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ORIGINAL ARTICLE

Ghrelin is independently associated with anti-mullerian hormone levels in obese but not non-obese women with polycystic ovary syndrome

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Abstract Ghrelin is an endogenous appetite stimulant that may have a role in ovarian function. Women with polycystic ovary syndrome have anovulation and frequently weight management issues; however the associations between ghrelin and hormonal markers in polycystic ovary syndrome have not been well studied. In order to characterize the association between total ghrelin levels and ovarian function and the possible modification of this relationship by obesity, we examined total ghrelin levels and anti-mullerian hormone, total testosterone, and insulin in obese and non-obese women with and without polycystic ovary syndrome. Total ghrelin levels were lower in obese women with polycystic ovary syndrome ($n = 45$) compared to obese controls ($n = 33$) ($p = 0.005$), but similar in non-obese women with polycystic ovary syndrome ($n = 20$) compared to non-obese controls ($n = 21$) ($p = \text{NS}$). In the obese polycystic ovary syndrome group, anti-mullerian hormone was associated with ghrelin levels independent of age, insulin, and total testosterone ($p = 0.008$). There was no association between total ghrelin and anti-mullerian hormone levels in non-obese women with polycystic ovary syndrome, non-obese controls, or obese controls ($p = \text{NS}$). Our results provide evidence for a potential relationship between ghrelin and ovarian function in obese women with polycystic ovary syndrome that was not observed in non-obese women with polycystic ovary syndrome or controls.

Keywords PCOS · Ghrelin · AMH · Obesity

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Introduction

The associations between weight, metabolism, and fertility have been clinically observed in women, but these relationships are not well-defined. Ghrelin has been suggested as a potential link between energy homeostasis and fertility. Ghrelin is an endogenous ligand specific for the GH secretagogue receptor (GHS-R) [1] that is secreted by the stomach and acts as an appetite stimulant, regulator of energy intake [2–4], and may affect fertility through suppression of the hypothalamic-pituitary-gonadal axis [5, 6]. Additionally, ghrelin and its receptor have been localized in the human and rodent ovary suggesting a direct role for ghrelin in ovarian function [7–11]. Obesity is an independent predictor of low ghrelin levels [12, 13], and is also associated with decreased fertility [14, 15].

Polycystic ovary syndrome (PCOS), is a condition characterized by frequent anovulation secondary to dysregulation of the hypothalamic pituitary ovarian axis which is commonly associated with obesity. It is unclear whether the pathophysiology resulting in decreased fertility in obese women with PCOS is similar to that in non-obese women with PCOS or healthy obese women. Given the known relationships between ghrelin levels and both energy homeostasis and fertility, characterization of ghrelin levels in women with PCOS is of interest. Several studies have

described lower serum ghrelin levels in obese women with PCOS compared to non-affected women [16–19]; however, no studies have extensively characterized these levels in a cohort comprised of obese and non-obese women with and without PCOS. Anti-mullerian hormone (AMH), a member of the TGFβ family, is produced by ovarian granulosa cells and its serum levels are positively associated with PCOS [20, 21] and inversely associated with obesity [22]. AMH has been proposed as a potential diagnostic criterion for PCOS and has been shown to correlate with multiple features of the syndrome [20]. Although ghrelin and AMH levels have been assessed in women with PCOS [23], only one study has reported the association between total ghrelin and AMH levels in obese women with PCOS and did not assess comparator groups [24].

Although PCOS is often associated with increased body mass, alterations in energy homeostasis [25], and dysregulation of appetite [18, 26, 27], not all women with PCOS are overweight or obese. To elucidate the potential association between ghrelin and ovarian function in PCOS, and to see if this relationship is modified by the obese disease state, we examined the relationship between total ghrelin and AMH and other hormones dysregulated in PCOS in both obese and non-obese women. We hypothesized that the relationship between total ghrelin and AMH would differ between women with PCOS and controls, and that this effect would be modified by obesity.

