Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome


ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a common condition associated with obesity and with reproductive and metabolic dysfunction. Abnormalities in appetite regulation in PCOS patients may contribute to difficulties in weight management.

Objective: We aimed to examine appetite, appetite hormones, and ad libitum food consumption before and after weight loss in overweight women with and without PCOS.

Design: Overweight age- and weight-matched women with (n = 14) and without (n = 14) PCOS undertook an 8-wk energy-restricted diet (5185.3 ± 141.6 kJ/d). At baseline and study end, subjects consumed a test meal (936 kJ; 25% of energy from protein, 9% from fat, and 67% from carbohydrate). Subjective appetite and circulating glucose, insulin, ghrelin, cholecystokinin, and peptide YY were assessed at 0, 15, 30, 45, 60, 90, 120, and 180 min. A mixed buffet lunch was then offered to assess ad libitum food intake.

Results: Weight loss (4.2 ± 3.9 kg) did not differ significantly between the 2 groups. Women with PCOS had significantly (P = 0.023) lower ghrelin concentrations before and after weight loss than did women without PCOS. The degree of postprandial ghrelin suppression was lower at weeks 0 (P = 0.048) and 8 (P = 0.069) in women with PCOS than in women without PCOS. There were no significant differences between the 2 groups in appetite responses, buffet consumption, or fasting or postprandial peptide YY and cholecystokinin before or after weight loss.

Conclusions: PCOS was associated with lower fasting ghrelin and a smaller postprandial ghrelin suppression both before and after weight loss but was not associated with other postprandial gut peptides, subjective satiety, or food intake. It is not clear whether appetite regulation is impaired in PCOS.

KEY WORDS Appetite, obesity, polycystic ovary syndrome, weight loss, ghrelin, cholecystokinin, peptide YY

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine condition in women of reproductive age; it is associated with menstrual dysfunction, hyperandrogenism, a greater risk of developing type 2 diabetes, and an adverse cardiovascular disease risk profile. Insulin resistance (IR) is implicated in its development through the insulin stimulation of ovarian androgen production and the reduction in hepatic synthesis of sex hormone–binding globulin (SHBG) (1). Obesity—in particular, central obesity—is present in 10–65% of Western women with PCOS (2), and its presence worsens the associated IR and metabolic and endocrine features; weight loss reduces abdominal fat and IR and improves menstrual function (3).

We previously showed that overweight women with PCOS have lower postprandial satiety and higher postprandial hunger before and after weight loss than do weight-matched controls (4). There is some evidence that disturbances in appetite regulation in PCOS could account for these reported discrepancies in hunger and satiety. Ghrelin is proposed as an acute meal initiator; ghrelin concentrations increase preprandially and decrease postprandially (5), and ghrelin administration stimulates hunger and food intake (6). The reduction in postprandial ghrelin was less in obese than in lean subjects (7–9) and in overweight PCOS patients than in weight-matched persons without PCOS (4). Cholecystokinin is released from the small intestine postprandially, primarily in response to duodenal protein and fat, and it inhibits gastric emptying and reduces meal size and calorie intake in humans (10). Postprandial cholecystokinin has been reported either to be greater in obese than in lean subjects or to be unaffected by body weight status (11, 12). In overweight women with PCOS, a lower postprandial cholecystokinin response than in weight-matched controls was observed, which further supports impaired appetite regulation in PCOS (13). Peptide YY (PYY), a peptide that is

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synthesized in the gastrointestinal tract, increases postprandially, increases satiety, and reduces food intake. It has additional functions, including inhibition or reduction of gallbladder secretion, gut motility and pancreatic secretion (14, 15). Reductions in total fasting PYY or impaired postprandial PYY (15, 16) or no changes in fasting PYY (17, 18) have been observed in overweight subjects, and it is not known whether PYY is differentially regulated in PCOS.

Although fasting ghrelin concentrations rise and the postprandial ghrelin response improves with weight loss, these changes may be impaired in women with PCOS (4). Fasting PYY increases (19) or decreases (17) after diet-induced weight loss, and fasting or postprandial cholecystokinin is unchanged by weight increases (19) or decreases (17) after diet-induced weight loss, and may be impaired in women with PCOS (4). Fasting PYY in-dial ghrelin response improves with weight loss, these changes in fasting PYY (17, 18) have been observed in overweight subjects, and it is not known whether PYY is differentially regulated in PCOS.

