Imbalance Between Postprandial Ghrelin and Insulin Responses to an Ad Libitum Meal in Obese Women With Polycystic Ovary Syndrome

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Abstract

Obese women with polycystic ovary syndrome (PCOS) may have impairment in the regulation of food intake associated with ghrelin and insulin. In order to compare postprandial ghrelin and insulin responses to an ad libitum meal, we assessed 30 obese women with PCOS and 23 obese women without PCOS (control group). Blood samples were taken under fasting conditions, preprandially, and 15, 45, 75, and 135 minutes after the beginning of an ad libitum meal and ghrelin and insulin concentrations were analyzed. Insulin resistance (IR) was classified using basal insulin, quantitative insulin sensitivity check index, and homeostasis model assessment index. Mean ad libitum food intake was similar between the groups (468 ± 150 vs 444 ± 165 g, $P = .60$). The IR was found in 56.6% in PCOS group compared with 30.4% in the control group ($P < .01$). The postprandial ghrelin response was similar in both the groups but the insulin area under the curve (AUC) tend to be greater in the PCOS group (12807 ± 8149.4 vs 8654.4 ± 7232.3 $\mu\text{IU/mL/min}$; $P = .057$). The ghrelin AUC was negatively correlated with the insulin AUC ($r = -.5138$; $P = .01$) only in the control group. The imbalance in the feedback mechanisms between insulin and ghrelin, present in obese women, especially those with IR, may affect food intake throughout the day and that could be a mechanism for the development of obesity in PCOS.

Keywords

ghrelin, insulin resistance, obesity, polycystic ovary syndrome, appetite regulation

Introduction

Women with polycystic ovary syndrome (PCOS) have high rates of obesity and insulin resistance (IR) and are at risk of developing impaired glucose tolerance, diabetes mellitus type 2, metabolic syndrome, anxiety, and depression. Although PCOS is associated with obesity, it remains unclear whether PCOS predisposes to obesity and the mechanisms involved are unknown. Obesity has been found to worsen the clinical and biochemical manifestations of PCOS.¹

Although some studies have suggested that women with PCOS have abnormalities in energy expenditure, due to reductions in resting metabolic rate² and postprandial thermogenesis,³ these changes have not been detected in other studies.^{4,5}

The postprandial response of gastrointestinal hormones, part of the neural control of food intake, was shown to be lower in obese women with than without PCOS.⁶⁻⁸ Ghrelin, an orexigenic hormone, has been found to increase during fasting periods (preprandially) and to decrease after the ingestion of a meal, indicating that this molecule plays a short-term role in signaling the beginning of a meal and a long-term role in regulating energy balance.^{9,10}

The nutritional status of an individual is an important regulator of ghrelin secretion. Obese people have lower fasting ghrelin concentrations than eutrophic individuals^{11,12} and these concentrations seem to be lower in obese women with PCOS than without PCOS.^{6,7,13} Obese individuals also show lower postprandial suppression in response to meals rich in carbohydrates or proteins and lipids¹⁴ or in response to mixed meals.¹² Impaired postprandial suppression of ghrelin may result in a

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Figure 1. Study design.

reduced sensation of satiety, leading to greater food intake.^{12,14,15} Reduced postprandial suppression of ghrelin in response to a standard meal (a fixed amount of energy) has also been observed in obese women with than without PCOS,^{6,7} suggesting that the ghrelin-associated regulation of appetite may be even more impaired when obesity is associated with PCOS. These studies, however, used test meals with a fixed quantity of energy. To date, no studies have used meals ad libitum, which better reflect daily food intake.

We hypothesized that obese women with PCOS consume a greater amount of food ad libitum and have a lower postprandial suppression of ghrelin in response to this ad libitum meal than do obese women without PCOS and that this may represent a mechanism for the development of obesity in PCOS. We therefore compared the postprandial ghrelin and insulin responses to ad libitum food intake in obese women with and without PCOS and we analyzed the interrelationship of ghrelin with insulin and the role of these hormones in ad libitum food intake.

