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RAPID COMMUNICATION

Factors associated with fasting plasma ghrelin levels in children and adolescents

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Abstract

AIM: To measure plasma ghrelin levels in children and adolescents, analyze the associated factors, and investigate the role of ghrelin in obesity, insulin resistance and reproductive physiology.

METHODS: A total of 283 subjects aged 4.8-15.8 year were enrolled. Fasting blood samples were collected and plasma ghrelin levels were measured by radioimmunoassay. Fasting glucose (FG), fasting insulin (FI), baseline testosterone (T), estradiol (E2), prolactin (PRL), luteinizing hormone (LH), follicle-stimulating hormone (FSH), serum total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT) and uric acid (UA) were measured. Body mass index (BMI), insulin resistance by homeostasis model (HOMA-IR) and beta cell function by homeostasis model (HOMA-β) were calculated.

RESULTS: The median ghrelin level was 290 ng/L (15.0-1325.0 ng/L). Bivariate correlation analysis showed that ghrelin levels were inversely correlated with BMI, ALT, TG, UA, LH, FI and HOMA-IR (all $P < 0.05$). No other significant correlation was found between ghrelin levels and age, gender, TC, E2, FSH, PRL, FG and HOMA-β. Stepwise multiple regression analysis showed that only BMI and FI were independent determinants of plasma ghrelin levels in these children and adolescents ($P = 0.018$ and $P = 0.046$, respectively), which explained 25.4% of the variance.

CONCLUSION: These data suggest that the lower ghrelin levels in obese subjects may be the result of obesity and hyperinsulinemia, which is very common in obese subjects. Moreover, ghrelin may regulate human reproductive physiology indirectly.

Key words: Ghrelin; Obesity; Body mass index; Insulin

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INTRODUCTION

Ghrelin, recently isolated from rat and human stomachs, is an endogenous ligand for growth hormone secretagogue receptor (GHSR) type 1a[1,2]. Recent studies have shown that ghrelin is secreted from hypothalamus, pituitary, endocrine pancreas islets, bowel, kidney, heart and testis[1,2]. Besides stimulating GH secretion, ghrelin has been reported to have other endocrine and non-endocrine actions, including stimulation of lactotroph and corticotroph secretion, effects on the gonadal axis, cardiovascular activity, influence on behavior and sleep, modulation of cell proliferation, repression of apoptosis, and orexigenic effects[3-7]. However, most of these studies used animal models and there were some discrepancies in the results. Thus, the regulation of ghrelin secretion and its specific role in endocrinology in humans, including the gonadal axis, insulin resistance, energy balance and obesity, are not completely understood.

In this study, we measured plasma ghrelin levels in Chinese children and adolescents, analyzed their associated factors, and investigated the role of ghrelin in obesity, insulin resistance and reproductive physiology.

MATERIALS AND METHODS

Materials

A total of 283 subjects aged 4.8-15.8 year (mean ± SD;

9.4 ± 2.4 year) were enrolled in this study. They included 133 males and 150 females. Body mass index (BMI) was 12.15-44.00 kg/m^2 (19.88 ± 6.23 kg/m^2) with a median BMI Z score of 0.52. Ninety-six individuals were prepubescent and 187 were pubescent, including 107 with Tanner Ⅱ stage breasts, 53 with Tanner Ⅲ, 19 with Tanner Ⅳ and eight with Tanner Ⅴ. Subjects with obesity were included and subjects with other endocrine diseases, viral hepatitis, kidney and infectious diseases were excluded. Written informed consent was obtained from the parents and the study was approved by the Ethics Committee of the Children's Hospital of Zhejiang University School of Medicine, China.

Plasma ghrelin measurement

Two-milliliter venous blood samples were obtained by venipuncture after an overnight fast and were placed in a tube with 30 μL 10% EDTA and 20 μL aprotinin. Plasma was isolated and stored at -70℃ until analysis. Plasma total ghrelin levels, including Ser 3-octanoyl and Ser 3-des-octanoyl ghrelin, were measured by radioimmunoassay. The commercially available kits were purchased from Phoenix Pharmaceuticals (Burlingame, CA, USA). The assay sensitivity and CV were 10 ng/L and 5%-10%, respectively.

Biochemistry markers

Fasting glucose (FG) and insulin (FI) levels were measured by glucose oxidase method and radioimmunoassay (Beijing North Biotechnology Invest, China). Insulin resistance by homeostasis model (HOMA-IR) and beta cell function by homeostasis model (HOMA-β) were calculated according to Matthews *et al*[8]: HOMA-IR = FG × FI/22.5 and HOMA-β = 20 × FI/(FG-3.5).

