The Journal of Clinical Endocrinology & Metabolism, 2021, Vol. XX, No. XX, 1–11
doi:10.1210/clinem/dgab028
Clinical Research Article

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Clinical Research Article

Changes in Ghrelin and Glucagon following a Low Glycemic Load Diet in Women with PCOS

Sarah E. Hoover, Barbara A. Gower, Yenni E. Cedillo, Paula C. Chandler-Laney, Sarah E. Deemer, and Amy M. Goss

Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ORCiD number: 0000-0003-4112-6438 (S. E. Hoover).

Abbreviations: AUC, area under the curve; BMI, body mass index; CHO, carbohydrate; CRU, Clinical Research Unit; CV, coefficient of variation; GL, glycemic load; GLP-1, glucagon-like peptide-1; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; PCOS, polycystic ovarian syndrome; PYY, peptide YY.

Received: 17 September 2020; Editorial Decision: 14 January 2021; First Published Online: 25 January 2021; Corrected and Typeset: 19 February 2021.

Abstract

Context: Altered satiety hormones in women with polycystic ovarian syndrome (PCOS) may contribute to obesity. Diets with a low glycemic load (GL) may influence appetite-regulating hormones including glucagon and ghrelin.

Objective: To test the hypothesis that following a 4-week, eucaloric low vs high GL diet habituation, a low vs high GL meal will increase glucagon and decrease ghrelin to reflect greater satiety and improve self-reported fullness.

Methods: Secondary analysis of a randomized crossover trial.

Participants: Thirty women diagnosed with PCOS.

Intervention: Participants were provided low (41:19:40% energy from carbohydrate:protein:fat) and high (55:18:27) GL diets for 8 weeks each. At each diet midpoint, a solid meal test was administered to examine postprandial ghrelin, glucagon, glucose, insulin, and self-reported appetite scores.

Results: After 4 weeks, fasting glucagon was greater with the low vs high GL diet ($P = .035$), and higher fasting glucagon was associated with lesser feelings of hunger ($P = .009$). Significant diet effects indicate 4-hour glucagon was higher ($P < .001$) and ghrelin was lower ($P = .009$) after the low vs high GL meal. A trending time × diet interaction ($P = .077$) indicates feelings of fullness were greater in the early postprandial phase after the high GL meal, but no differences were observed the late postprandial phase.

Conclusion: These findings suggest after low GL diet habituation, a low GL meal reduces ghrelin and increases glucagon in women with PCOS. Further research is needed to determine the influence of diet composition on ad libitum intake in women with PCOS.

Key Words: ghrelin, glucagon, glycemic load, satiety, hunger, appetite

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA
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Polycystic ovarian syndrome (PCOS) is a common endocrine disorder affecting over 10% of premenopausal women (1). Features of the syndrome include ovulatory dysfunction, hyperandrogenemia, and/or polycystic ovaries (2, 3). Metabolic disturbances, including insulin resistance and hyperinsulinemia, occur with higher prevalence in women with PCOS than in women without PCOS of a similar body mass index (BMI) (4-6). These metabolic impairments increase susceptibility to obesity and make weight maintenance uniquely challenging. While the exact mechanisms that underly this propensity to weight gain are not entirely clear, satiety has been shown as a critical contributor to weight management (7).

Women with PCOS have lower postprandial satiety and higher postprandial hunger than weight-matched controls (8). This may be due in part to dysregulation of appetite-regulating hormone ghrelin. Elevated fasting ghrelin has been reported among women with PCOS when compared with weight-matched controls (9), and has been related to increased adiposity (10) and elevated androgens (11). Moreover, impaired postprandial ghrelin suppression has also been shown in PCOS, which has been related to poor insulin sensitivity (12, 13), and shown to improve with weight loss (8). Insulin and glucagon are thought to regulate appetite hormones, and may be implicated in ghrelin secretion and action. Studies have shown exogenous administration of glucagon reduces postprandial appetite scores (14) and suppress postprandial ghrelin (15, 16). Thus, it is possible that interventions designed to alter glucagon and insulin secretion may also influence appetite-regulating hormones in women with PCOS.

Glycemic load (GL), a measure of carbohydrate (CHO) quality and quantity in food (17), may help manage satiety. Consumption of a low GL meal results in a reduced postprandial glucose excursion, thus reducing postprandial insulin and increasing postprandial glucagon secretion (18, 19). In the postprandial state, a reduced insulin to glucagon ratio has been suggested to increase circulating energy availability and decrease late phase feelings of hunger (18, 20). Monitoring changes in these regulatory hormones throughout the postprandial period could provide insight into whether diet composition changes physiological appetite regulation with potential to promote healthy eating patterns in women with PCOS.

Therefore, the objective of this study was to test the hypothesis that following a 4-week habituation to a low vs high GL diet, a low GL meal will increase glucagon and decrease ghrelin to reflect greater satiety and improve self-reported fullness compared with a high GL meal. Additionally, we aim to explore the relationships among ghrelin, glucagon, and self-reported appetite in women with PCOS.