Materials and Methods

This prospective clinical study involved 53 women aged 20 to 45 years with obesity (body mass index [BMI], 30–40 kg/m²); of these, 30 had been diagnosed with PCOS according to the Rotterdam criteria¹⁶ and 23 were non-PCOS controls. Women were excluded if they were receiving treatment with oral hypoglycemic agents, contraceptives and/or hormones, or anti-psychotic drugs (washout of 3 months); had been diagnosed with diabetes mellitus, a gastrointestinal disease, or an eating disorder; did not like the foods used in the study (bread, margarine, coffee, sugar, pasta, tomato sauce, onion, and ground meat); were current smokers; had undergone nutritional monitoring during the previous 3 months; were pregnant or nursing; were in a climacteric period; or had thyroid dysfunction, infectious-contagious diseases, or other causes of hyper-androgenism such as Cushing syndrome or adrenal hyperplasia.

Patients were selected from women seen at the Gynecologic Endocrinology Outpatient Clinic of the University Hospital, Ribeirão Preto Medical School, University of São Paulo (HCFMRP-USP). Data from eumenorrheic women were collected outside the 10 days preceding the menstrual period, due to evidence that there is an increase in food intake during this period,¹⁷ whereas data were collected from amenorrheic women on any day of the month.

The study was approved by the Research Ethics Committee of HCFMRP-USP. The patients received 2 consent forms for signature, one before and the other after the study. Only after the study were the women informed that ad libitum food intake would be quantified, thus preventing them from being influenced by this information regarding the amount of food to be consumed. Figure 1 shows the study design.

Sample Characterization

Anthropometric and body composition data. Weight and height were measured to calculate BMI. Body composition was determined by bioelectric impedance (Biodynamics BIA 450 Bioimpedance Analyzer, Biodynamics Corporation Shoreline, WA). Fat-free mass (FFM) was calculated using a specific predictive equation for obese women¹⁸ and fat mass was calculated as the difference between weight and FFM.

Biochemical Analyses

Blood was collected into tubes containing sodium fluoride and EDTA for the analysis of plasma glucose concentrations and into tubes containing EDTA K2 for the analysis of plasma ghrelin concentrations; the latter samples were immediately stored on ice in a Styrofoam container (Dimensions: 19.4 cm × 10.5 cm × 14.8 cm [Length × Width × Height]; Thickness: 1.8 cm; Capacity: 3 liters). Blood was collected into tubes containing a clot activator and a separating gel for the analysis of

serum insulin, testosterone, and sex hormone-binding globulin (SHBG) concentrations. All blood samples were centrifuged at 2000 rpm for 15 minutes within 90 minutes after collection and serum and plasma samples were stored at -20°C .

Testosterone and SHBG concentrations were measured in samples taken at 08:00 hours after an overnight fast. Concentrations of ghrelin, insulin, and glucose were measured in samples taken under fasting conditions, preprandially at 12:00 before the ad libitum meal, and postprandially 15, 45, 75, and 135 minutes after the beginning of the ad libitum meal.

Glucose was analyzed by a final point photometric method (Glucose PAP Liquiform, Labtest Diagnóstica SA, Minas Gerais), insulin by chemiluminescence (Immulin 2000 kit; Siemens Healthcare Diagnostics Ltd, United Kingdom), and total ghrelin (Ghrelin - Total - RIA kit, Millipore, Missouri), testosterone (Diasource Testo-RIA-CT Kit, Diasource ImmunoAssays SA, Nivelles, Belgium), and SHBG (IRMAZENco SHBG, ZenTech SA, Angleur, Belgium) by radioimmunoassay. Insulin resistance was based on 3 criteria: a quantitative insulin sensitivity check index (QUICKI) ≤ 0.33 , a homeostasis model assessment (HOMA) index ≥ 2.6 , and a basal insulin concentration ≥ 12 mU/L.¹⁹ The ghrelin–insulin ratio was calculated at each time point. Maximum ghrelin suppression was defined as the greatest postprandial decline, compared with the basal (preprandial) period, in ghrelin concentration.

