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Sex-related differences in the association of ghrelin levels with obesity in adolescents

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Abstract

Background: The utility of ghrelin as a biomarker may be different depending on gender. The aim of this study was to assess ghrelin levels in a population-based sample of adolescents, and to evaluate their association with obesity and obesity-related parameters depending on sex.
Methods: The studied population included 601 randomly selected 14-to 16-year-old children. Anthropometrical data were measured and body mass index (BMI) and waist to hip ratio calculated. Body composition was assessed using an impedance body composition analyzer. Total serum ghrelin levels were determined using a multiplexed bead immunoassay. Serum leptin and adiponectin levels were determined by ELISA and insulin by RIA.
Results: Ghrelin levels were significantly higher in girls than in boys. Serum ghrelin concentrations were significantly lower ($p<0.01$) in obese than in normal weight (NW) girls, but showed no differences by weight category in boys. Ghrelin showed a significant negative relationship with waist circumference (WC), waist to hip ratio and fat mass ($p<0.05$) in both genders, and with weight and BMI ($p<0.01$) in girls, and insulin ($p<0.01$) and HOMA ($p<0.05$) in boys. Ghrelin also correlated negatively with leptin levels in girls ($p<0.01$).
Conclusions: Our study describes serum ghrelin levels in adolescents, showing a sexual dimorphism in ghrelin levels in these 14-to 16-year-old children, and a different association of ghrelin levels with obesity by gender that suggests a different appetite and energy expenditure control depending on sex at this age.

Keywords: adolescents; anthropometric data; fat mass; ghrelin levels.

Dedicated to the late Prof. Manuel de Oya, as the warmest homage to his memory.

Introduction

Ghrelin is an orexigenic peptide derived from the stomach [1], that stimulates appetite, in humans, increasing food intake and regulating body weight maintenance [2]. It seems that ghrelin has a role in glucose homeostasis affecting insulin sensitivity [3] by influencing insulin secretion through the AMPK-UPC2 pathway in β cells [4].

Studies in adults suggest a negative association between ghrelin and obesity and obesity-related phenotypes. In different ethnic groups, ghrelin levels have been observed to be decreased in obese adults [5, 6] and have been negatively correlated with body mass index (BMI) [6–8] and waist circumference (WC) [9]. Ghrelin levels have also been associated with fat mass in some studies [5, 9] but not in others [7]. In addition, a negative association of ghrelin concentrations with insulin levels and insulin resistance has been reported [7, 10–12].

The association between ghrelin and obesity has also been analyzed in different populations of children and adolescents. Some studies have found decreased ghrelin levels in obese children [13, 14] and a negative correlation between BMI and ghrelin concentrations [15–19]. However, the results are inconsistent, since these studies were not derived from population-based samples, but rather from either relatively small population samples, or studies including a broader range of age groups. Furthermore, the reason behind different ghrelin levels depending on sex remains unclear.

The aim of our study was to investigate a potential sexual dimorphism in ghrelin levels in pubertal children, and the possible different relationship of ghrelin with obesity and obesity-related parameters in pubertal boys

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and girls. Thus, our study examines total ghrelin levels by gender in Spanish adolescents between the ages of 14 and 16, and analyzes their relationship with anthropometric measurements, body composition, insulin, leptin and adiponectin levels in a large population-based cohort of healthy adolescents.

Materials and methods

Subjects

The studied population included 601 14- to 16-year-old children (285 boys and 316 girls). Children were participants in a cross-sectional study designed to analyze cardiovascular risk factors in Spanish schoolchildren, and were selected by means of random cluster sampling in schools. Parents were required to provide written consent for their children to participate in the study. All children reported by their parents to be suffering from chronic diseases, including pubertal delay, were excluded. The study protocol complied with Helsinki Declaration guidelines and Spanish legal provisions governing clinical research on humans, and was approved by the Clinical Research Ethics Committee of the Fundación Jiménez Díaz in Madrid.