Subjects and methods

Subjects

Subjects between the ages of 21–50 years meeting well-defined criteria for PCOS based on evidence of clinical (Ferriman-Gallwey score ≥8) or biochemical (total testosterone level ≥50 ng/dL) hyperandrogenism and oligomenorrhea were included in this study [28]. All PCOS subjects were recruited from the PCOS clinic and screened with testing for TSH, prolactin, DHEAS and 17 hydroxy progesterone to exclude alternative causes of oligomenorrhea and hyperandrogenism. Control participants were recruited simultaneously from a random sample of healthy women receiving routine gynecologic care and had no history of irregular menstrual cycles, or evidence of hyperandrogenism such as hirsutism or acne. Demographic and medical information was collected on all subjects. None of the subjects had existing cardiovascular disease or current symptoms of cardiovascular disease such as shortness of breath and chest pain [29, 30]. Other exclusion criteria for all subjects included pregnancy, lactation, hysterectomy, menopause, and chronic illnesses such as asthma and inflammatory bowel disease, missing laboratory values or demographic characteristics. Only subjects with complete data were included in this study. The University of Pennsylvania Institutional Review Board approved the study and written informed consent was obtained from all participants.

Methods

Fasting blood serum samples collected at random and stored at −70 °C were used for analysis. Glucose was measured using standard enzymatic methods. Insulin and testosterone were measured by radioimmunoassay using human specific kits (EMD Millipore, Billerica, MA, USA). AMH levels were measured using the Gen II enzyme linked immunosorbent assay (Beckman Colter, Brea, CA, USA) and total ghrelin was measured using a radioimmunoassay kit (EMD Millipore, Billerica, MA, USA). Body mass index (BMI) was calculated as kilograms per meter squared. Women with obesity were defined as BMI ≥30 kg/m^2. Non-obese women were defined as BMI <30 kg/m^2.

Statistical analysis

Continuous data were reported as medians with interquartile ranges. Univariate associations between continuous variables were tested using Spearman's correlations, Kruskal–Wallis tests, Wilcoxon rank sum tests and simple linear regression as appropriate. Ghrelin and AMH were log transformed to smooth the variable distributions for analysis. Multivariable linear regression was performed to determine the relationship between log transformed ghrelin and log transformed AMH, PCOS status, and obese disease state, adjusting for relevant covariates. We pursued a rational approach to model building that incorporated forward selection of covariates with significant associations in univariate testing. All tests were considered significant at the $p < 0.0125$ level to account for multiple comparisons. Statistical tests were performed using STATA 12 software (College Station, TX).

Results

The data included four groups: 45 women with obesity and PCOS, 33 control women with obesity, 20 non-obese women with PCOS, and 21 non-obese controls. Baseline characteristics are shown in Table 1. Between group differences in ghrelin were investigated, and obese women with PCOS had significantly lower total ghrelin levels compared to obese controls ($p = 0.005$). Non-obese women with PCOS and non-obese controls had similar ghrelin levels ($p =$ NS).