Although fasting ghrelin concentrations rise and the postprandial ghrelin response improves with weight loss, these changes may be impaired in women with PCOS (4). Fasting PYY increases (19) or decreases (17) after diet-induced weight loss, and fasting or postprandial cholecystokinin is unchanged by weight loss (20). The effect of weight loss on cholecystokinin and PYY has not yet been examined in persons with PCOS. The aim of this study was therefore to examine fasting and postprandial subjective appetite, appetite hormones (ie, ghrelin, PYY, and cholecystokinin), and ad libitum buffet meal consumption before and after weight loss in overweight women with and without PCOS.

SUBJECTS AND METHODS

Subjects and recruitment

Overweight age- and weight-matched women with (n = 14) and without (n = 14) PCOS (age: 32.3 ± 5.9 and 36.2 ± 4.5 y, respectively; weight: 94.5 ± 19.8 and 94.9 ± 15.4 kg, respectively) were recruited through public advertisement. PCOS was diagnosed according to the Rotterdam consensus group as previously described (21, 22). Exclusion criteria were pregnancy, breastfeeding, body mass index (BMI; in kg/m²) < 25, type 2 diabetes mellitus, and the use of oral contraceptives, endocrine hormonal treatment, or insulin-sensitizing agents (subjects were required to cease oral contraceptives 4 wk and hormonal treatment or insulin-sensitizing agents 2 wk before commencement of the study). The PCOS patients and control subjects were matched for age, BMI, and smoking status.

All subjects gave written informed consent. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The study was approved by the human ethics committee of the Commonwealth Scientific and Industrial Research Organisation Division of Human Nutrition at The Royal Adelaide Hospital and of the Women’s and Children’s Hospital of South Australia.

Study design and dietary treatment

The study was conducted on an outpatient basis over a span of 8 wk. Subjects followed an energy-restricted diet in which 2 meals/d were replaced with commercially available meal replacements (Slimfast; Unilever Australasia, Epping, Australia), a supply of which was provided every 2 wk (21). The aim of the dietary intervention was to induce an energy deficit of ≈30% through daily consumption of 2 meal replacements (1800 kJ), a low-fat evening meal, and ≥5 servings of fruit and vegetables (3500 kJ). The dietary composition of the intervention is described in Table 1. Nutrient intakes were calculated with the use of DIET 4/NUTRIENT CALCULATION software (version 4; Xyris Software, Highgate Hill, Australia) by using data from Australian food composition tables. Nutritional intake was assessed from 3-d consecutive dietary food records (1 weekday and 2 weekend days) collected every 2 wk and daily dietary check-lists. Dietary compliance was determined by subject adherence to the meal replacement regimen. Subjects attended the clinic every 2 wk and were weighed while wearing light clothes but no shoes (model AMZ14 Mettler scales; A&D Mercury, Kinomoto, Japan). At weeks 0 and 8, waist circumference and total fat mass and total fat-free mass (determined by bioelectrical impedance

| TABLE 1 | Dietary intake data for subjects at baseline and during the study intervention1 |
|-----------------|-----------------|-----------------|-----------------|
|                | PCOS patients   | Control subjects |                |
|                | (n = 13)        | (n = 13)         | (n = 13)        |
| Energy (MJ)    | 7.3 ± 0.9       | 5.2 ± 0.2        | 8.1 ± 0.9       |
| Protein        | 93.6 ± 10.8     | 75.8 ± 3.1       | 101.5 ± 11.2    |
| (% of total energy) | 21.0 ± 0.7      | 23.3 ± 0.5       | 20.1 ± 0.8      |
| Carbohydrate   | 176.0 ± 22.0    | 159.6 ± 6.0      | 200.7 ± 21.1    |
| (% of total energy) | 40.8 ± 1.2      | 52.2 ± 1.2       | 42.7 ± 1.9      |
| Fat            | 73.6 ± 9.4      | 32.3 ± 42.4      | 82.4 ± 10.9     |
| (% of total energy) | 37.4 ± 1.2      | 22.8 ± 1.4       | 36.8 ± 1.5      |
| SFA (% of total energy) | 15.3 ± 0.5      | 12.5 ± 0.9       | 15.2 ± 0.9      |
| MUFA (% of total energy) | 13.3 ± 0.5      | 10.9 ± 1.0       | 13.0 ± 0.5      |
| PUFA (% of total energy) | 5.3 ± 0.5       | 6.4 ± 0.6        | 5.2 ± 0.4       |
| Fiber          | 17.3 ± 2.4      | 20.0 ± 1.1       | 21.4 ± 2.1      |
| Cholesterol    | 319.9 ± 33.0    | 147.6 ± 9.5      | 318.6 ± 48.4    |

1 All values are ± SEM. PCOS, polycystic ovary syndrome; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Measurements were made at the week 0 visit (food-frequency questionnaire) or during the study (3-d food records) and were assessed by using a one-factor ANOVA with PCOS status as the fixed factor.

