tract, and the brain. Endokrynol Pol. 2010; 61:194–206. PMid:20464707
4. Tschöp M, Weyer C, Tataranni PA, et al. Circulating ghrelin levels are decreased in human obesity. Diabetes. 2001; 50:707–709. https://doi.org/10.2337/diabetes.50.4.707 PMid:11289032
5. Schöff C, Horn R, Schill T, et al. Circulating ghrelin levels in patients with polycystic ovary syndrome. J Clin Endocrinol Metab. 2002; 87:4607–4610. https://doi.org/10.1210/jc.2002-020505 PMid:12364442
6. Ikezaki A, Hosoda H, Ito K, et al. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. Diabetes. 2002; 51:3408–3411. https://doi.org/10.2337/diabetes.51.12.3408 PMid:12453893
7. Marzullo P, Caumo A, Savia G, et al. Predictors of postabsorptive ghrelin secretion after intake of different macronutrients. J Clin Endocrinol Metab. 2006; 91:4124–4130. https://doi.org/10.1210/jc.2006-0270 PMid:16882748
8. Moesgaard SG, Ahren B, Carr R, et al. Effects of high-fat feeding and fasting on ghrelin expression in the mouse stomach. Regul Pept. 2004; 120: 261–267. https://doi.org/10.1016/j.regpep.2004.03.018 PMid:15177945
9. Greenman Y, Golani N, Gilad S, et al. Ghrelin secretion is modulated in a nutrient- and gender-specific manner. Clin Endocrinol. 2004; 60: 382–388. https://doi.org/10.1111/j.1365-2265.2004.01993.x
10. Purnell JQ, Weigle DS, Breen P, et al. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab. 2003; 88: 5747–5752. https://doi.org/10.1210/jc.2003-030513 PMid:14671163
11. Cole TJ, Bellizzi CM, Flegal MK, et al. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ. 2000; 320: 1240–1243. https://doi.org/10.1136/bmj.320.7244.1240 PMid:10797032 PMcid:PMC27328
12. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis

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model assessment: IR and beta-cell function from fasting plasma glucose and insulin concentration in man. Diabetologia. 1985; 28: 412-419. https://doi.org/10.1007/BF00280883 PMid:3899825

13. Lucidi P, Murdolo G, Di Loreto C, et al. Ghrelin is not necessary for adequate hormonal counterregulation of insulin-induced hypoglycemia. Diabetes. 2002; 51:2911–2914. https://doi.org/10.2337/diabetes.51.10.2911 PMid:12351426

14. Flanagan DE, Evans ML, Monsod TP, et al. The influence of insulin on circulating ghrelin. American Journal of Physiology, Endocrinology and Metabolism. 2003; 284: E313–E316. https://doi.org/10.1152/apendo.00569.2001 PMid:12531744

15. Qader SS, Lundquist I, Ekelund M, et al. Ghrelin activates neuronal constitutive nitric oxide synthase in pancreatic islet cells while inhibiting insulin release and stimulating glucagon release. Regul Pept. 2005; 128: 51–56. https://doi.org/10.1016/j.regpep.2004.12.018 PMid:15721487

16. Soriano-Guillén L, Barrios V, Martos G, et al. Effect of oral glucose administration on ghrelin levels in obese children. Eur J Endocrinol. 2004; 151: 119–121. https://doi.org/10.1530/eje.0.1510119 PMid:15248831

17. Anderwald C, Brabant G, Bernroider E, et al. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. Diabetes. 2003; 52: 1792–1798. https://doi.org/10.2337/diabetes.52.7.1792 PMid:12829648

18. Covasa M, Swartz T. The role of ghrelin in eating behaviour. In: Preedy VR, Watson RR, Martin CR (eds), Handbook of behavior, food and nutrition. Springer Science, New York, 2011; 175-188. https://doi.org/10.1007/978-0-387-92271-3_13

19. Sato T, Ida T, Nakamura Y, et al. Physiological roles of ghrelin on obesity. Obes Res Clin Pract. 2014; 8: e405-413. https://doi.org/10.1016/j.orcp.2013.10.002 PMid:25263830