Baseline testosterone (T), estradiol (E2), prolactin (PRL), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by chemiluminescence (Siemens, Gwynedd, UK). Serum total cholesterol (TC), triglyceride (TG), alanine aminotransferase (ALT) and uric acid (UA) were measured in the clinical laboratories of the Children's Hospital of Zhejiang University School of Medicine.

Body composition measurements

Body composition was measured in the morning after an overnight fast. Body weight was measured to the nearest 0.1 kg on an electronic scale, and body height was measured to the nearest 0.1 cm, with the subjects in underwear and barefoot. BMI was calculated as weight (kg) divided by height (m) squared.

Statistical analysis

Statistical analyses were conducted by using SPSS software (11.5 Version) (SPSS, Chicago, IL, USA). Quantitative data with normal distribution were presented as the mean ± SD, and data with skewness distribution were presented as the median (min-max). The T and E2 levels, which were lower than measured limits (both 20 ng/dL), were treated as the lower limit. The relation between fasting ghrelin levels and other factors was analyzed by bivariate correlation using the Pearson test. Stepwise multiple linear regression models were used to examine the determinants of plasma ghrelin levels. Differences were considered statistically significant at $P < 0.05$.

Table 1 Bivariate correlation analysis between fasting ghrelin levels (ng/L) and other factors

	r value	*P* value
Age (yr)	-0.107	0.073
Gender (male 1, female 0)	0.082	0.167
BMI Z score	-0.259	< 0.001
FI (mIU/L)	0.283	< 0.001
FG (mmol/L)	-0.108	0.085
HOMA-IR	0.266	< 0.001
HOMA-β	-0.049	0.447
ALT (U/L)	-0.192	0.002
TG (mmol/L)	-0.136	0.029
TC (mmol/L)	-0.051	0.258
UA (μmol/L)	-0.210	0.001
T (ng/L)	-0.149	0.016
E2 (ng/L)	-0.075	0.226
LH (IU/L)	-0.120	0.053
FSH (IU/L)	-0.063	0.281
PRL (μg/L)	-0.003	0.963

RESULTS

In 283 subjects, the levels of FG, FI, HOMA-IR and HOMA-β were 4.55 ± 0.64 mmol/L, 7.60 mIU/L (2.0-56.90 mIU/L), 1.57 (0.29-12.39) and 156.80 (-5240 to 10 640), respectively. The levels of ALT, TG, TC and UA were 18.0 U/L (6.0-369.0 U/L), 1.42 mmol/L (0.22-8.64 mmol/L), 4.18 mmol/L (2.12-7.81 mmol/L) and 297.75 ± 82.09 μmol/L, respectively. The levels of T, E2, LH, FSH and PRL were < 200.0 ng/L (< 200.0-2700 ng/L), < 20.0 ng/L (< 20-98.6 ng/L), 0.30 IU/L (0.10-98.60 IU/L), 2.40 IU/L (0.10-100.0 IU/L) and 13.30 μg/L (2.90-69.30 μg/L). The median ghrelin level was 290 ng/L (15.0-1325.0 ng/L).

Bivariate correlation analysis was performed between fasting ghrelin levels and other factors, including age, gender (female as 0, male as 1), BMI Z score, ALT, TG, TC, UA, T, E2, LH, FSH, PRL, FG, FI, HOMA-IR, and HOMA-β (Table 1). The results showed the ghrelin levels were inversely correlated with BMI, ALT, TG, UA, FI and HOMA-IR (all $P < 0.05$). LH, age and FG had a marginal relationship with ghrelin (P ranged from 0.053 to 0.085). No other significant correlation was found between ghrelin levels and gender, TC, E2, FSH, PRL and HOMA-β.

Stepwise multiple regression analysis included all the factors which showed $P < 0.1$ in the bivariate correlation analysis. We found that only FI and BMI were independent determinants of plasma ghrelin levels in these children and adolescents, which explained 22.7% of the variance (Table 2). Plasma ghrelin levels were inversely associated with FI and BMI Z score ($P = 0.026$ and $P = 0.048$, respectively). HOMA-IR, T, LH, ALT, TG, UA and age were excluded in the equations (all $P > 0.05$).