Materials and Methods

Participants

Thirty women diagnosed with PCOS were recruited from the community and enrolled in the study. The criteria for diagnosis of PCOS were consistent with the National Institutes of Health 1990 criteria, including (1) hyperandrogenism and/or hyperandrogenemia, (2) oligo-ovulation, and (3) the exclusion of any existing disorders such as Cushing's syndrome, hyperprolactinemia, or congenital (nonclassical) adrenal hyperplasia. Specific inclusion and exclusion criteria have been described elsewhere (21). Briefly, inclusion criteria were BMI ≤45 kg/m^2, age 21-50 years, nondiabetic as determined by an oral glucose tolerance test at screening, and no weight change >2.3 kg over the previous 6 months. Exclusion criteria included exercise >2 hours per week, pregnancy, current breastfeeding, medication affecting body composition or glucose metabolism (including oral contraceptives, cholesterol medication, and blood pressure medications), current tobacco use, use of illegal drugs in the past 6 months, major food allergies or food dislikes, and a medical history that contraindicated inclusion in the study. Participants were informed of study protocol and oral and written consent were obtained prior to enrollment. The protocol for this study was approved by the Institutional Review Board for Human Use at the University of Alabama at Birmingham.

Study Design

A randomized crossover, controlled feeding design was used for this study (21). After enrollment, participants were assigned to 1 of 2 eucaloric diet orders using a randomization scheme. Comprehensive metabolic testing was conducted before and after each 8-week arm, with a 4-week washout period. Original to this report, a solid meal test was administered to each participant at the 4-week midpoint of each diet arm to examine postprandial insulin, glucose, glucagon, ghrelin, peptide YY (PYY), and glucagon-like Peptide-1 (GLP-1) responses (described below). The UAB Clinical Research Unit (CRU) provided all food and participants visited the CRU several times each week to be weighed and to collect the meals for off-site consumption. Participants were asked to maintain their baseline physical activity throughout the duration of the study. Energy needs were estimated from baseline energy expenditure assessed by indirect calorimetry using an activity factor of 1.35 by

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the CRU Research Dietitian. Energy provided to each individual was adjusted when necessary to maintain body weight.

Diets

Participants were blinded to diet order and consumed a low GL diet (41% energy from CHO, 19% energy from protein, and 40% energy from fat) and the high GL diet (55% energy from CHO, 18% energy from protein, and 27% energy from fat) for 8 weeks in randomized order with a 4-week washout period (21). All food was provided throughout each diet intervention arm. The specific composition of the test diets have been reported (21, 22). The glycemic index was ≈50 and ≈60 for the low GL and high GL diet respectively. Intervention diet menus were designed using Nutrition Data System for Research (NDSR) software versions 2009 and 2011 (Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN).

Solid Meal Test

Following a 12-hour fast, participants presented at the CRU and consumed a breakfast composed of the assigned diet menu items for that day in a solitary setting. Examples for each meal are provided in Table 1. The average GL of the high GL breakfast meal was 83.31/1000 kcal, and average GL of the low GL breakfast meal was 48.27/1000 kcal. To perform the test, a flexible intravenous catheter was placed in the antecubital space of the left arm. Blood samples were collected at –15 and –5 minutes prior to and 15, 60, 90, 120, 180, and 240 minutes after initiation of meal consumption (time "zero"). Plasma was collected in vacutainers with EDTA and protease inhibitors were added immediately after blood collection. Sera and plasma were stored at –85°C. Samples of sera were analyzed for insulin and glucose. Plasma was analyzed for GLP-1, ghrelin, PYY, cortisol, and glucagon.

Table 1. Sample breakfast menus

High GL breakfast meal		Low GL breakfast meal	
Meal/Food	Amount	Meal/Food	Amount
Frosted flakes	0.75 oz (1 bowl)	Cheerios	0.75 oz (1 bowl)
Skim milk	8 fl oz	Skim milk	8 fl oz
White bread	2 slices	Rye bread	2 slices
Peanut butter	21 g	Peanut butter, regular	21 g
Jelly	0.5 oz	Promise 60% spread margarine	5 g

Visual Analog Scale

Participants were asked to rate hunger, fullness, and desire to eat on a visual analog scale 15 and 5 minutes before meal initiation, as well as 15, 60, 90, 120, 180, and 240 minutes after meal initiation (time "zero"). Self-reported values were quantified by measuring the distance in mm from the left anchor to the mark.