2 Assessed with a food-frequency questionnaire.

3 Assessed with 3-d food records.
Insulin sensitivity

weight change of

weighed themselves daily to ensure weight stability, defined as a

(24). For the 2 wk before study commencement, subjects

assessed at weeks 0 and 8 by using a 7-d physical activity record

previous 6 mo) was assessed with the use of a food-frequency

questionnaire from the Anti-Cancer Foundation. Exercise was

assessed at weeks 0 and 8 by using a 7-d physical activity record

(24). For the 2 wk before study commencement, subjects

weighed themselves daily to ensure weight stability, defined as a

weight change of ≤2% of initial body weight.

At weeks 0 and 8, subjects underwent a meal tolerance test

(MTT). All subjects consumed the same meal the evening before

the test (3820 kJ; 20% of energy from protein, 17% from fat, and

62% from carbohydrate) and refrained from consuming alcohol

for 24 h. A cannula was inserted into a forearm vein, and an

overnight fasting venous blood sample was taken between 0800

and 1000 for measurement of plasma glucose and insulin con-

centrations and serum ghrelin, cholecystokinin, and PYY con-

centrations. Subjects then completed a validated visual analogue

scale (VAS) questionnaire to assess subjective hunger as previ-

ously described (25). The change in ratings from baseline was

quantified. Subjects consumed a liquid preload of Slimfast [325

mL, 936 kJ, 12 g protein (25% of energy from protein), 2 g fat

(9% of energy from fat), and 35 g carbohydrate (67% of energy

from carbohydrate)] within 5 min; additional blood samples were

taken and VAS questionnaires were completed at 15, 30, 45, 60,

90, 120, and 180 min after meal consumption. At 180 min, sub-

jects were given a mixed buffet-style lunch (12.1 MJ; 15% of

energy from fat), and 35 g carbohydrate (67% of energy

from carbohydrate) 5% of energy from fat, and 41% from carbohydrate); each subject served his or her own meal from designated portions

of the foods and ate until satisfied over a 30-min period. Each

food was weighed to the nearest gram with the use of digital

scales before and after eating. Total (glucose, insulin, cholecys-

tokinin, and PYY), incremental (ghrelin), and net (VAS) areas

under the curve (AUCs) during the 3-h MTT were calculated

geometrically by using the trapezoidal rule (26).

Biochemical measurements

Blood for measurement of serum concentrations was collected

in tubes with no additives and allowed to clot at room temperature

for 30 min. Blood for measurement of plasma concentrations was

collected in tubes containing sodium fluoride and EDTA (glu-

cose) or potassium and EDTA and aprotinin (aprotinin concen-

tration: 500 KIU/mL blood; Roche Diagnostics, Indianapolis,

IN) (ghrelin, cholecystokinin, and PYY) and stored on ice. Se-

rum and plasma were stored at −80 °C. Serum SHBG and total

testosterone (bound and unbound) (3), total ghrelin (4), insulin

and glucose (21), cholecystokinin (11), and total PYY (1–36 and

3–36) (16) were measured as described previously. The homeo-

static model assessment (HOMA) was used as a surrogate mea-

sure from which to calculate insulin sensitivity according to the

following equation (27):

\[
\text{Insulin sensitivity} = \frac{[\text{fasting insulin (mU/L)}]}{[\text{fasting glucose (mmol/L)}]/22.5}
\]  

The free androgen index (testosterone/SHBG × 100) and equilibrium-binding equations were used for estimation of free

testosterone (28). Biochemical assays were performed in a single

assay at the completion of the study, and all samples for individ-

uals were analyzed in the same assay.

Statistical analysis

Parametric data were presented as means ± SEMs except where

specified. Nonparametric data (TFEQ) were presented as median, minimum, and maximum. When data were nonnormally

distributed, data were log transformed for analysis. Results are

presented for 28 subjects (n = 14 for both the PCOS patients and

control subjects); for postprandial glucose, insulin, ghrelin, PYY,

and cholecystokinin, the numbers differed slightly (n = 14 and

13 in the PCOS patients and control subjects, respectively)

because of incomplete data. Two-tailed statistical analysis was

performed with the use of SPSS for WINDOWS software (ver-

sion 14.0; SPSS Inc, Chicago, IL) with statistical significance set

at an α level of P < 0.05. Baseline data were assessed by using

a one-factor analysis of variance for parametric data and a

Kruskal-Wallis test for nonparametric data (TFEQ). Compari-

sons between time points were assessed by using a general linear

model repeated-measures analysis of variance with PCOS diag-

nosis as the between-subject factor. In the event of an interaction,

Bonferroni post hoc pairwise comparisons were performed. Re-

lations between variables were examined by using bivariate and

partial correlations and analysis of covariance after adjustment

for weight, fasting and postprandial insulin, and total energy

intake. This study had 80% power to detect a preweight-loss

difference of 75.9 pmol/L in fasting ghrelin and a change of 31.3

pmol/L in fasting ghrelin during weight loss between subjects

with and without PCOS (P < 0.05 for both) (4).