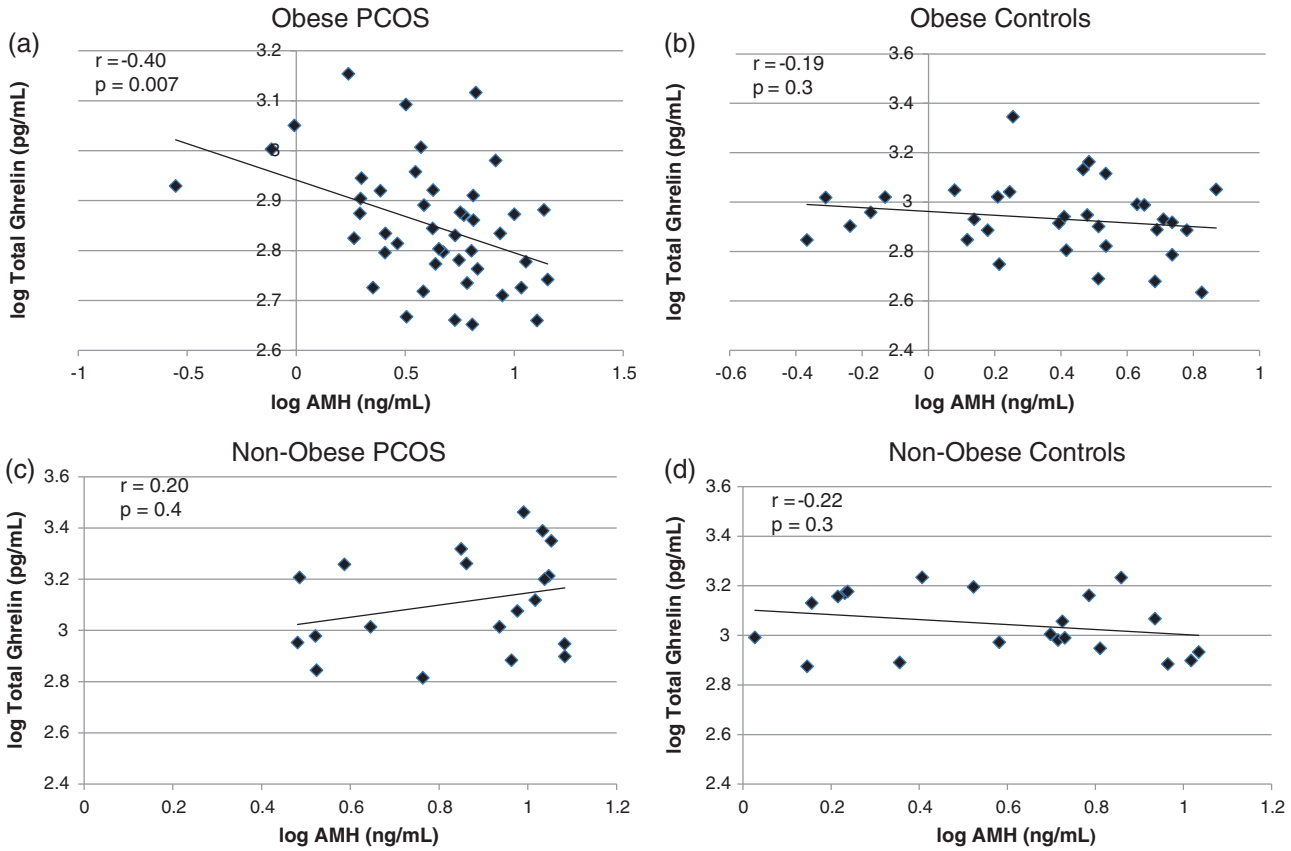

Fig. 1 Correlations between total ghrelin and AMH in **a** obese women with PCOS, **b** obese control women, **c** non-obese women with PCOS, and **d** non-obese control women

In a univariate analysis (Table 2, Fig. 1), total ghrelin had a significant negative correlation with AMH in obese women with PCOS only ($p = 0.007$). In non-obese women with PCOS, BMI was associated with ghrelin levels ($p = 0.02$). Ghrelin and age were associated in healthy obese women ($p = 0.04$). Age, BMI, TT, and insulin correlated with ghrelin in the total cohort ($p < 0.05$).

Multiple regressions

A multivariable model of the whole cohort with PCOS as a dichotomous variable and BMI as a continuous variable, adjusted for age, total testosterone and insulin, failed to demonstrate a significant inverse association between log ghrelin and log AMH ($p = 0.1$). In this model, only BMI ($p < 0.001$) and insulin ($p = 0.021$) had negative associations with log ghrelin. When the analysis was stratified by PCOS status and obese disease state, a significant inverse relationship between log ghrelin and log AMH was found in the obese women with PCOS ($p = 0.008$), adjusted for age, TT, BMI, and insulin (Table 3). This association was not found in obese controls, non-obese women with PCOS, or non-obese controls (Table 3).

Discussion

This is the first study assessing the relationship between endogenous serum total ghrelin levels and AMH, independent of androgens and insulin resistance, in both obese and non-obese women with PCOS and controls. Ghrelin levels were lowest in obese women with PCOS compared to obese controls, non-obese controls, and non-obese women with PCOS. Moreover AMH levels are associated with total ghrelin levels independent of androgens, insulin resistance, and age in obese women with PCOS but not in the other groups of the cohort. Total ghrelin levels were similar in non-obese PCOS women and non-obese controls, as has been shown before [31].