Hormone and Glucose Analysis

Analyses were conducted in the Core Laboratory of the Center for Clinical and Translational Science, Nutrition and Obesity Research Center, and Diabetes Research Center. GLP-1 was measured using Millipore (Billerica, MA) Human GPL-1 (active) enzyme-linked immunosorbent assay kits: intra-assay coefficient of variation (CV) 2.58%, interassay CV 4.1%. Total ghrelin was measured using a Millipore total ghrelin enzyme-linked immunosorbent assay kit: intra-assay CV 4.92%, interassay CV 7.35%. PYY was measured using Millipore PYY (3-36 active) radioimmunoassay kits: intra-assay CV 6.56%, interassay CV 9.06%. Cortisol was measured with immunofluorescence using the TOSOH A1A-900 (TOSOH, San Francisco, CA): intra-assay CV 5.19%, interassay CV 1.66%. Glucose was measured using the glucose oxidase method (Stanbio Laboratory, Boerne, TX): intra-assay CV 1.2%, interassay CV 3.1%. Insulin was assayed with immunofluorescence technology on a TOSOH AIA-II analyzer (TOSOH): intra-assay CV 1.5%, interassay CV 4.4%. Glucagon was measured using Millipore Glucagon RIA kits: intra-assay CV 2.18%, interassay CV 5.42%.

Calculations

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index (23) was calculated using a formula based on fasting glucose and fasting insulin: fasting glucose (mg/dl) × fasting insulin (μU/mL)/405. Fasting insulin and HOMA-IR for 2 meals were excluded from the analyses because hemolysis of the serum samples invalidated the insulin measures. During the solid meal test, fasting values were calculated as the average of the –15 and –5 minutes time points prior to consumption of the solid meal. Areas under the curve (AUCs) were calculated using the trapezoidal method.

Statistical Analysis

Participant characteristics at baseline are expressed as mean ± SD. The difference in BMI at the time of the meal test was evaluated using a 2-tailed paired t-test with an alpha level of 0.05 denoting statistical significance. Statistical

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assumptions were tested using Levene's test for equality of variance and the Kolmogorov–Smirnov and Shapiro–Wilk tests for normal distribution, which was confirmed visually with QQ-plot observation.

Linear mixed models were used to examine main effects of diet (high GL and low GL) and time (0, 15, 60, 90, 120, 180, and 240 minutes), and the diet by time interaction for each hormone during the postprandial period. Log-transformation for cortisol, ghrelin, GLP-1, glucagon, glucose, and insulin was used because they did not conform to a normal distribution. Four individuals experienced glucagon exceeding the normal range of 50 to 100 pg/mL at fasting (24): 1 during both diet interventions, and 3 during the low GL diet. Since circulating glucagon exceeding the normal range has been reported in women with PCOS and may be related to abnormalities in androgens (25, 26), and, as an exaggerated response to hypoglycemia (27), glucagon measurements exceeding normal range were excluded from all analyses. Further linear mixed model analysis was used to examine the relationship between hunger scores and glucoregulatory hormones at fasting in both diets combined. Insulin and glucagon were tested as fixed effects, while fullness scores and diet were tested as covariates. The final model reflects only variables that were significantly related to hunger. Fasting values of hunger, insulin, glucagon, and fullness were normally distributed and were not log-transformed. In all mixed model analyses, compound symmetry covariance structure was used and subject ID was set as a repeated effect. Studentized residuals for data points extending past 3 standard deviations were considered outliers and removed from analyses. Significant findings were determined by an alpha level of 0.05. Tukey post hoc correction was used where significant effects were observed; however, due to possible overcorrection because of small sample size, significance reported are based on unadjusted pairwise comparisons.

Multiple regression was used to examine the relationship between postprandial glucagon and ghrelin AUCs by diet. Both glucagon AUC and ghrelin AUC were log-transformed prior to analysis. Data were analyzed using SAS (version 9.3; SAS Institute, In., Cary, NC, USA).

Results

Of the 30 women enrolled, 27 completed the solid meal test for both arms of the study. As shown in Table 2, participants were ethnically diverse (N = 13, 16, 1 African American, Caucasian, and Hispanic, respectively) with an average age of 31.2 ± 5.8 years. Although energy intake was calculated to maintain weight, fluctuations in body weight were observed in some women. During the time of the solid meal test, BMI did not differ by diet.

Fasting and Postprandial Hormone Responses

Fasting and postprandial hormones are reported by diet in Table 3 and Fig. 1. For GLP-1, cortisol, glucose, and insulin there were significant effects of time ($P < .001$ all), but not of diet. On average, PYY trended higher in the high vs low GL meal (93.39 ± 45.13 vs 85.47 ± 46.70, $P = .050$) but did not reach statistical significance at any time point. There was no significant effect of time for PYY.

Independent of time, participants experienced higher ghrelin after the high GL meal (776.43 ± 422.23) than after the low GL meal (711.37 ± 353.39, $P = .009$). This difference reached statistical significance at 180 and 240 minutes after meal initiation in the high GL meal compared with the low GL meal. Differences in ghrelin did not reach statistical significance during any other time point.

Glucagon was higher after the low GL meal (69.35 ± 19.28) than after the high GL meal independent of time (63.11 ± 15.89, $P < .001$). This difference was attributable to glucagon being significantly higher at fasting among those consuming the low vs high GL diet, and throughout most of the postprandial period: 60 minutes, 120 minutes, 180 minutes, and 240 minutes. There was no significant difference in glucagon at 15 and 90 minutes. There were no significant time × diet interactions in the hormone responses.