Table 2 Multiple linear regression analysis for determinant of plasma ghrelin levels

Variable	Coefficient (Beta)	*r* value	*P* value
FI (mIU/L)	-1.208	2.244	0.026
BMI Z score	-1.107	1.985	0.048
FG (mmol/L)	-0.203	1.857	0.065
ALT (U/L)	-0.099	1.252	0.212
T (ng/L)	-0.081	1.125	0.262
HOMA-IR	0.099	1.251	0.277
UA (μmol/L)	-0.046	0.574	0.567
Age (yr)	0.026	0.371	0.711
LH (IU/L)	-0.017	0.237	0.813
TG (mmol/L)	0.002	0.029	0.977

r^2 for the model = 0.227.

DISCUSSION

Ghrelin has recently been identified as a ligand of GHSR type 1a. It has been reported to have endocrine and nonendocrine actions[3-7], although there have been some contrary results. Also, the regulation of ghrelin secretion and its exact role have not yet been fully elucidated. In this study, we measured the plasma ghrelin levels in 283 Chinese children and adolescents and investigated the associated factors. We found that the median ghrelin level was 290 ng/L (15.0-1325.0 ng/L), which was similar with that reported previously[9,10].

We also found that BMI Z scores were inversely correlated with fasting plasma ghrelin levels, both by bivariate correlation and multiple regression analyses. This confirmed the view that ghrelin is closely associated with obesity. Ghrelin has been considered as a cause of obesity in some studies, on account of the fact that ghrelin has orexigenic effects in both rats and humans[11,12]. However, the results of most studies in humans, similar to our own, have demonstrated that ghrelin levels are negatively correlated with BMI Z score[9,13]. Moreover, Marzullo *et al* have reported that active ghrelin levels in subjects with obesity are lower than those in lean subjects[14]. These findings are contrary to the view that ghrelin is a factor in obesity. We speculate that the lower ghrelin levels in obesity are part of negative feedback to inhibit appetite and body weight, but not the primary cause of obesity[15]. This is also supported by the fact that circulating ghrelin levels increase in anorexia and cachexia[16-18]. However, animals without ghrelin do not have significantly altered body weight or food intake when compared with their wild-type littermates[19,20]. This suggests that it is a part of a reversible feedback mechanism, but not a determinant factor. Further studies are required to investigate the effect and the mechanism of ghrelin deficiency in individuals with obesity.

We also report that FI, HOMA-IR, ALT, UA and TG (but not glucose or HOMA-β) were negatively associated with plasma ghrelin levels. Moreover, FI was an independent factor of ghrelin levels by multiple regression analysis. These findings are supported by several previous studies[21,22] and suggest that insulin suppresses circulating ghrelin, independently of glucose. Hence, we speculate that hyperinsulinemia, which is very common in children and adolescents with obesity[23-25], might partly explain their lower ghrelin levels.

Some previous studies have suggested that ghrelin plays a role in the regulation of reproductive physiology. Expression of ghrelin in rat and human testes has been investigated[26,27], and some studies have reported that ghrelin levels are related to GnRH and androgens[28-31]. However, some animal studies have shown that ghrelin stimulates GnRH and/or androgens *in vitro*[28,29], but inhibits these hormones *in vivo*[30,31]. However, similar studies investigating the relationship between ghrelin and the gonadal axis in humans are rare, and the actual role of ghrelin in reproductive physiology is still unclear. In this study, we found that T and LH (but not E2 and FSH) were negative associated with fasting plasma ghrelin levels, although T and LH were not independent factors for ghrelin levels in stepwise regression analysis. This finding suggests that ghrelin regulates reproductive physiology in humans indirectly.

There are several limitations in this current study. Firstly, the sample may have been too small to find major differences. Secondly, we measured total circulating ghrelin, including Ser 3-octanoyl and Ser 3-des-octanoyl ghrelin, but not the active form of ghrelin. Thirdly, the actual correlation coefficients between serum ghrelin levels and BMI Z scores and FI were low. These imply that some other factors play a role in regulating circulating ghrelin levels.

In summary, our data suggest the lower ghrelin levels in subjects with obesity are the result of obesity and hyperinsulinemia, which is very common in these subjects. Moreover, ghrelin may regulate human reproductive physiology indirectly. Further studies are required to improve our understanding of the ghrelin effect and regulation of ghrelin secretion.

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COMMENTS

Background

Ghrelin is an endogenous ligand for growth hormone secretagogue receptor type 1a and has some endocrine and non-endocrine actions. However, the regulation of ghrelin secretion and its specific role in endocrinology in humans, including the gonadal axis, insulin resistance, energy balance and obesity, are not completely understood.

