Relation of nutrients and hormones in polycystic ovary syndrome¹–³

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ABSTRACT

Background: Insulin resistance, infertility, and hirsutism, common characteristics of polycystic ovary syndrome (PCOS), improve with even modest weight loss. Optimal dietary treatment for PCOS is not known.

Objective: We compared the effects of acute protein administration with those of glucose challenges on hormones related to obesity and insulin resistance (ie, cortisol and insulin), hirsutism (ie, dehydroepiandrosterone [DHEA] and androstenedione), and hunger (ie, ghrelin).

Design: Patients with PCOS (n = 28; aged 26 ± 2 y) were tested with a 5-h oral-glucose-tolerance test (OGTT) and a euvolemic, euenergetic protein challenge.

Results: Glucose ingestion caused larger fluctuations in blood glucose and more hyperinsulinemia than did protein (P < 0.01, overall treatment-by-time interaction). During the protein challenge, cortisol and DHEA declined over 5 h. During OGTT, cortisol and DHEA increased after the third hour and began to show significant divergence from protein in the fourth hour (P < 0.01). During OGTT, 18 patients who had a blood glucose nadir of <69 mg/dL had elevated cortisol (baseline: 10.4 ± 0.4; nadir: 5.9 ± 0.1; peak: 12.7 ± 0.9 μg/dL) and DHEA (baseline: 15.6 ± 1.3; nadir: 11.2 ± 1.0; peak: 24.6 ± 1.6 ng/mL) (P < 0.01), whereas the remaining 10 patients with a glucose nadir of 76 ± 2 mg/dL had no increase in adrenal steroids. Both glucose and protein suppressed ghrelin (from 935 ± 57 to 777 ± 51 pg/mL and from 948 ± 60 to 816 ± 61 pg/mL, respectively). After glucose ingestion, ghrelin returned to baseline by 4 h and increased to 1094 ± 135 pg/mL at 5 h. After the protein challenge, ghrelin remained below the baseline (872 ± 60 pg/mL) even at 5 h. The overall treatment effect was highly significant (P < 0.0001).

Conclusions: Glucose ingestion caused significantly more hyperinsulinemia than did protein, and it stimulated cortisol and DHEA. Protein intake suppressed ghrelin significantly longer than did glucose, which suggested a prolonged satietogenic effect. These findings provide mechanistic support for increasing protein intake and restricting the simple sugar intake in a PCOS diet. Am J Clin Nutr 2007;85:688–94.

KEY WORDS  Polycystic ovary syndrome, PCOS, whey protein, adrenal steroids, ghrelin

INTRODUCTION

Polycystic ovary syndrome (PCOS) affects 6% of women; in the United States, ≈6.8 million women have PCOS. The cardinal features of PCOS are androgen excess, ovarian dysfunction, and infertility (1). Most patients with PCOS are obese and insulin resistant; almost 50% of them meet the criteria of the metabolic syndrome (2, 3) as defined by the National Cholesterol Education Program Adult Treatment Panel III (4). Their risk of type 2 diabetes is significantly increased (1). Gestational diabetes may also be more common in PCOS (5–7), although the evidence is not conclusive (8).

It is important to recognize that even modest amounts of weight loss improve all of the manifestations of PCOS: weight loss decreases insulin resistance, serum androgen concentrations, ovarian size, and the number of ovarian cysts; it increases ovulation and fertility; and it improves the concentrations of plasma lipids (9–11). Despite the importance of weight loss, the optimal dietary treatment for PCOS is not known. High-protein diets are being promoted (12) because of their beneficial effects on satiety (13, 14), lean body mass (15–17), weight maintenance (18, 19), and lipid markers (10). High-fat diets are being used to reduce insulin response (20).

These desirable outcomes may partly be due to concomitant decreases in dietary carbohydrates, glycemic index, and glycemic load (21–23). Unfortunately, the mechanisms underlying the differential effects of nutrients are not known. Thus, the overall aim of this research was to compare the acute hormonal effects of eucaloric, euvolemic glucose with those of protein ingestion, with a long-term goal of defining the optimal dietary treatment strategies for persons with PCOS. We focused on the hormones influencing clinical features of PCOS. These hormones included insulin, adrenal steroids, cortisol—which causes insulin resistance, dyslipidemia, central obesity, and hypertension (24)—and dehydroepiandrosterone (DHEA) and androstenedione—which constitute the substrates for peripheral testosterone synthesis (25). The hunger signal ghrelin was

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measured to compare the potential effects of glucose with those of protein on satiety (26, 27).

Changes in these hormones were compared by using the oral-glucose-tolerance test (OGTT) and a eucaloric, euvoletic whey protein challenge. OGTT was used because it is well standardized to identify insulin resistance, impaired glucose tolerance, and diabetes (28). Whey protein was used because it is considered a “fast protein” that does not coagulate in the stomach and that rapidly increases plasma concentrations of amino acids, exerts an insulinoitropic effect, and reduces postprandial glycermia in healthy subjects and persons with type 2 diabetes (13, 29).