Anthropometric variables

Measurements were taken with children wearing light clothing and barefoot. Weight was determined to the nearest 0.1 kg using a standardized electronic digital scale, and height was measured to the nearest 0.1 cm using a portable stadiometer. WC was measured at the narrowest point between the lowest rib and the uppermost lateral border of the right iliac crest. Hip circumference (HC) was measured at the widest point of the hips with the subject standing with both feet together. The waist to hip ratio was calculated based on these two circumference values. In a subgroup of children, body composition [expressed as fat mass (kg) and % of body fat] was assessed using a Tanita (Arlington Heights, IL, USA) TBF-300MA impedance body composition analyzer. BMI (weight in kg/height in m^2) was calculated and BMI z-score was also calculated using reference data [20]. According to Cole et al. criteria [21], we considered children to be overweight (OW) if their BMI exceeded the cut-off points between 22.6 and 23.9 kg/m^2 for boys and between 23.3 and 24.4 kg/m^2 for girls depending on age (half a year intervals). We considered children to be obese if their BMI exceeded the cut-off points between 27.6 and 28.9 kg/m^2 for boys and between 28.6 and 29.4 kg/m^2 for girls. Biochemical data: Fasting (12 h) venous blood samples were obtained by venipuncture and collected in Vacutainer tubes. Total serum ghrelin levels were quantified using multiplex assay kits that utilize fluorescent microbead technology, allowing simultaneous quantification of several target proteins within a single serum sample. Serum without protease inhibitor was used for ghrelin determination, which may result in lower ghrelin levels than using samples with preservative. A customized panel from BioRad was used (Bio-PlexPro™ Human Diabetes Standard; Bio-Rad, Hercules, CA, USA) in the platform Luminex 200 System (Luminex corporation, Invitrogen; Caramillo, CA, USA). As previously reported, in our panel, we analyzed visfatin and resistin in addition to ghrelin. Serum leptin and adiponectin levels were determined by ELISA using commercially available kits (Leptin EIA-2395, DRG Instruments GmbH, Marburg, Germany and Adiponectin E-09, Mediagnost® Reutlingen, Germany, respectively). Serum insulin concentrations were measured by radioimmunoassay (RIA) using a commercial kit (BI-Insulin IRMA, Bio-Rad, France).

Statistical analysis

Statistical analyses were performed using the SPSS software package, version 9.0 (SPSS, Inc., Chicago, IL, USA). The results are expressed as mean (95% confidence interval). Given that serum ghrelin levels did not follow a normal distribution, the data were normalized prior to the statistical analysis. Gender differences in the variables being studied were assessed using a t-test. Differences in variables by weight category in boys and girls by age were evaluated by one-factor ANOVA. Partial correlation analyses were performed to evaluate the relationships between ghrelin and anthropometric variables adjusted by age, and between ghrelin and insulin levels and adipokines adjusted by age and BMI z-score. To ascertain the independent predictors of ghrelin levels by gender, stepwise multiple regression analysis was performed, including fat mass, insulin and leptin as independent variables.

Results

Serum ghrelin levels and anthropometric variables in 14- to 16-year-old boys and girls in our study are shown in Table 1. Serum ghrelin levels were significantly higher in girls ($p<0.01$) than in boys.

Serum ghrelin levels in normal weight (NW), OW and obese children by sex are shown in Table 2. Serum ghrelin concentrations were significantly lower in obese than in NW 14- to 16-year-old girls ($p<0.01$). No significant

Table 1: Serum ghrelin levels and anthropometrical characteristics (mean (95% confidence interval) in 14- to 16-year old boys and girls).

	Boys	Girls	p-Value
n	285	316	
Age, years	14.9 (14.8–15.0)	14.8 (14.7–14.9)	–
Ghrelin, pg/mL	362 (337–387)	424 (397–450)	0.001
Weight, kg	65.2 (63.7–66.8)	56.8 (55.7–57.8)	0.001
Height, cm	170 (169–171)	161 (160–162)	0.001
BMI, kg/m^2	22.2 (21.8–22.7)	21.8 (21.5–22.2)	–
z-score BMI	0.18 (0.06–0.30)	0.14 (0.04–0.23)	–
n	163	176	
Fat mass, %	17.0 (15.6–18.2)	28.2 (27.2–29.2)	0.000
Fat mass, kg	12.7 (11.2–15.0)	16.8 (15.7–17.8)	0.001

Table 2: Serum ghrelin levels (pg/mL) in NW, OW and obese children by sex.