Ad Libitum Food Intake

Each patient consumed a snack, containing 286 kcal and 73% carbohydrate at 09:00 or 3 hours before the ad libitum meal in order to standardize the last meal before the ad libitum intake. The ad libitum meal consisted of pasta bolognese, a dish typical of the Southeast region of Brazil, with which each patient would be familiar. Moreover, pasta is a good source of carbohydrate, the main nutrient that stimulates ghrelin suppression.²⁰ The preparation of the noodles and sauce was standardized and rigorously applied on each day of administration. Each ad libitum meal consisted of 1600 g (2100 kcal) and comprised 58.8% carbohydrates, 22.1% lipids, and 17.8% proteins. Each individual ate alone ad libitum in a kitchen that simulated a domestic environment, with no time restriction. At the end of the meal, the patient completed a questionnaire, consisting of 100-mm visual analog scales (VAS), to assess familiarity (“How much do you like pasta with sauce?”) and palatability (“How good is this pasta?”). The quantity of pasta consumed was evaluated by the difference between the starting amount and the leftovers on the pan and the plate.

Statistical Analysis

Demographic and anthropometric characteristics, ad libitum food intake, and the areas under the curve for ghrelin, insulin, and glucose were compared between the PCOS and the non-PCOS groups by linear regression models. For ad libitum food intake, the model was adjusted for BMI. A mixed-effect model was used to compare the concentrations of ghrelin, insulin, and

Table 1. Baseline Characteristics of the Study Patients.^a

	PCOS (n = 30)	Control (n = 23)	P Value
Sociodemographic data			
Age, years	29.1 $\pm$ 5.8	33.2 $\pm$ 5.4	.01
Schooling, years	10.3 $\pm$ 2.8	10.4 $\pm$ 3.4	.80
Anthropometry and body composition			
Weight, kg	92.1 $\pm$ 12.0	91.7 $\pm$ 10.7	.94
Height, m	1.61 $\pm$ 0.08	1.63 $\pm$ 0.07	.44
BMI, kg/m ²	35.3 $\pm$ 2.7	34.6 $\pm$ 2.8	.32
Fat-free mass, kg	51.3 $\pm$ 5.9	51.3 $\pm$ 4.9	.98
Fat-free mass, %	55.8 $\pm$ 1.6	56.1 $\pm$ 1.9	.68
Fat mass, kg	40.8 $\pm$ 6.3	40.4 $\pm$ 6.1	.86
Fat mass, %	44.1 $\pm$ 1.6	43.9 $\pm$ 1.9	.65
Fasting biochemical analyses			
Glucose, mg/dL	88.6 $\pm$ 9.7	90.3 $\pm$ 12.2	.73
Insulin, $\mu\text{U/mL}$	16.6 $\pm$ 12.2	12.6 $\pm$ 11.0	.58
Ghrelin, pg/mL	542.9 $\pm$ 142.6	600.3 $\pm$ 224.6	.91
Total testosterone, ng/mL	0.57 $\pm$ 0.29	0.34 $\pm$ 0.21	<.01
SHBG, nmol/L	19.8 $\pm$ 11.0	24.2 $\pm$ 11.0	.09
HOMA-IR	3.69 $\pm$ 2.77	2.95 $\pm$ 2.80	.32
QUICKI	0.34 $\pm$ 0.05	0.35 $\pm$ 0.05	.25

Abbreviations: BMI, body mass index; HOMA-IR, homeostasis model assessment insulin resistance; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin sensitivity check index; SD, standard deviation; SHBG, sex hormone-binding globulin.