RESULTS

Subjects, physical activity, dietary intake, weight loss, body composition, and reproductive hormones

Baseline characteristic of the subjects are shown in Table 1.

For the TFEQ data, there were no differences in the baseline

scores for dietary restraint (median: 9.5; range: 3–16), disini-

bition (10; 3–16), or hunger (7; 0–14) between the PCOS patients

and the control subjects. Activity levels did not differ signifi-

cantly between the 2 groups at week 0 and did not change

throughout the study. There were no significant differences in

energy or macronutrient intake between the 2 groups at baseline

or during the study intervention (Table 2). Decreases in weight

(± SEM: 4.2 ± 3.9 kg or 4.3 ± 3.8%), waist circumference,

total fat mass, total fat-free mass, free testosterone, free androgen

index, and testosterone and increases in SHBG occurred in all

subjects with no differential effect of PCOS status (Table 2).

Insulin and glucose homeostasis

There was no effect of PCOS status on changes in fasting

glucose and no change in fasting glucose over the study duration.

There was an interaction between postprandial glucose and

PCOS status (P = 0.017) so that the postprandial glucose re-

sponse decreased significantly (P = 0.043) only in the PCOS

patients, and no changes in postprandial glucose occurred with

weight loss in the control subjects (Figure 1). Decreases in

HOMA (13.6%; P = 0.010), fasting insulin (15.9%; P = 0.004),

AUC insulin (20.0 ± 3.9%; P < 0.001), and postprandial insulin

(P < 0.001) did not differ significantly between the 2 groups.