Self-reported Appetite Scores

Self-reported appetite scores are reported in Table 4 and Fig. 2. For self-reported hunger and self-reported desire to eat, there were significant time effects ($P < .001$ all) but no significant effects of diet or time x diet interactions. For self-reported fullness, the time × diet interaction approached significance ($P = .077$), suggesting that fullness over time differed by diet, such that in the high GL condition participants reported greater fullness at 15 through 120 minutes but in the low GL condition, participants reported slightly greater fullness at fasting and 180 and 240 minutes after

Table 2. Baseline characteristics of study population

Variable	Mean ± standard deviation[a]
Ethnicity (N = European American/ African American/Hispanic)	13/16/1
Age (yr)	31.2 ± 5.8
Body mass index (kg/m^2)	31.57 ± 5.83
% fat baseline	41.67 ± 7.01
Fasting Glucose (mg/dL)	96.0 ± 8.98
Fasting Insulin (µIU/mL)	8.65 ± 6.60
HOMA-IR	2.13 ± 1.71

[a]Unless otherwise indicated.

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Table 3. Fasting and postprandial hormones by meal during solid meal test

	Time (min)	High GL	Low GL	Pairwise comparisons	Time (*P*)	Diet (*P*)	Time × Diet (*P*)
GLP-1 (pM)	0	2.61 ± 2.15	3.09 ± 2.87	0.252	<.0001	.738	.728
	15	3.81 ± 3.07	3.58 ± 3.50	0.174			
	60	3.64 ± 3.55	4.35 ± 4.00	0.777			
	90	4.84 ± 5.27	4.40 ± 3.83	0.575			
	120	3.63 ± 2.83	4.04 ± 3.97	0.717			
	180	3.49 ± 2.64	3.90 ± 3.13	0.948			
	240	2.72 ± 1.42	3.54 ± 3.23	0.952			
Ghrelin (pg/mL)	0	880.88 ± 502.83	825.44 ± 393.31	0.51	< .0001	.009	.742
	15	840.17 ± 486.35	803.65 ± 389.19	0.819			
	60	725.00 ± 394.63	644.81 ± 315.91	0.217			
	90	690.67 ± 351.15	663.50 ± 347.84	0.313			
	120	666.91 ± 337.54	644.62 ± 341.49	0.253			
	180	793.23 ± 377.89	688.04 ± 323.67	0.085			
	240	830.57 ± 469.96	709.54 ± 348.20	0.036			
PYY (pg/mL)	0	89.88 ± 49.97	80.21 ± 44.04	0.301	.595	.05	.448
	15	87.94 ± 39.99	78.28 ± 40.59	0.196			
	60	84.70 ± 38.10	92.57 ± 79.06	0.271			
	90	88.49 ± 25.94	87.42 ± 34.31	0.929			
	120	100.47 ± 42.00	87.42 ± 47.55	0.171			
	180	99.68 ± 49.57	84.55 ± 38.23	0.134			
	240	102.32 ± 63.46	87.47 ± 31.99	0.141			
Cortisol (µg/dL)	0	12.25 ± 2.90	11.93 ± 3.20	0.653	<.0001	.939	.804
	15	10.77 ± 2.49	11.12 ± 4.03	0.912			
	60	9.69 ± 3.00	9.32 ± 2.83	0.612			
	90	9.22 ± 3.16	9.27 ± 3.91	0.821			
	120	8.26 ± 2.92	8.38 ± 3.18	0.672			
	180	8.18 ± 2.89	7.87 ± 2.76	0.49			
	240	7.76 ± 2.73	8.39 ± 2.76	0.179			
Glucose (mg/dL)	0	92.73 ± 9.40	94.08 ± 8.54	0.681	<.0001	.341	.992
	15	98.63 ± 10.66	99.17 ± 12.26	0.936			
	60	112.59 ± 25.07	113.23 ± 23.80	0.92			
	90	100.61 ± 21.52	99.83 ± 21.73	0.92			
	120	96.42 ± 19.41	96.72 ± 16.12	0.799			
	180	94.04 ± 12.38	97.06 ± 12.64	0.366			
	240	92.79 ± 11.78	95.25 ± 9.41	0.42			
Insulin (µIU/mL)	0	8.07 ± 6.40	9.24 ± 5.78	0.476	<.0001	.796	.928
	15	24.84 ± 19.68	25.06 ± 19.71	0.997			
	60	61.88 ± 51.38	56.65 ± 32.67	0.754			
	90	36.95 ± 31.17	38.43 ± 30.08	0.613			
	120	31.78 ± 40.87	33.88 ± 31.56	0.566			
	180	19.38 ± 22.08	22.53 ± 20.08	0.473			
	240	14.23 ± 13.60	15.03 ± 14.52	0.626			
Glucagon (pg/mL)	0	63.66 ± 15.09	71.92 ± 23.22	0.035	.059	<.001	.524
	15	66.16 ± 18.00	68.71 ± 16.72	0.373			
	60	56.27 ± 10.12	66.17 ± 19.85	0.006			
	90	65.54 ± 19.39	64.93 ± 14.86	0.729			
	120	65.54 ± 21.38	72.19 ± 21.54	0.025			
	180	59.30 ± 8.69	70.51 ± 21.18	0.008			
	240	64.75 ± 12.62	70.85 ± 17.34	0.076			

Data analyzed using linear mixed models. Hormones were modeled as a function of time, diet, and the time × diet interaction. Unadjusted pairwise comparisons reported. Data reported Mean ± SD unless otherwise noted. Cortisol, GLP-1, ghrelin, glucose, insulin, and glucagon were log-transformed for analysis, back-logged data is reported.