Many hormones and neuropeptides in the energy homeostasis pathway, including insulin, kisspeptin, leptin, neuropeptide Y, and ghrelin, have been identified as possible connections between appetite, weight, and fertility [32]. Due to the high prevalence of obesity and anovulatory infertility in PCOS, the interactions of these hormones are particularly relevant. Ghrelin was first noted as an appetite stimulant in rats [2, 3] and humans [33, 34] but has more recently been found to possibly have central effects on fertility in healthy women by suppressing LH and FSH

Table 1 Demographics and laboratory values in women with PCOS and controls, median and interquartile range (IQR, 25th–75th percentile)

	Total ($n = 119$)	Obese PCOS ($n = 45$)	Obese control ($n = 33$)	p-value obese PCOS vs obese control	Non-obese PCOS ($n = 20$)	Non-obese control ($n = 21$)	p-value non-obese PCOS vs non-obese control	p-value all groups compared
Age (years)	31 (26, 36)	32 (26, 36)	34 (30, 38)	0.2	29 (24, 33)	28 (26, 32)	0.9	0.02
BMI (kg/m^2)	34.5 (26.1, 40.4)	39.3 (35.1, 43.5)	38.7 (33.5, 41.5)	0.4	25.9 (23.2, 27.9)	22.3 (20.1, 24.3)	<0.001	<0.0001
Total ghrelin (pg/mL)	850 (683, 1123)	683 (587, 833)	852 (704, 1049)	0.005	1253 (889, 1821)	1012 (873, 1466)	0.3	<0.0001
AMH (ng/mL)	4.3 (2.4, 6.7)	4.5 (2.6, 6.6)	2.9 (1.4, 4.7)	0.002	8.9 (4.0, 10.9)	5.0 (1.7, 6.9)	0.005	<0.0001
Total testosterone (ng/dL)	43 (27, 62)	58 (40, 86)	35 (22, 45)	<0.001	55 (37, 70)	21 (10, 35)	<0.001	<0.0001
Insulin (uU/mL)	14.8 (7.9, 22.1)	20.8 (15.7, 31.3)	15.2 (11.6, 19.7)	0.007	9.4 (6.7, 13.2)	1.0 (1.0, 8.4)	0.004	<0.0001

Table 2 Univariate analysis, correlations between total ghrelin and other variables

	Obese PCOS, $n = 45$		Obese controls, $n = 33$		Non-obese PCOS, $n = 20$		Non-obese controls, $n = 21$		Total, $n = 119$	
Predictors	Correlation (r)	p-value	Correlation (r)	p-value	Correlation (r)	p-value	Correlation (r)	p-value	Correlation (r)	p-value
Total ghrelin × AMH	−0.40	0.007	−0.19	0.3	0.20	0.4	−0.22	0.3	−0.09	0.3
Total ghrelin × age	0.22	0.1	0.37	0.04	0.06	0.8	0.22	0.3	0.7	0.04
Total ghrelin × BMI	−0.04	0.8	−0.3	0.09	−0.5	0.02	0.26	0.3	−0.51	<0.0001
Total ghrelin × total T	0.07	0.6	−0.08	0.6	0.2	−0.3	−0.41	0.07	−0.22	0.01
Total ghrelin × insulin	−0.2	0.2	−0.3	0.09	−0.4	0.09	−0.36	0.1	−0.5	<0.0001

Table 3 Associations between Ghrelin and AMH, simple and multivariable regression models

	Association between Ghrelin and AMH unadjusted	Association in multivariable linear regression
Model with full cohort	β −0.02 95% CI −0.1, 0.07 $p = 0.7$	β −0.07 95% CI −0.16, 0.005 $p = 0.1$*
Model with obese PCOS only	β −0.15, 95% CI −0.24, −0.05 $p = 0.005$	β −0.14 95% CI −0.25, −0.03 $p = 0.008$**
Model with obese controls only	β −0.08 95% CI −0.23, 0.08 $p = 0.3$	–
Model with non-obese PCOS only	β 0.23 95% CI −0.18, 0.65 $p = 0.2$	–
Model with non-obese controls only	β −0.1 95% CI −0.28, 0.08 $p = 0.8$	–

*Adjusted for BMI, age, PCOS status, insulin, and total testosterone

**Adjusted for BMI, age, insulin, and total testosterone

secretion after repeated, but not single doses, of exogenous ghrelin [5, 6]. Ghrelin may also have direct effects on ovarian steroidogenesis and oocyte maturation through local expression of ghrelin and the GHS-R [7–11]. Our study focused on total ghrelin levels in women with PCOS as previous reports suggested that total ghrelin levels were lower in obese adolescents [31] and adults [16, 17, 19, 35, 36] with PCOS compared to controls.