There was no difference between the 2 groups in the magnitude
<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 8</th>
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</thead>
<tbody>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS patients</td>
<td>94.5 ± 5.3</td>
<td>90.6 ± 4.8</td>
</tr>
<tr>
<td>Control subjects</td>
<td>94.9 ± 4.1</td>
<td>90.2 ± 3.9</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PCOS patients</td>
<td>35.3 ± 1.5</td>
<td>34.8 ± 1.5</td>
</tr>
<tr>
<td>Control subjects</td>
<td>35.3 ± 1.3</td>
<td>34.4 ± 1.7</td>
</tr>
<tr>
<td><strong>Waist circumference (cm)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PCOS patients</td>
<td>113.9 ± 4.3</td>
<td>106.9 ± 4.4</td>
</tr>
<tr>
<td>Control subjects</td>
<td>111.0 ± 3.1</td>
<td>103.9 ± 3.1</td>
</tr>
<tr>
<td><strong>Total fat-free mass (kg)</strong></td>
<td></td>
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<tr>
<td>PCOS patients</td>
<td>59.5 ± 3.4</td>
<td>58.3 ± 3.2</td>
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<tr>
<td>Control subjects</td>
<td>61.1 ± 2.5</td>
<td>58.9 ± 2.3</td>
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<tr>
<td><strong>Total fat mass (kg)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>34.5 ± 2.5</td>
<td>31.8 ± 2.1</td>
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<tr>
<td>Control subjects</td>
<td>34.5 ± 2.0</td>
<td>31.3 ± 1.8</td>
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<tr>
<td><strong>Fasting glucose (mmol/L)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCOS patients</td>
<td>5.3 ± 0.2</td>
<td>5.3 ± 0.1</td>
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<tr>
<td>Control subjects</td>
<td>5.1 ± 0.2</td>
<td>5.1 ± 0.2</td>
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<tr>
<td><strong>Fasting insulin (mU/L)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>22.4 ± 3.8</td>
<td>18.3 ± 3.8</td>
</tr>
<tr>
<td>Control subjects</td>
<td>11.8 ± 1.8</td>
<td>8.5 ± 1.5</td>
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<tr>
<td><strong>HOMA</strong></td>
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<tr>
<td>PCOS patients</td>
<td>5.5 ± 1.0</td>
<td>4.4 ± 0.9</td>
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<tr>
<td>Control subjects</td>
<td>2.8 ± 0.5</td>
<td>2.0 ± 0.4</td>
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<tr>
<td><strong>Testosterone (nmol/L)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>3.2 ± 0.3</td>
<td>2.7 ± 0.2</td>
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<tr>
<td>Control subjects</td>
<td>2.1 ± 0.1</td>
<td>1.8 ± 0.1</td>
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<td><strong>SHBG (nmol/L)</strong></td>
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<td>PCOS patients</td>
<td>20.5 ± 2.9</td>
<td>21.8 ± 3.0</td>
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<tr>
<td>Control subjects</td>
<td>24.9 ± 2.1</td>
<td>28.7 ± 3.0</td>
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<tr>
<td><strong>Free androgen index</strong></td>
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<tr>
<td>PCOS patients</td>
<td>22.0 ± 5.0</td>
<td>17.3 ± 4.9</td>
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<tr>
<td>Control subjects</td>
<td>9.3 ± 1.2</td>
<td>7.3 ± 1.0</td>
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<tr>
<td><strong>Free testosterone (pmol/L)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>81.4 ± 9.9</td>
<td>64.6 ± 9.0</td>
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<tr>
<td>Control subjects</td>
<td>45.0 ± 3.9</td>
<td>36.5 ± 3.3</td>
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<tr>
<td><strong>Glucose AUC (mmol/L/180 min)</strong></td>
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</tr>
<tr>
<td>PCOS patients</td>
<td>989 ± 36</td>
<td>954 ± 26</td>
</tr>
<tr>
<td>Control subjects</td>
<td>935 ± 31</td>
<td>912 ± 35</td>
</tr>
<tr>
<td><strong>Insulin AUC (mU·L⁻¹·180 min⁻¹)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>11842 ± 2543</td>
<td>8818 ± 1466</td>
</tr>
<tr>
<td>Control subjects</td>
<td>6702 ± 890</td>
<td>5039 ± 690</td>
</tr>
<tr>
<td><strong>Ghrelin AUC (pmol/L/180 min)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>2118 ± 480</td>
<td>1943 ± 693</td>
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<tr>
<td>Control subjects</td>
<td>3941 ± 919</td>
<td>2446 ± 422</td>
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<tr>
<td><strong>CCK AUC (pmol·L⁻¹·180 min⁻¹)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>369 ± 112</td>
<td>412 ± 94</td>
</tr>
<tr>
<td>Control subjects</td>
<td>290 ± 69</td>
<td>304 ± 91</td>
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<tr>
<td><strong>PYY AUC (pmol·L⁻¹·180 min⁻¹)</strong></td>
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<tr>
<td>PCOS patients</td>
<td>3748 ± 279</td>
<td>4036 ± 390</td>
</tr>
<tr>
<td>Control subjects</td>
<td>3295 ± 194</td>
<td>3139 ± 256</td>
</tr>
</tbody>
</table>

1 All values are ± SEM. PCOS, polycystic ovary syndrome; SHBG, sex hormone–binding globulin; HOMA, homeostasis model assessment; AUC, area under the curve; CCK, cholecystokinin; PYY, peptide YY. n = 14 in each group except for weight; waist circumference; total fat-free mass; total fat mass; and AUC glucose, insulin, ghrelin, CCK and PYY (n = 14 and 13 in the PCOS patients and control subjects, respectively. Data were assessed by using a one-factor ANOVA for week 0 or week 8 data with PCOS status as the fixed factor and repeated-measures ANOVA for changes with time as the within-subject factor and PCOS status as the between-subject factor. For conversion of glucose values from mmol/L to mg/dL, multiply by 18. For conversion of insulin values from mU/L to pmol/L, multiply by 6.95. For conversion of ghrelin values from pmol/L to pg/mL, multiply by 3.38.

2 P < 0.05 for effect of time for all subjects (weeks 0–8).

3 P ≤ 0.05 for difference between subjects with and without PCOS at week 0 and week 8. There was no time × PCOS status interaction for any variable.
APPETITE HORMONES IN POLYCYSTIC OVARY SYNDROME

Fasting ghrelin

Fasting ghrelin was significantly higher in the PCOS patients than in the control subjects at week 0 (486.2 ± 41.1 and 318.1 ± 44.0 pg/mL, respectively; \( P = 0.01 \)), and there was a trend for it to be higher at week 8 (463.5 ± 36.8 and 347.0 ± 56.0 pg/mL, respectively; \( P = 0.099 \)). There was no change in fasting ghrelin after weight loss in either group. At weeks 0 and 8, fasting ghrelin in the PCOS patients and control subjects was correlated with fasting insulin (\( r = -0.518, P = 0.006 \) and \( r = -0.515, P = 0.006 \), respectively), HOMA (\( r = -0.488, P = 0.008 \) and \( r = -0.508, P = 0.007 \), respectively), free androgen index (\( r = -0.553, P = 0.003 \) and \( r = -0.489, P = 0.010 \), respectively), and free testosterone (\( r = -0.538, P = 0.004 \) and \( r = -0.471, P = 0.013 \), respectively). After control for weight, all of the above relations remained except fasting ghrelin and HOMA at week 0 (\( r = -0.369, P = 0.063 \)).