^aData reported as mean $\pm$ SD.

glucose between the 2 groups at different time points and between time points within each group. The IR frequencies in the 2 groups were compared using the chi-square test, with correlations determined by Spearman correlation coefficient. All statistical analyses were performed using SAS/STAT® Version 9 (SAS Institute Inc., Cary, NC, 2004), and the level of significance was set at 95% ($P < .05$).

Results

Patient Characteristics

The baseline characteristics of the 2 groups did not differ significantly, except for testosterone concentrations and age (Table 1). Fasting ghrelin and insulin concentrations were similar in the groups of obese women with and without PCOS.

Ghrelin was positively correlated with age ($r = .48$; $P = .02$) and negatively correlated with BMI ($r = -.48$; $P = .02$) in the control group but not in the PCOS group (ghrelin vs age: $r = -.10$; $P = .59$; ghrelin vs BMI: $r = .01$; $P = .95$). Neither the PCOS ($r = .12$; $P = .50$) nor the control ($r = -.15$; $P = .48$) group showed a significant correlation between ghrelin and testosterone.

Ad Libitum Food Intake

Obese women with PCOS consumed, on average, 468 ± 150 g (615 ± 197 kcal) of pasta in the ad libitum meal, similar to the amount consumed by obese women without PCOS, 444 ± 165 g

Figure 2. Postprandial (A) ghrelin, (B) insulin, and (C) glucose responses after an ad libitum meal, relative to baseline, in the polycystic ovary syndrome (PCOS) and control groups.

(583 ± 217 kcal; $P = .60$). Mean intake time was 9 ± 3 minutes for the PCOS group and 8 ± 2 minutes for the control group ($P = .17$).

The VAS measurements of palatability (85 ± 23 vs 83 ± 23 mm; $P = .28$) and familiarity (88 ± 20 vs 76 ± 25 mm; $P = .25$) were also similar in the PCOS and the control groups.

Hormonal Responses (Ghrelin and Insulin)

We observed small increases in plasma ghrelin concentrations in both groups, relative to preprandial levels, 15 minutes after the start of the ad libitum meal, with subsequent decreases started within 30 minutes after the end of the meal (Figure 2). There were no between-group differences in the absolute ghrelin concentrations at all time points ($P = .91$) or in the area under the ghrelin curve between the pre- and the postprandial periods ($P = .33$). The between-group differences at each time point were also not statistically significant (Figure 2).

Neither the PCOS ($r = .19$; $P = .32$) nor the control ($r = -.23$; $P = .29$) group showed a significant correlation between preprandial ghrelin concentration and ad libitum food intake nor was ad libitum food intake significantly correlated with maximum ghrelin suppression in either the PCOS ($r = -.25$; $P = .18$) or the control ($r = -.07$; $P = .75$) group.

Although the curve for the insulin response to the ad libitum meal was greater for the PCOS than for the control group in

absolute values (12807 ± 8149.4 vs 8654.4 ± 7232.3 μ U/mL/min), this difference was not significant ($P = .057$). The area under the glucose curve also did not differ in the 2 groups ($P = .18$).

Concentrations of insulin increased by about 50% postprandially, with the greatest increase occurring within 30 minutes after the end of the meal, decreasing between 45 and 75 minutes (Figure 2).

The correlations between preprandial insulin and ad libitum intake were not significant in either the PCOS ($r = .19$; $P = .32$) or the control ($r = .07$; $P = .75$) group. However, the change in insulin from 0 to 15 minutes was positively and significantly correlated with ad libitum intake in the control group ($r = .56$, $P < .01$) but not in the PCOS group ($r = .17$; $P = .36$). The area under the curve (AUC) for insulin was positively and significantly correlated with ad libitum food intake in both the PCOS ($r = .4982$; $P = .005$) and the control ($r = .4439$; $P = .03$) groups.