Research frontiers

Worldwide, obesity has reached epidemic proportions. Obesity also increases the risk of developing a number of chronic diseases including insulin resistance, type 2 diabetes, hypertension, hypercholesterolemia, stroke, and heart attack. Hence, analysis of the relationship between ghrelin levels, obesity and insulin resistance is necessary.

Related publications

The prevalence of obesity has increased in China, which is closely associated with

metabolic syndrome (*Int J Obes* 2007; 31: 15-22). Some studies have reported the relationship between obesity, adipocytokines and cytokines (*Endocrin J* 2005; 52: 519-524. *Indian Pediatr* 2007; 44: 275-279).

Innovations and breakthroughs

The lower ghrelin levels in subjects with obesity may be the result of obesity and hyperinsulinemia, which is very common in these subjects. Moreover, ghrelin might regulate human reproductive physiology indirectly.

Applications

Regulating ghrelin levels may be beneficial for improving insulin sensitivity, controlling body weight, or even reproductivity.

Terminology

Insulin resistance is a condition in which the cells of the body become resistant to the effects of insulin, that is, the normal response to a given amount of insulin is reduced. As a result, higher levels of insulin are needed in order for insulin to have an effect. Almost all individuals with type 2 diabetes mellitus and many with hypertension, cardiovascular disease, and obesity are insulin resistant.

Peer review

This study is of particular importance to childhood health and obesity, and correlates many factors with plasma ghrelin in the fasting state. It suggests an interesting hypothesis linking hyperinsulinemia and obesity to lower ghrelin levels.

REFERENCES

1 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

2 **Volante M**, Allia E, Gugliotta P, Funaro A, Broglio F, Deghenghi R, Muccioli G, Ghigo E, Papotti M. Expression of ghrelin and of the GH secretagogue receptor by pancreatic islet cells and related endocrine tumors. *J Clin Endocrinol Metab* 2002; **87**: 1300-1308

3 **Broglio F**, Prodam F, Me E, Riganti F, Lucatello B, Granata R, Benso A, Muccioli G, Ghigo E. Ghrelin: endocrine, metabolic and cardiovascular actions. *J Endocrinol Invest* 2005; **28**: 23-25

4 **Allison KC**, Ahima RS, O'Reardon JP, Dinges DF, Sharma V, Cummings DE, Heo M, Martino NS, Stunkard AJ. Neuroendocrine profiles associated with energy intake, sleep, and stress in the night eating syndrome. *J Clin Endocrinol Metab* 2005; **90**: 6214-6217

5 **Abiko Y**, Suzuki H, Masaoka T, Nomura S, Kurabayashi K, Hosoda H, Kangawa K, Hibi T. Enhanced plasma ghrelin levels in Helicobacter pylori-colonized, interleukin-1-receptor type 1-homozygous knockout (IL-1R1-/-) mice. *World J Gastroenterol* 2005; **11**: 4148-4153

6 **Taub DD**. Novel connections between the neuroendocrine and immune systems: the ghrelin immunoregulatory network. *Vitam Horm* 2008; **77**: 325-346

7 **Leite-Moreira AF**, Rocha-Sousa A, Henriques-Coelho T. Cardiac, skeletal, and smooth muscle regulation by ghrelin. *Vitam Horm* 2008; **77**: 207-238

8 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419

9 **Shiiya T**, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; **87**: 240-244

10 **Bunt JC**, Salbe AD, Tschop MH, DelParigi A, Daychild P, Tataranni PA. Cross-sectional and prospective relationships of fasting plasma ghrelin concentrations with anthropometric measures in pima Indian children. *J Clin Endocrinol Metab* 2003; **88**: 3756-3761

11 **Tang-Christensen M**, Vrang N, Ortmann S, Bidlingmaier M, Horvath TL, Tschop M. Central administration of ghrelin and agouti-related protein (83-132) increases food intake and decreases spontaneous locomotor activity in rats. *Endocrinology* 2004; **145**: 4645-4652

12 **Haqq AM**, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ. Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. *J Clin Endocrinol Metab* 2003; **88**: 174-178

13 **Tritos NA**, Kokkinos A, Lampadariou E, Alexiou E, Katsilambros N, Maratos-Flier E. Cerebrospinal fluid ghrelin is negatively associated with body mass index. *J Clin Endocrinol Metab* 2003; **88**: 2943-2946