Postprandial ghrelin

The PCOS patients had significantly lower ghrelin concentrations at all time points after preload consumption before and after weight loss than did the control subjects (\( P = 0.023 \) for between-subject effect of PCOS) (Figure 1). There was a trend for an effect of weight loss on changes in test meal ghrelin (\( P = 0.097 \)) and a significant effect of PCOS status on test meal ghrelin (minute-by-PCOS status effect, \( P = 0.023 \)). In the PCOS patients, the postprandial ghrelin response was more impaired (as indicated by a lesser postprandial decrease) at week 0 and tended to be more impaired at week 8 than in the control subjects (\( P = 0.048 \) and 0.069, respectively, for time-by-PCOS status effect). The above differences in ghrelin between subjects with and without PCOS were removed on adjustment for fasting or postprandial insulin at week 0 or 8.

Fasting and postprandial PYY and cholecystokinin

There was no significant effect of PCOS status on baseline cholecystokinin in either the PCOS patients or the control subjects (1.1 ± 0.3 and 0.7 ± 0.2 pmol/L, respectively; \( P = 0.276 \)) and PYY (17.2 ± 1.6 and 14.0 ± 0.7 pmol/L, respectively; \( P = 0.091 \)); on post-weight-loss cholecystokinin (1.0 ± 0.3 and 0.7 ± 0.2 pmol/L, respectively; \( P = 0.302 \)) and PYY (15.9 ± 2.8 and 13.6 ± 1.0 pmol/L, respectively; \( P = 0.381 \)); or on the changes in fasting cholecystokinin and PYY with weight loss. PCOS status had no significant effect on postprandial cholecystokinin or PYY or on changes in postprandial cholecystokinin or PYY with weight loss (Table 1). There was no significant effect of weight loss on fasting cholecystokinin (\( P = 0.919 \)) or PYY (\( P = 0.404 \)) or postprandial cholecystokinin (\( P = 0.440 \)) or PYY (\( P = 0.210 \)) in either group. Cholecystokinin and PYY increased in both groups after test meal consumption at both week 0 and week 8 (\( P < 0.001 \)). The change in weight was significantly and negatively correlated with the change in AUC PYY (\( r = -0.453, P = 0.018 \)) or CCK (\( r = -0.443, P = 0.021 \)) with weight loss.

Visual analogue scores

PCOS patients and control subjects had an increase in their sensation of fasting fullness with weight loss (29.4 ± 3.5 and of the change, although subjects without PCOS had lower fasting, AUC insulin, and postprandial insulin responses at all time points (Figure 1, Table 1).

**FIGURE 1.** Mean (±SEM) glucose, insulin, and ghrelin concentrations at baseline and 15, 30, 45, 60, 90, 120, and 180 min after the ingestion of a test meal at weeks 0 (—) and 8 (⋯⋯). Week 0 and week 8 data were compared by repeated-measures ANOVA with week and blood sampling time as within-subject factors and polycystic ovary syndrome (PCOS) status as the between-subject factor. †, subjects with PCOS, \( n = 14 \); □, subjects without PCOS, \( n = 13 \). To convert glucose values from mmol/L to mg/dL, multiply by 18. To convert insulin values from mU/L to pg/mL, multiply by 6.95. To convert ghrelin values from pmol/L to pg/mL, multiply by 3.38. *Significant week × PCOS status effect, \( P = 0.017 \). ††Significant effect of time from week 0 to week 8 for fasting (\( P = 0.004 \)) and postprandial (\( P < 0.001 \)) insulin. ††Significant between-subject effect of PCOS status, \( P < 0.05 \). ††Significant difference at 0 min between subjects with and subjects without PCOS at week 0 (\( P = 0.042 \)) and week 8 (\( P = 0.009 \)). ††Significant min × PCOS status effect (\( P = 0.023 \)).
38.4 ± 4.8 mm, respectively; P = 0.033) and a decrease in their sensation of postprandial fullness with weight loss (P = 0.005), as reflected in the AUC (3326.5 ± 630.0 and 1130.9 ± 686.4 mm/180 min, respectively; P = 0.009). No significant reduction in fullness after weight loss was observed with adjustment for the change in the total amount of energy consumed at the buffet meal. There were no other significant differences in fasting or postprandial VAS measures before or after weight loss or between the PCOS patients and control subjects (data not shown). None of the postprandial appetite hormones correlated with each other or with VAS.