14-to16-year old boys (n=285)				14-to16-year old girls (n=316)			
NW (n=198)	OW (n=64)	Obese (n=23)	p-Value	NW (n=254)	OW (n=49)	Obese (n=13)	p-Value
367 (335–398)	345 (294–396)	380 (310–449)	NS	442 (413–472)	373 (302–444)	250 (157–344)	NW-OB[a] OW-OB[b]

p: ANOVA, Post Hoc Tukey. [a]p<0.01, [b]p<0.05.

differences in ghrelin levels by weight category were observed in boys.

Partial correlation analyses were performed between ghrelin levels and anthropometric parameters adjusted by age (Table 3), and between ghrelin levels and insulin, HOMA, leptin and adiponectin levels adjusting by age and BMI z-score (Table 4). The analysis showed significant negative correlations of ghrelin levels with measures of central obesity (WC and waist to hip ratio) and with fat mass in both boys and girls. Furthermore, negative correlations between ghrelin levels and weight and BMI were observed in girls (Table 3). After adjusting by age and BMI z-score, ghrelin correlated negatively with insulin levels and HOMA in boys but not in girls. On the other hand, negative correlations between ghrelin levels and leptin were observed in girls but not in boys, while no significant correlations were found between ghrelin and adiponectin levels. Stepwise multiple regression analysis revealed that insulin accounts for 7.1% of the variance in ghrelin in boys, and fat mass accounts for 4.5% of its variance in girls.

Table 3: Correlation analysis between ghrelin and anthropometric data and body composition in boys and girls adjusted by age.

	Ghrelin	
	Boys	Girls
n	285	316
Weight, kg	−0.086	−0.190[b]
BMI, kg/m^2	−0.053	−0.154[b]
BMI z-score	−0.055	−0.154[b]
Waist circumference, cm	−0.151[a]	−0.280[c]
Waist-to-hip ratio	−0.216[b]	−0.268[c]
n	163	176
Fat mass, %	−0.197[a]	−0.192[a]
Fat mass, kg	−0.174[a]	−0.228[b]

[a]p<0.05, [b]p<0.01, [c]p<0.001.

Table 4: Correlation analysis between ghrelin and insulin related variables and adipokines in boys and girls adjusted by age and BMI z-score.

	Ghrelin	
	Boys (n=285)	Girls (n=316)
Insulin	−0.166[b]	−0.079
HOMA	−0.136[a]	−0.064
Leptin, ng/mL	−0.105	−0.181[b]
Adiponectin, ng/mL	0.012	0.022

[a]p<0.05, [b]p<0.01.

Discussion

Data on ghrelin levels in healthy populations coming from prospective studies in our area are scarce. Our study provides data on ghrelin levels from a large sample of Mediterranean children analyzed by gender.

In our population of 14- to 16-year-old children, we have observed significantly higher ghrelin levels in girls than in boys. In the literature, the findings of different studies analyzing gender differences in serum ghrelin levels are inconsistent. Although some authors failed to find any differences between sexes [5–7, 22], some studies in adults have also reported higher ghrelin levels in females than in males [23–25], suggesting that there are important sex-based differences in the regulation and action of ghrelin in humans. A possible explanation for this sexual dimorphism could be related to sex steroid effects, however, no relationship between ghrelin levels and sexual hormones has been observed in our population (data not shown). As previously suggested [25], the higher serum ghrelin concentration in females could be related, at least partially, with the different body fat content and distribution in women compared to men. Girls in our study have significantly higher fat mass than boys.