PCOS is a complex disorder characterized by androgen excess, ovulatory dysfunction and polycystic appearing ovaries and has been associated with an increased risk of impaired glucose tolerance and impaired fertility [37]. AMH levels are elevated in women with PCOS [20, 21] and associated with increased preantral and antral ovarian follicles [38]. Many women with PCOS are overweight or obese, supporting the hypothesis that appetite dysregulation [18, 26] or impaired energy homeostasis [25] may play a role in this condition; however, not all women with PCOS experience excess adiposity. Although ghrelin plays a role in appetite and weight regulation, and lower fasting and post prandial levels of ghrelin are reported in PCOS, there is limited information on whether obesity might modulate the hormonal associations of ghrelin levels in women with PCOS. To our knowledge only one prior study has examined the relationship between AMH and total ghrelin levels in PCOS, and it reported that ghrelin was associated with AMH levels in women with obesity and PCOS [24] but did not assess this relationship in comparison to non-obese women with PCOS or controls. The only other study of correlates of ghrelin levels in non-obese PCOS did not assess AMH levels [39].

Taken as a group, obese women with PCOS appear to have differences in their energy homeostasis-reproduction axis compared to non-obese women with PCOS. Clearly, weight loss is not an acceptable therapy for women with PCOS who are already at a healthy weight, but weight loss is an efficacious therapy for restoring cyclicity in obese women [24]. Some studies have shown that weight loss may decrease AMH and increase ghrelin levels in women with or without PCOS [40–42]. However, obese women with PCOS may not be homogenous in this respect, and it is possible that some individual obese women with PCOS have hormonal profiles that mirror those of non-obese women. Perhaps the ovarian dysfunction in non-obese PCOS is more intrinsic, whereas the dysfunction in some women with obese PCOS is modifiable. This might explain why approximately 40% of obese women do not respond to weight loss with improved ovarian function [24]. It is unclear why only the subgroup of obese women with PCOS has an inverse relationship between ghrelin and AMH; additionally, the directionality of the relationship is still undetermined.

There is more evidence that might explain why we find low ghrelin levels in obese patients. Previous studies in women with PCOS suggest that low ghrelin levels contribute to obesity. Diminished ghrelin response to a meal may result in failure of the satiety signal and contribute to appetite dysregulation in women with PCOS [17, 43] and this blunting may be more pronounced in obese rather than non-obese PCOS [44]. These results suggest a plausible mechanism for how low baseline ghrelin levels and ghrelin dysregulation could contribute to obesity in patients predisposed to the obese PCOS phenotype. But it is unclear, then, why decreases in adiposity would increase ghrelin levels if low ghrelin is the driver of adiposity. A negative feedback loop might exist, and might explain why weight loss is often difficult to achieve in patients with PCOS.

This study is limited by the smaller sample size of the non-obese subgroups. However, the aim of this study was to describe trends in total ghrelin levels in obese and non-obese women with PCOS, to add to current understanding, and to inform future study design. A relationship between total ghrelin and AMH in obese PCOS, and a difference in this relationship between obese PCOS and non-obese PCOS are evident from this data. These results showed that further study of this area with larger sample sizes is warranted. The strengths of this study include use of the NIH criteria to define PCOS [28] and inclusion of non-obese women, and women without PCOS.

We report for the first time that total ghrelin levels are inversely associated with AMH levels in obese women with PCOS independent of total testosterone, insulin, and age.

Overall, these results indicate that obesity may be a significant modifier of the association between metabolic factors such as ghrelin and markers of ovarian function and reserve such as AMH in women with PCOS. This relationship appears to differ between obese and non-obese women with PCOS and may partially explain differing responses to weight loss therapy between individual patients.

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Compliance with ethical standards

Conflict of interest D.B.S. had consulting relationships with BaroNova and Enteromedics at the time the study was conducted. M.C.G. declares that she has no conflict of interest. S.F.B declares that she has no conflict of interest. K.C.A declares that she has no conflict of interest. S.S declares that she has no conflict of interest. A.D. declares that she has no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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