Although standard OGTT is conducted over 2 h, this test was extended to 5 h because, in a previous study in patients with PCOS, we observed that several participants experienced symptoms suggestive of hypoglycemia after a 2-h OGTT (30). Therefore, a 5-h OGTT was used to document postprandial hypoglycemia, if it occurred (31).

The 5-h testing also allowed us to determine the changes in ghrelin during a period that is similar to the usual interval between meals. Previously published reports showed changes in ghrelin in 2–3 h, which is a shorter time than the usual between-meal interval (32–34).

SUBJECTS AND METHODS

Subjects

Twenty-eight patients with PCOS aged 18–45 y and with a body mass index (in kg/m²) of 25–40 were recruited. All participants were examined by the principal investigator (SEK-K), who is the director of the PCOS program at the Medical Center of the University of California, Davis. The participants fulfilled the Rotterdam criteria for PCOS (35) by having ovarian dysfunction, as evidenced by amenorrhea (no periods for >6 mo) or oligomenorrhea (<8 periods/y, cycle length >45 d, or both), and clinical (hirsutism) or laboratory evidence for hyperandrogenemia (total testosterone >54 ng/dL or free testosterone >9.2 pg/mL). Ultrasound evaluation of the ovarian structure was not carried out because most ultrasound reports do not provide the detailed information required by the Rotterdam criteria (presence of ≥12 follicles in each ovary, with each follicle measuring 2–9 mm in diameter; increased ovarian volume > 10 mL; or a combination, although the subjective appearance of PCO is not adequate).

Adult-onset 21-hydroxylase deficiency was ruled out by measuring basal concentrations of 17-hydroxyprogesterone in the morning. Although this measurement is routinely made during the follicular phase of the cycle, most patients had oligomenorrhea or amenorrhea, and, thus, the follicular phase could not be defined. The mean basal concentration of 17-hydroxyprogesterone was 53.0 ± 3.4 ng/dL; the highest value was 106.0 ng/dL. Because all of the basal morning concentrations of 17-hydroxyprogesterone were <200 ng/dL, none of the participants required a cortisyn-stimulation test. Prolactinoma was ruled out by measuring the serum prolactin concentration, and androgen-secreting tumors were ruled out based on serum testosterone (36–38). Cushing’s disease was ruled out clinically because the Rotterdam criteria require biochemical testing only when there is clinical suspicion. Patients were excluded if they used oral contraceptives, antiandrogen medications, insulin sensitizers, d-chiro inositol, or any other medications or supplements that affect weight or insulin sensitivity during the preceding 2 mo; have impaired glucose tolerance, diabetes mellitus, untreated hypothyroidism, and any other systemic illness such as renal, hepatic, and gastrointestinal disease; smoke; or drink > 2 alcoholic drinks/wk.

Written informed consent was obtained from all subjects. The study protocol was approved by the Human Subjects Committee of the University of California, Davis.

Oral-glucose-tolerance and protein challenge tests

The OGTT and protein challenge test studies were performed at the General Clinical Research Center of the University of California, Davis. The subjects were following their habitual diets and were weight stable. Their average carbohydrate intake was 255 g/d before testing. The studies were initiated between 0700 and 0800, after an overnight fast. An intravenous catheter was placed in the forearm. The OGTT and the protein challenge test were performed 7–10 d apart, in random order. For the OGTT, the participants ingested 75 g glucose (Glucola; Allegiance Healthcare Corp, McGaw Park, IL) at 0 min. For the protein challenge, they ingested 75 g 98% pure, intact whey protein isolate containing no carbohydrate (Glambria Foods, Twin Falls, ID). The glucose and the protein drinks were euvoletic and euvoletic. The blood samples were obtained at baseline and every 30 min thereafter for 5 h. The subjects remained supine in bed throughout the testing to avoid confounding effects of physical activity on blood glucose. The samples for glucose were collected in sodium fluoride–containing tubes on ice. Other samples were collected in either serum separation tubes or tubes containing EDTA or heparin. The nursing staffs who collected the samples and the laboratory personnel who carried out the assays were blinded. Before data analysis, a glucose concentration <69 mg/dL was defined as hypoglycemia.

Laboratory assays

Glucose was measured with the use of the hexokinase method in a clinical chemistry analyzer (Poly-Chem System, Cortlandt Manor, NY). Insulin and total ghrelin concentrations were measured with the use of radioimmunoassay kits (Linco Research Inc, St Charles, MO); the CVs were 8.2% and 7%, respectively. Cortisol, DHEA, and androstenedione were measured with the use of radioimmunoassay kits (Diagnostic System Laboratories, Webster, TX); the CVs were 5.3%, 7.8%, and 4.3%, respectively.