14 **Suematsu M**, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Matsumoto K, Kitagawa N, Akatsuka H, Hori Y, Nakatani K, Togashi K, Yano Y, Adachi Y. Decreased circulating levels of active ghrelin are associated with increased oxidative stress in obese subjects. *Eur J Endocrinol* 2005; **153**: 403-407

15 **McLaughlin T**, Abbasi F, Lamendola C, Frayo RS, Cummings DE. Plasma ghrelin concentrations are decreased in insulin-resistant obese adults relative to equally obese insulin-sensitive controls. *J Clin Endocrinol Metab* 2004; **89**: 1630-1635

16 **Misra M**, Miller KK, Stewart V, Hunter E, Kuo K, Herzog DB, Klibanski A. Ghrelin and bone metabolism in adolescent girls with anorexia nervosa and healthy adolescents. *J Clin Endocrinol Metab* 2005; **90**: 5082-5087

17 **Nagaya N**, Itoh T, Murakami S, Oya H, Uematsu M, Miyatake K, Kangawa K. Treatment of cachexia with ghrelin in patients with COPD. *Chest* 2005; **128**: 1187-1193

18 **Janas-Kozik M**, Krupka-Matuszczyk I, Malinowska-Kolodziej I, Lewin-Kowalik J. Total ghrelin plasma level in patients with the restrictive type of anorexia nervosa. *Regul Pept* 2007; **140**: 43-46

19 **Wortley KE**, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox HJ, Yancopoulos GD, Wiegand SJ, Sleeman MW. Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. *Proc Natl Acad Sci USA* 2004; **101**: 8227-8232

20 **Sun Y**, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol* 2003; **23**: 7973-7981

21 **Flanagan DE**, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV, Sherwin RS. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab* 2003; **284**: E313-E316

22 **Mohlig M**, Spranger J, Otto B, Ristow M, Tschop M, Pfeiffer AF. Euglycemic hyperinsulinemia, but not lipid infusion, decreases circulating ghrelin levels in humans. *J Endocrinol Invest* 2002; **25**: RC36-RC38

23 **Zou CC**, Liang L, Hong F, Fu JF, Zhao ZY. Serum adiponectin, resistin levels and non-alcoholic fatty liver disease in obese children. *Endocr J* 2005; **52**: 519-524

24 **Murdolo G**, Kempf K, Hammarstedt A, Herder C, Smith U, Jansson PA. Insulin differentially modulates the peripheral endocannabinoid system in human subcutaneous abdominal adipose tissue from lean and obese individuals. *J Endocrinol Invest* 2007; **30**: RC17-RC21

25 **Sobhonslidsuk A**, Jongjirasiri S, Thakkinstian A, Wisedopas N, Bunnag P, Puavilai G. Visceral fat and insulin resistance as predictors of non-alcoholic steatohepatitis. *World J Gastroenterol* 2007; **13**: 3614-3618

26 **Miller DW**, Harrison JL, Brown YA, Doyle U, Lindsay A, Adam CL, Lea RG. Immunohistochemical evidence for an endocrine/paracrine role for ghrelin in the reproductive tissues of sheep. *Reprod Biol Endocrinol* 2005; **3**: 60

27 **Gaytan F**, Barreiro ML, Caminos JE, Chopin LK, Herington AC, Morales C, Pinilla L, Paniagua R, Nistal M, Casanueva FF, Aguilar E, Dieguez C, Tena-Sempere M. Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. *J Clin Endocrinol Metab* 2004; **89**: 400-409

28 **Pagotto U**, Gambineri A, Pelusi C, Genghini S, Cacciari M,

Otto B, Castaneda T, Tschop M, Pasquali R. Testosterone replacement therapy restores normal ghrelin in hypogonadal men. *J Clin Endocrinol Metab* 2003; **88**: 4139-4143

29 **Unniappan S**, Peter RE. In vitro and in vivo effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R1093-R1101

30 **Barreiro ML**, Gaytan F, Castellano JM, Suominen JS, Roa J, Gaytan M, Aguilar E, Dieguez C, Toppari J, Tena-Sempere M. Ghrelin inhibits the proliferative activity of immature Leydig cells in vivo and regulates stem cell factor messenger ribonucleic acid expression in rat testis. *Endocrinology* 2004; **145**: 4825-4834

31 **Fernandez-Fernandez R**, Tena-Sempere M, Aguilar E, Pinilla L. Ghrelin effects on gonadotropin secretion in male and female rats. *Neurosci Lett* 2004; **362**: 103-107

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