**Buffet dietary intake**

The energy intake at the buffet meal did not differ significantly between the PCOS patients and control subjects before (4625.8 ± 458.6 and 5476.3 ± 354.6 kJ, respectively) or after (3921.4 ± 355.3 and 4314.6 ± 330.1 kJ, respectively) weight loss. Macronutrient intake and fatty acid profile also did not differ significantly before or after weight loss (data not shown). After weight loss, the amount of food eaten at the buffet meal decreased equivalently, by 919.0 ± 260.0 kJ, for all subjects (P = 0.002); there were consequent reductions in total macronutrient intake but no significant changes in proportional macronutrient intake. The amount of food eaten at the buffet correlated significantly with weight (r = 0.489, P = 0.008), BMI (r = 0.447, P = 0.017), total FFM (r = 0.498, P = 0.007), and total FM (r = 0.513, P = 0.005) at week 0 but not at week 8.

**DISCUSSION**

We have confirmed reports of lower fasting ghrelin concentrations (4, 29), and we also reported a smaller postprandial reduction in ghrelin in overweight women with PCOS than in control subjects. In contrast to previous findings (4), we observed no significant change in fasting ghrelin and only a trend for a change in postprandial ghrelin with weight loss. This discrepancy likely is due to the modest weight loss (4.2 kg), because no significant changes in ghrelin were observed after weight losses of 3% to 5% (30). Postmeal ghrelin suppression is related to the energy content of the meal (9), and it correlates with postmeal decreases in hunger and increases in satiety (31). The impairment in postprandial ghrelin secretion observed in obesity (7–9) may be related to the impairment of appetite regulation in overweight humans, which is consistent with reports of delayed satiation (32). Furthermore, positive energy balance may decrease the sensitivity of the central nervous system to ghrelin (33), which suggests that postprandial ghrelin and its regulatory role on appetite may be blunted in obesity. The improvement in postprandial ghrelin that follows weight loss suggests an improvement in appetite regulation, and this possibility is supported by the post-weight-loss reduction in food consumption observed here and by other investigators (34).

We observed an impaired postprandial ghrelin response in women with PCOS that was partially normalized by weight loss; this same observations was previously made by our group in conjunction with increased postprandial hunger (4). One proposed regulator of ghrelin is insulin, which could acutely suppress ghrelin secretion postprandially (35) and could chronically suppress it in hyperinsulinemic conditions such as obesity or PCOS (4, 36). A recent study found that, in obese children, postprandial ghrelin decreases were positively correlated with insulin sensitivity and negatively correlated with postprandial insulin (35). A dose-dependent suppression of total ghrelin by hyperinsulinemia was also observed in subjects without type 2 diabetes, whereas supraphysiological insulin concentrations were required to suppress ghrelin concentrations in subjects with type 2 diabetes (37). The ability of insulin to suppress ghrelin may thus be altered by IR. This possibility is consistent with the lower ghrelin concentrations observed in the present study in persons with PCOS and in all subjects before weight loss and with amelioration of the differences in ghrelin between women with and without PCOS after adjustment for insulin concentrations. Furthermore, women with PCOS but without IR have fasting ghrelin concentrations similar to those of control subjects and higher than those of women with PCOS and IR (29). Thus, it is possible that abnormalities in appetite regulation will be more prevalent in women with PCOS and IR than in women with PCOS but not IR. Conversely, our previous study showed lower fasting ghrelin concentrations in women with PCOS than in weight- and insulin-matched control subjects, which suggests a role for PCOS independent of IR in regulating ghrelin (4). A variety of contributory factors to ghrelin regulation in PCOS—including adiponectin (38), fat-free mass (39), and androgens (40)—also warrant investigation. A greater degree of weight loss may be necessary to reduce insulin, androgen, or other factors sufficiently to induce changes in ghrelin.