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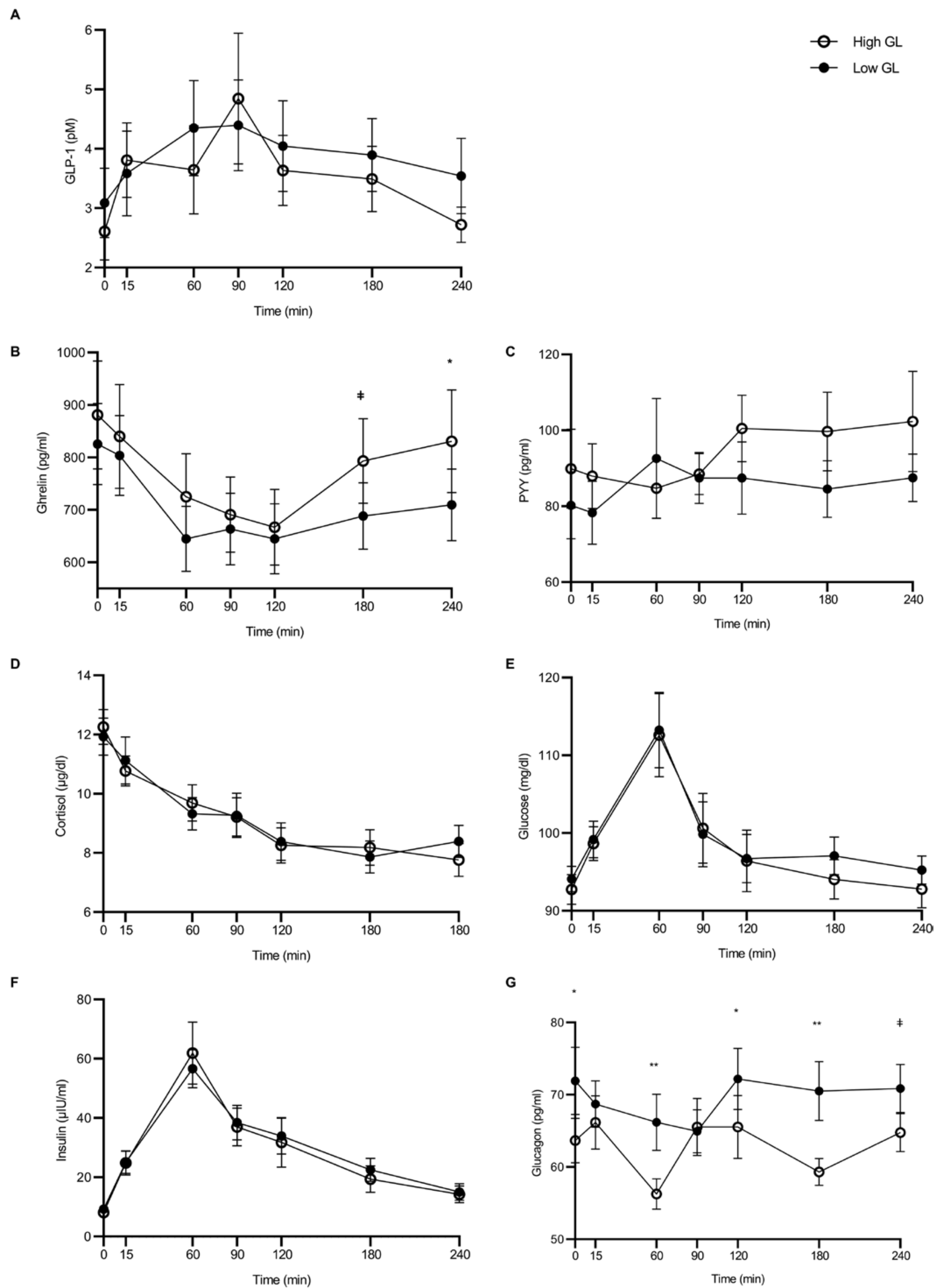

Figure 1. Postprandial gut and glucoregulatory hormone responses to a solid meal test for low GL and high GL diet groups. $^{**}P \le .01$, $^{*}P \le .05$, ‡$P \le .10$ for paired t-test result.