The preprandial ghrelin–insulin ratio was significantly lower ($P = .04$) in the PCOS group (median: 28.3 [minimum: 4.0 – maximal: 247.4]) than in the control group [median: 43.4 [minimum: 7.7 – maximal: 498.7]], but this variable was not significantly correlated with ad libitum food intake in either the PCOS ($r = -.017$; $P = .37$) or the control ($r = -.11$; $P = .61$) group. That is, neither the preprandial concentrations of ghrelin and insulin nor the ghrelin–insulin ratio influenced ad libitum food intake.

There was a negative and significant correlation between ghrelin and insulin concentrations in both the PCOS ($r = -.3110$; $P < .0001$) and the control ($r = -.4512$; $P < .0001$) groups. However, a negative correlation between fasting ghrelin and HOMA-IR ($r = -.6787$; $P = .0004$) and a positive correlation between fasting ghrelin and QUICKI ($r = .6683$; $P = .0005$) were only seen in the control group. Similarly, the AUC for ghrelin was negatively correlated with the areas under the curve for insulin ($r = -.5138$; $P = .01$) and glucose ($r = -.4437$; $P = .034$) only in the control group. Percentage of IR was 56.6 in the PCOS group and 30.4 in the control group ($P < .01$).

When the women were divided into 2 groups according to IR independently of PCOS, the area under the ghrelin curve was smaller in the group with than without IR ($P < .01$), and the correlation between insulin and ghrelin was negative and significant in the group without ($r = -.3404$; $P < .0001$), but not with ($r = -.1535$; $P = .066$), IR.

Discussion

Based on the studies showing that obese women with PCOS had lower fasting ghrelin concentrations^{6,7,13} and impaired postprandial responses to a test meal compared with obese women without PCOS,^{6,7} we hypothesized that obese women with PCOS would have greater food intake and lower postprandial ghrelin suppression in response to an ad libitum meal than obese women without PCOS. This hypothesis was not confirmed in the present study because the ad libitum food intake and the postprandial ghrelin response were similar in obese women with PCOS and those without PCOS as were fasting and preprandial ghrelin concentrations. This finding is consistent with those of 2 previous studies, which found no differences in fasting ghrelin concentration between women with and without PCOS although these studies did not evaluate only obese women.^{21,22}

Exogenous ghrelin infusion has been reported to increase food intake in both eutrophic and obese patients,^{10,15} indicating that this hormone is involved in the quantity of food consumed during a meal. Thus, our finding that ad libitum food intake was similar in our PCOS and control groups may be associated with the similar preprandial ghrelin concentrations in the 2 groups. However, preprandial ghrelin concentration was not significantly correlated with ad libitum food intake in either group. This is consistent with findings showing that an increase in preprandial ghrelin concentration was not a determinant of the energy content consumed during a meal, suggesting that absolute ghrelin concentration may not be primarily responsible for meal size.²³

A previous study suggested that impaired postprandial suppression of ghrelin in women with PCOS may be responsible for greater food intake.⁶ Both groups showed an 8% increase in ghrelin concentration during the ad libitum meal (ie, between 0 and 15 minutes), with suppression starting to occur during the first half hour after the end of the meal, amounting to 9% decrease in each group. Similarly, obese patients showed an increase in ghrelin during a meal (between 0 and 15 minutes) whereas eutrophic patients showed a

decrease.¹² Moreover, eutrophic individuals receiving a simulated meal (in which they smelled, chewed, and tasted the food but did not swallow it) showed an immediate ghrelin suppression in response to the cephalic phase of feeding.²⁴ These findings suggest that, in contrast to its effect in eutrophic individuals, ghrelin does not play a primary role in satiation in obese patients because it is not suppressed during a meal.