We report for the first time that fasting and postprandial PYY do not differ significantly in age- and weight-matched women with or without PCOS before or after weight loss. Postprandial PYY profiles are proposed to play a role in regulating both acute satiation and longer-term satiety (16, 41), and the similar postprandial PYY may account for the similar food intake between women with and without PCOS. We also report for the first time that postprandial PYY is not altered after diet-induced weight loss. Lower fasting and postprandial PYY concentrations observed previously in obese persons—potentially caused by decreased PYY synthesis or release—could cause reduced postprandial satiety and therefore potentially contribute to obesity (15, 16). Fasting total PYY increases with weight loss, and the increase is positively related to the degree of weight loss (19). The lack of a change in fasting or postprandial PYY with weight loss seen in the present study was unexpected; however, the intervention duration and degree of weight loss (4.2 kg) may have been inadequate to induce changes. Alternatively, this lack of a change may reflect increased central nervous system sensitivity to the effects of PYY or an adaptive response to attempts to restore energy balance. Food intake is similarly reduced after PYY 3–36 infusions in obese and lean subjects (15), which indicates that obese subjects may not suffer PYY resistance. The negative correlation between the degree of weight loss and a change in AUC PYY could also indicate that subjects with the greatest degree of weight loss potentially had a relative compensatory decrease in postprandial PYY; this possibility is supported by recent studies showing decreases in fasting (17) or postprandial (42) PYY after diet- or gastric bypass–induced weight loss.

Unlike other investigators (13), we observed no differences in fasting or postprandial cholecystokinin between women with and without PCOS either before or after weight loss. It is not clear whether the similar cholecystokinin profiles of the women with and without PCOS are related to the consequences of the diet meal energy intake, because the primary effects of cholecystokinin are an inhibition of gastric emptying, an increase in satiation, and a
and ad libitum food consumption, but we confirmed alterations in subjective appetite, PYY and cholecystokinin concentrations, diverse conditions such as obesity, weight loss, and PCOS (55). May not be optimal for the assessment of ghrelin regulation in eaters (54). Other potential limitations of the current study in menstrual cycle (51–53) or between restrained and unrestrained glucose homeostasis have been reported over the course of the hormones (specifically, cholecystokinin), insulin sensitivity, or stages of the menstrual cycle would further strengthen our observations. The lack of observed differences in PYY may also be affected by the preload macronutrient composition, because postprandial PYY is stimulated more strongly by protein than by fat or carbohydrate (45). Furthermore, PYY is released in proportion to calories ingested (14), and the energy content of the preload (936 kJ) may not have been adequate to allow detection of subtle differences.

Whereas altered ghrelin regulation in PCOS potentially indicates impaired appetite regulation and difficulty with weight management, these possibilities are not supported by the findings of similar cholecystokinin and PYY concentrations, subjective appetite, and buffet food intake in women with and without PCOS. In addition to its proposed role in appetite regulation, ghrelin has a variety of functions—including endocrine pancreatic function, glucose metabolism, inflammation, vasodilation, and ovarian function (46–48). It is possible that the lower ghrelin concentrations commonly observed in persons with PCOS represent the increased metabolic, diabetic, and reproductive dysfunction associated with the condition rather than abnormalities in appetite regulation. The preprandial increase in ghrelin may also occur as an anticipatory response to feeding rather than as a physiologic meal initiator (49, 50); this possibility is supported by a later onset of the postprandial ghrelin decrease in obese than in lean males, but similar energy intakes in the 2 groups (11). The observed differences in ghrelin may induce subtle alterations in appetite, which would be more detectable in a free-living environment with ad libitum energy intake. Selection of subjects with similar eating behaviors and performance of the MTT at defined stages of the menstrual cycle would further strengthen our observations, because variations in appetite or food intake, appetite hormones (specifically, cholecystokinin), insulin sensitivity, or glucose homeostasis have been reported over the course of the menstrual cycle (51–53) or between restrained and unrestrained eaters (54). Other potential limitations of the current study include the measurement of total ghrelin, which comprises biologically active (Ser3 octanoylated) and inactive ghrelin and which may not be optimal for the assessment of ghrelin regulation in diverse conditions such as obesity, weight loss, and PCOS (55).

In conclusion, we observed no differences in postprandial subjective appetite, PYY and cholecystokinin concentrations, and ad libitum food consumption, but we confirmed alterations in fasting and postprandial ghrelin concentrations in women with PCOS. Ad libitum buffet consumption was decreased after modest weight loss in conjunction with an improved postprandial ghrelin profile, but no changes were seen in subjective appetite or circulating cholecystokinin or PYY concentrations. It is not clear whether women with PCOS have abnormal regulation of energy homeostasis or appetite hormones, and, despite improving some aspects of appetite regulation, the effect of weight loss on postprandial anorexigenic hormones is unclear in overweight women with or without PCOS.

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