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Table 4. Fasting and postprandial self-reported satiety scores by meal during solid meal test

	Time (min)	High GL	Low GL	Pairwise comparisons	Time (*P*)	Diet (*P*)	Time × Diet (*P*)
Self-reported hunger	0	59.08 ± 25.39	50.69 ± 26.68	0.110	<.001	.752	.382
	15	16.63 ± 19.15	22.22 ± 25.76	0.250			
	60	17.33 ± 17.82	22.54 ± 22.94	0.302			
	90	25.38 ± 24.15	26.65 ± 25.29	0.749			
	120	31.08 ± 24.96	29.59 ± 25.42	0.820			
	180	46.42 ± 21.76	40.74 ± 27.54	0.298			
	240	57.54 ± 21.20	54.67 ± 25.40	0.620			
Self-reported desire to eat	0	60.44 ± 25.92	58.25 ± 22.84	0.710	<.0001	.822	.480
	15	16.71 ± 17.27	18.07 ± 21.15	0.694			
	60	20.75 ± 19.43	28.35 ± 25.66	0.103			
	90	28.71 ± 24.47	29.42 ± 25.79	0.766			
	120	36.42 ± 22.81	32.81 ± 27.71	0.503			
	180	49.25 ± 22.04	42.93 ± 29.16	0.211			
	240	58.00 ± 21.77	54.41 ± 26.24	0.504			
Self-reported fullness	0	21.10 ± 20.11	25.62 ± 19.40	0.480	<.0001	.140	.077
	15	74.54 ± 17.54	64.67 ± 33.32	0.033			
	60	73.75 ± 18.31	65.92 ± 29.87	0.091			
	90	66.54 ± 23.22	60.88 ± 27.27	0.190			
	120	63.92 ± 25.83	56.96 ± 28.32	0.118			
	180	46.50 ± 23.86	53.00 ± 28.19	0.272			
	240	33.48 ± 21.62	38.93 ± 24.80	0.431			

Data analyzed using linear mixed models. Self-reported appetite scores were modeled as a function of time, diet, and the time × diet interaction. Unadjusted pairwise comparisons reported. Data reported mean ± SD unless otherwise noted.

the meal. These differences reached statistical significance at 15 and 60 minutes after meal initiation. Statistical significance was not observed at any other time point.

Associations among Hunger, Glucagon, and Ghrelin

The association between hunger and glucagon at fasting is shown in Fig. 3, indicating participants with greater fasting glucagon experienced less hunger prior to meal intake (parameter estimate for glucagon = –0.8032 ± 0.1460, P = .009) independent of fasting fullness score (parameter estimate for fullness score = –0.8984 ± 0.1416 P < .001). Insulin and diet were not significant and therefore not included in the final model.

The relationship between glucagon AUC and ghrelin AUC by diet indicated that during the low GL meal, individuals who experienced greater glucagon AUC tended to experience lower ghrelin AUC (R = –0.81, P = .060). This relationship was not observed in the high GL meal (R = –0.22, P = .694) (28).

Discussion

To our knowledge, this is the first study to show lower postprandial ghrelin and higher postprandial glucagon in response to a low vs high GL solid meal challenge following diet habituation in women with PCOS. Furthermore, data from this study provide information about potential mechanisms explaining the satiating effect of a low GL meal. Specifically, we found that greater glucagon was associated with lesser hunger during fasting following habituation to the low vs high GL diet. Additionally, greater postprandial glucagon was associated with lesser postprandial ghrelin following the low vs high GL meal. The high GL meal led to greater fullness in the early postprandial phase while no significant differences were observed in the late postprandial phase. Collectively, these findings suggest that a low GL diet may influence hunger/satiety through alterations in fasting and postprandial appetite-regulating hormones in women with PCOS with overweight or obesity.

A novel finding of this study was that individuals experienced lower ghrelin, particularly at 180 and 240 minutes, after the low vs high GL meal. These data complement previous literature (11-13), in which postprandial ghrelin reduction is associated with fat loss, improved insulin sensitivity, and reduction in testosterone. We have previously reported that a low GL diet is likewise associated with fat loss, improved insulin sensitivity, and reduction in testosterone in this sample (21, 22). Taken together, these observations suggest that the positive metabolic effects of the low GL diet may be mediated to some extent by changes in gut hormones. Since impaired postprandial ghrelin