Reduced postprandial suppression of ghrelin may impair satiety, thereby increasing food intake by obese individuals during a subsequent meal.¹⁵ Moreover, plasma ghrelin response to test meals in eutrophic individuals was not a determinant of time for spontaneous requisition or of the energy content of a subsequent meal, suggesting that a postprandial decrease in ghrelin concentration may be more important for the “not eating” process that a preprandial increase to initiate a subsequent meal.²³

Although energy load and ghrelin suppression are related to eutrophic patients,²³ our findings suggest that this relationship does not occur in obese individuals. We found that the quantity of the ad libitum meal ingested by the obese women in this study was not significantly correlated with maximum ghrelin suppression, a finding consistent with that reported previously.²⁵

There are still gaps in our understanding of the role of ghrelin as a mediator of appetite and food intake, especially in obese individuals. Further studies are needed to assess the effects of preprandial ghrelin concentrations on energy intake during a meal and the effects of energy intake on the postprandial suppression of ghrelin.

Insulin and glucose are considered dynamic modulators of the ghrelin response because hyperinsulinemia and hyperglycemia tend to reduce circulating ghrelin concentrations.²⁶ Studies in humans have shown that ghrelin can be suppressed, at least in part, by intravenous infusion or oral administration of glucose^{27,28} and other studies have shown an inverse relationship between insulin and ghrelin concentrations.^{11,29} Insulin was found to suppress ghrelin concentration in hypoglycemic individuals, showing that ghrelin suppression can occur independently of glucose.³⁰ A synergistic effect of glucose and insulin may occur in euglycemic and hyperglycemic individuals. Furthermore, since ghrelin remains suppressed even after the reestablishment of preprandial concentrations of insulin and glucose,²⁰ the regulation of ghrelin may be influenced by glucose and insulin, although other factors may interfere with this response.³¹

Lower ghrelin concentrations in obese individuals may be due to hyperinsulinemia,^{9,11} since IR was negatively correlated with plasma ghrelin concentrations.³² The abnormal regulation of ghrelin in women with PCOS may be the consequence of IR,⁶ and hyperinsulinemia has been reported to reduce ghrelin concentrations, regardless of the degree of IR.¹³ The ability of insulin to suppress ghrelin may therefore be altered by IR.⁷

Insulin resistance may therefore be the key point of imbalance between ghrelin and insulin. We found that the negative correlation between insulin and ghrelin was absent in patients with IR but present in women without IR, regardless of the presence or absence of PCOS. Thus, prolonged exposure to

IR, common in obese individuals, may affect the sensitivity of gastric cells to the transmembrane flow of glucose and/or to signaling by insulin, impairing the postprandial suppression of ghrelin.³³

The high prevalence of IR among women with PCOS may explain the reported differences in ghrelin concentrations compared to women without PCOS.^{6,7,13} The presence of PCOS in obese women increases the prevalence of IR.³⁴ We found that IR was present in 56.6% of the PCOS group, but in only 30.4% of the control group ($P < .01$), which may explain the between-group difference in ghrelin–insulin ratio. The higher mean concentrations of insulin in the PCOS group were certainly due to their higher prevalence of IR. This finding was corroborated by the results obtained when the women were divided into groups with and without IR, regardless of PCOS, in that the AUC for ghrelin was smaller in the IR than in the non-IR group ($P < .01$).

Thus, the presence of IR leads to an increase in insulin concentrations, along with a reduction in ghrelin concentrations, due to the impairment of the control mechanisms exerted by insulin on ghrelin-producing cells in the digestive tract.³³ Furthermore, in obese patients, while plasma insulin levels are elevated, these concentrations seem to be reduced in the brain. This disorder may be associated with the fact that insulin crosses the blood–brain barrier via active transport to reach the hypothalamus, and this mechanism is saturable. In patients with hyperinsulinemia, such as obese individuals, and especially obese women with PCOS, the blood–brain barrier gradually becomes resistant to the penetration of insulin, which can result in decreased satiety signals mediated by insulin.³⁵

The imbalance in the feedback mechanisms between insulin and ghrelin, present in obese women, especially those with IR, may affect food intake throughout the day. However, further studies are needed to better clarify the complex control of food intake by mechanisms involving interactions between insulin and ghrelin. The sample size could be considered a limitation in this study since some analysis showed no conclusive results but rather borderline P values.

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