In both sexes, ghrelin levels were related to WC and waist-to-hip ratio and body composition, evaluated as total fat mass or % fat mass. The finding of an inverse association of serum ghrelin levels with anthropometric measurements, such as WC and waist to hip ratio, is in agreement with other studies in adults [9, 23], and with studies in children in which an association between ghrelin and measures of central obesity has been reported [26, 27]. In children, some studies have analyzed the association between ghrelin and body fat composition

previously reported in adults [5, 9]. A negative association between ghrelin levels and percentage of body fat has been previously reported in Pima Indian children [16], obese prepubescent children [26] and children with metabolic syndrome [27]. To our knowledge, the analysis of the association between these ghrelin concentrations and fat mass has not been addressed in previous population-based studies in Caucasian pubertal children.

Serum ghrelin levels showed an inverse association with weight and BMI in girls but not in boys. Also, ghrelin levels were lower in obese compared to OW and NW 14- to 16-year-old girls, without showing significant differences across BMI categories in 14- to 16-year-old boys. The association of lower ghrelin levels with obesity [5, 6], and the finding of decreased ghrelin levels across BMI categories has been described in previous studies considering males and females together [25]. Studies in children have also found an association between ghrelin and obesity [13, 14] and BMI [16–19], however, none of these studies analyzed boys and girls separately. In our study, in addition to finding decreased levels of ghrelin associated with obesity in girls but not in boys, we observed gender differences in the associations between ghrelin and BMI, which have not been reported previously in children. In girls, serum ghrelin levels had a relationship with weight, BMI and BMI z-score that was not present in boys. Our results, showing significant correlations of ghrelin levels with WC and fat mass but not with BMI in boys, suggest that it is the amount of fat and the body fat distribution that is related to ghrelin concentrations, rather than BMI. Different body fat mass composition and distribution between sexes could contribute to explain the different ghrelin levels between sexes, and the gender differences in the association of ghrelin with anthropometric variables.

In our study, we observed a negative association of ghrelin with insulin levels and insulin resistance in boys independent of anthropometric variables, which has been reported in some studies in children [6, 19, 28], but not in others [18], although none of these studies analyzed boys and girls separately.

We also found a negative association between ghrelin and leptin levels in girls. The regulation of ghrelin secretion and its biological effects appear opposite to those of leptin, and a negative association between ghrelin and leptin has been reported in the literature [28], although not all studies have found this association [25]. In our population, we have previously described a negative association of fat mass with leptin [29] that could contribute to explain the indirect association between ghrelin levels with these adipokines, as in our children the relationship of serum ghrelin with fat measures appears even stronger than with BMI.

The peptides acyl ghrelin (AG) and des-acyl ghrelin (DAG) are both encoded by the prepro-ghrelin gene. Acylation is catalyzed by the enzyme ghrelin O-acyltransferase. DAG represents more than 90% of human plasma ghrelin immunoreactivity. While AG acts on the GH secretagogue receptor (GHSR) in the CNS to promote feeding and adiposity and also acts on GHSR in the pancreas to inhibit glucose-stimulated insulin secretion, among other actions, DAG has long been considered an inert degradation product of AG. Recent evidence, however, indicates that DAG behaves like a separate hormone, acting as a potent functional inhibitor of ghrelin [30].

The main limitation of our study is the absence of pubertal stage assessment that prevents us from using Tanner stage as a covariate in our analysis. However, in our study, we have ruled out delayed puberty, defined as the absence of testicular enlargement in boys after 14 years or breast development in girls after age 13. A further adjustment of our analysis by age contributed to control this aspect. Other important limitations of our study, mainly attributed to methodological issues, are the lack of information about active ghrelin concentrations and the variability of the assays used to determine ghrelin, which make comparison difficult. Furthermore, the fact that serum without protease inhibitor has been used may contribute to the lower ghrelin levels observed in our study comparing with other studies using samples with preservative.

Conclusions

In our study, we reported that in healthy 14- to 16-year-old children levels of ghrelin are higher in girls than in boys, which suggests a sexual dimorphism in appetite and energy expenditure control at this age. Serum ghrelin levels were related to obesity, but were mainly influenced by body composition measures such as fat mass more so than by BMI.

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