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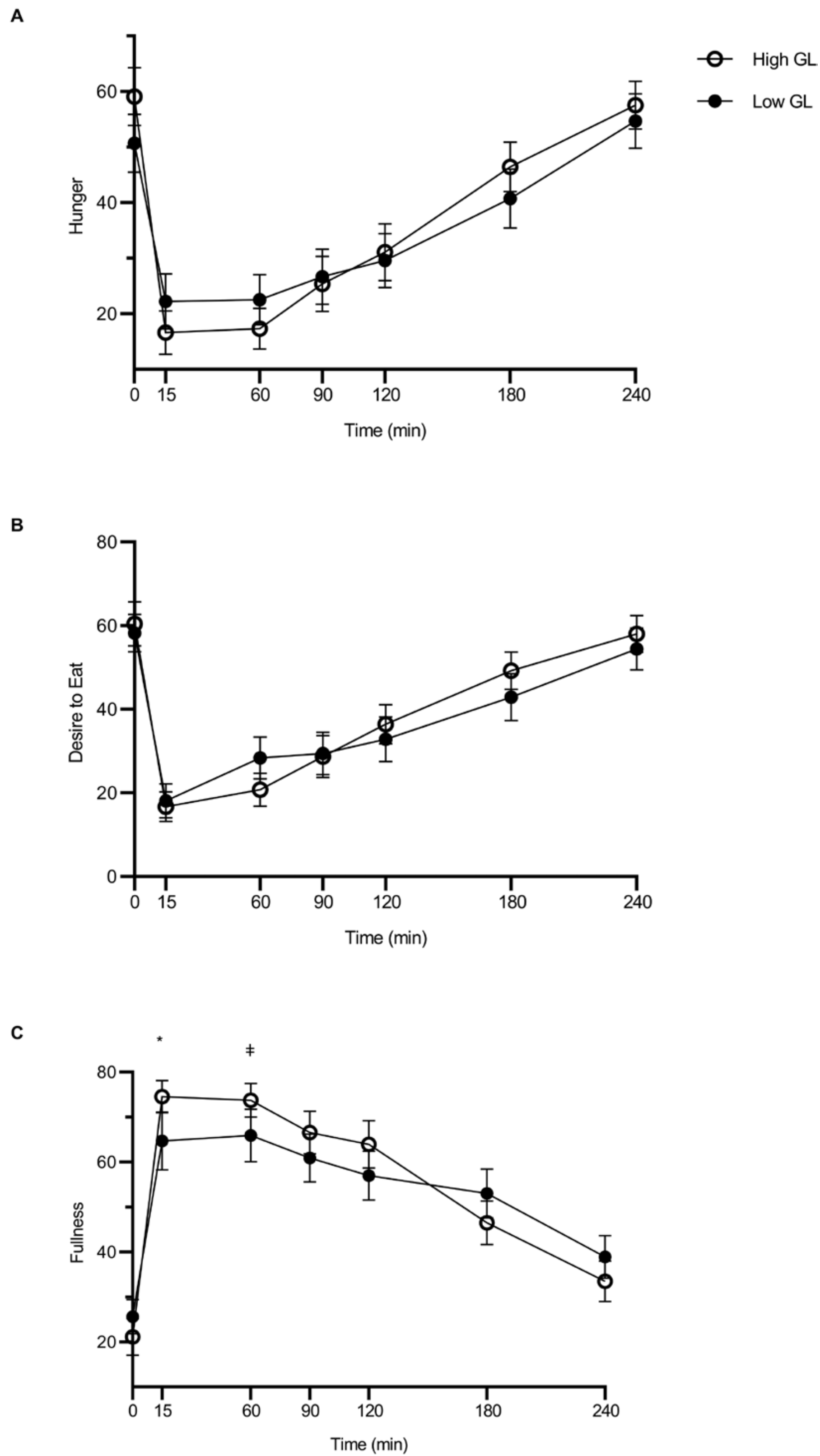

Figure 2. Postprandial self-reported satiety responses to a solid meal test for low GL and high GL diet groups. $^{**}P \le .01$, $^{*}P \le .05$, ‡$P \le .10$ for paired t-test result.

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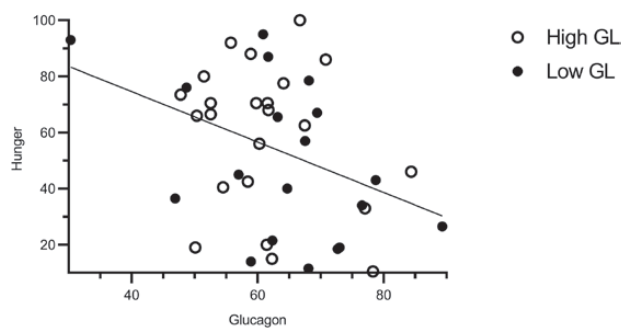

Figure 3. Individuals with greater fasting glucagon experienced less hunger prior to meal intake in both diets combined.

suppression is commonly observed in women with PCOS when compared with non-PCOS controls, and may contribute to some of the characteristic features of PCOS (29, 30), this finding supports the use of a low GL diet in PCOS treatment.

An additional finding in this study was greater plasma glucagon observed at fasting and in the postprandial phase of the low vs high GL meal. The higher fasting glucagon likely represents the effect of diet habituation. Considering the counter regulation between glucagon and insulin as well as the lipolytic effect of glucagon (31), this observation is consistent with greater insulin sensitivity and fat loss following the low GL diet reported in this cohort (21, 22). The higher postprandial glucagon observed after the low GL meal is also consistent with responses seen in animal models, (32) and is similar to other CHO restriction interventions in overweight and obese adult populations (18). Moreover, this observation provides further insight into the acute satiety-promoting effects of a low GL meal. Glucagon has been suggested to suppress appetite and has been shown to reduce meal size (14, 33). This relationship is supported by our observation that individuals with greater fasting glucagon experienced less hunger prior to meal intake, independent of fullness. Further studies are needed to determine the relationships between fasting hunger and glucagon and whether it influences ad libitum food intake in women with PCOS.

The mechanisms through which glucagon regulates hunger are complex and have not been fully elucidated; however, ghrelin may partially contribute to this relationship. Reduced circulating ghrelin after glucagon administration has been shown in humans (15, 16), and is supported in the observed inverse association between higher glucagon AUC and lower ghrelin AUC in the present study. The mechanism through which glucagon affects ghrelin is not clear. However, evidence suggests that ghrelin, like glucagon, plays a role in the regulation of glucose homeostasis and energy balance (34, 35). Thus, it is possible that some aspect of glucoregulatory control links glucagon and ghrelin mechanistically. It is also possible that the observed relationship between glucagon AUC and ghrelin AUC may reflect the improved insulin sensitivity observed during the low GL diet habituation (21), since increased rate of postprandial ghrelin suppression has been associated with improved insulin sensitivity (36). Further studies are needed to determine the precise mechanisms through which glucagon and ghrelin interact to affect perceived hunger and the degree to which insulin sensitivity influences these relationships in women with PCOS.

The finding that fullness tended to be experienced in the early postprandial phase by the high GL meal but differences disappeared in the late postprandial phase may be due to effects of a low GL meal on the metabolic milieu. Circulating fuel availability has been associated with late postprandial hunger and voluntary energy intake, and is responsive to glycemic load (18, 20, 37). It has been suggested that meals with a high GL elicit a high insulin to glucagon ratio during the early postprandial phase, thereby stimulating uptake of glucose and fatty acids in peripheral tissues (20). This reduces circulating metabolic fuels during the late postprandial phase, resulting in increased feelings of hunger. Conversely, a low GL meal may elicit a lower insulin to glucagon ratio and increases circulating fuel availability during the late postprandial phase, thereby reducing feelings of hunger. We have previously observed a similar phenomenon, in which 64 relatively healthy, overweight adults experienced lower insulin iAUC, and lower reported appetite in the late postprandial phase after a low vs high GL solid meal (19). However in the present study we only observed greater reported fullness following the high GL meal during the early postprandial phase, and following the low GL meal fullness was not different in the late postprandial phase. Moreover, insulin, glucose, hunger, and satiety did not differ by diet, which is likely due to the lack of measurement of the 30-minute peak of insulin and glucose. Thirty-minute peak insulin and glucose has been reported in response to diets differing in GL in a previous study (18), and it is possible that more robust differences in insulin may be necessary to demonstrate the relationship between these hormones and reduced feeling of hunger in the late postprandial phase following consumption of a low GL meal. Further research is needed to determine the late postprandial effect of a low GL meal on overall energy availability and if this contributes to increased satiety in women with PCOS.

The study was limited by a relatively small sample size, which inhibited correction for multiple comparisons. An additional limitation was the absence of a 30-minute time point after meal consumption, which may have increased the ability to detect differences in postprandial glucose and insulin peaks in response to low vs high GL meals. Strengths of this study included the crossover study design in addition to provision of food for the entire study. Furthermore,

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the macronutrient distribution utilized was within acceptable macronutrient distribution range, making results generalizable to typical eating patterns.

In conclusion, consumption of a low glycemic load meal moderately reduced in CHO resulted in lower postprandial ghrelin, and higher fasting and postprandial glucagon when compared to a high glycemic load meal among women with PCOS and overweight/obesity. Additionally, our data suggest that glucagon may influence appetite, and this relationship may, in part, be explained by the suppression of ghrelin. Collectively, these findings support the hypothesis that a low GL meal following a 4-week habituation to a low GL diet provides improvements in satiety-regulating hormones when compared with a high GL meal in women diagnosed with PCOS.

Acknowledgments

The authors gratefully acknowledge the help of Maryellen Williams and Cindy Zeng of the UAB Metabolism/Human Physiology Core Laboratory (Nutrition Obesity Research Center, Diabetes Research and Training Center, Center for Clinical and Translational Science) with laboratory analyses, and of Betty Darnell and Suzanne Choquette of the UAB Center for Clinical and Translational Science with experimental design and diet development.

Financial Support: Research reported in this publication was supported by the National Institute of Child Health and Human Development (RO1HD054960), the National Center for Advancing Translational Sciences of the National Institutes of Health (UL1TR003096), and the National Institute of Diabetes and Digestive and Kidney Diseases P30DK056336, and P60DK079626. S.E.H. was supported by a T32 Predoctoral Award from the UAB Predoctoral Training Program in Obesity-Related Research (T32HL105349). S.E.D. was supported by a T32 Post-doctoral Award from the UAB Postdoctoral Training Program in Obesity-Related Research (T32DK062710).

Trial Information: ClinicalTrial.gov NCT01028989 (registered December 8, 2009).

Additional Information

Correspondence: Sarah E. Hoover, Department of Nutrition Sciences, The University of Alabama at Birmingham (UAB), 1675 University Blvd., Webb 513, Birmingham, AL 35233, USA. Email: stucker5@uab.edu.

Disclosures: The authors have no conflicts of interest.

Data Availability: The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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