Statistical analysis

Statistical analysis was programmed with the R 2.1.1 language and environment (The R Foundation for Statistical Computing, Auckland University, Auckland, New Zealand; Internet: http://www.r-project.org/). Data were presented as means ± SEs, and P < 0.05 was considered significant. Linear mixed models with random intercept were used as a primary tool for the statistical analysis. The random intercept was shared by measurement on the same subject to take into account the subject-specific effect and to adjust for correlated error structure of observations. Time was treated as a categorical factor to allow enough flexibility in reproducing the complex longitudinal patterns observed in marker measurements. Interaction terms of type of challenge (glucose compared with protein) with time were introduced to address the effect at each particular time point as well as overall. Symbolically, the model can be represented in the form of Marker = intercept + main effect (time) + interaction effect (time × treatment)/subject, where intercept models mean marker
value at time zero before treatment is applied. Main effect (time) models the mean marker response over time under the OGTT challenge. Interaction effect (time × treatment) models the difference in mean marker response by time point between protein challenge and OGTT. All terms are adjusted for the subject-specific effect that contributes to between-subject variability of measurements and a correlation of measurements over time. Validity of the model was assessed with the use of residual plots. Testing for treatment differences was done for every time point as well as overall; the latter testing involved testing all coefficients in the treatment by block of time. A model-based analysis that is resistant to type I error inflation resulting from multiple comparisons was used. All model-based hypothesis testing was based on the so-called Wald test, which is equivalent to the likelihood ratio test for large samples. Akaike information criterion was monitored to prevent inflated type I error and overfitting.

As a descriptive analysis, the area under the curve (AUC) summary measure was computed for each subject. A paired t test was used to assess the treatment effect on the AUC. AUC summary measures were computed with the use of the Symponent quadrature method. In numeric computation of the AUC integrals, missing values were treated according to “last observation carried forward” principle. AUC analysis was performed for descriptive and interpretation purposes, and no adjustment was made for multicomparisons.

Baseline, lowest, and peak values for cortisol and DHEA were defined on the basis of the longitudinal trajectory of the marker in each subject. The baseline was the value obtained at 0 min before the administration of glucose or protein challenge; the nadir was the lowest value; the peak was the highest value after the nadir. These definitions were adopted before (and without the knowledge of the results of) the statistical analysis. A statistical linear mixed model was then applied to such clinically assessed measurements.

RESULTS
Clinical characteristics of the participants
The mean age was 26 ± 2 y, the weight was 97.5 ± 4.1 kg, and the body mass index was 35.9 ± 1.2. The mean serum concentrations were 0.84 ± 0.12 ng/mL for testosterone, 37.2 ± 6.5 nmol/L for sex hormone–binding globulin, and 215 ± 32 ng/mL for DHEA sulfate (DHEAS). (The reference values in 19 women with regular menstrual cycles were 0.27 ± 0.03 ng/mL for testosterone, 68.5 ± 6.6 mmol/L for sex hormone–binding globulin, and 116 ± 24 ng/mL for DHEAS.) The mean morning 17-hydroxyprogesterone concentration was 53.0 ± 3.4 ng/dL, and the highest value was 106.0 ng/dL.

Changes in plasma glucose and insulin
Twenty-eight subjects completed the 5-h OGTT, and 23 subjects completed both the 5-h OGTT and the protein challenge test (Figure 1). The order of the tests was randomized, and no effect of the treatment order on test results was observed. Protein challenge did not significantly affect plasma glucose (baseline: 97 ± 2 mg/dL; peak at 30 min: 103 ± 3 mg/dL; nadir at 240 min: 91 ± 2 mg/dL; \( P = 0.30–0.75 \) when glucose concentrations at different time points were compared with the baseline concentration). During the OGTT, plasma glucose concentrations were higher during the first 3 h but below the baseline afterward (baseline: 98 ± 2 mg/dL; peak at 60 min: 156 ± 8 mg/dL; nadir at 240 min: 76 ± 2 mg/dL). Except \( t = 0 \) (no treatment at this point) and \( t = 180 \) min (crossing point), a significant treatment effect was observed at all time points (\( P < 0.001 \)). Although whey protein also increased insulin secretion (as is consistent with the known stimulatory effects of amino acids), plasma insulin concentrations were significantly higher after glucose ingestion. For example, the peak insulin concentrations were 132.1 ± 12.5 \( \mu \)U/mL at 30 min after glucose ingestion and 97.2 ± 12.2 \( \mu \)U/mL at 60 min after protein (\( P < 0.001 \)). Overall, a highly significant difference was observed in insulin concentrations by treatment (\( P < 0.0001, \) model-based). However, timepoint differences at 3 h and thereafter showed no significant difference. As seen in Figure 1, it was evident that the insulin response during the first 2.5 h was responsible for the overall significance, when glucose concentrations were above the baseline; the glucose group had significantly higher insulin concentrations than did the protein group. Because of the crossing curves, overall comparison of insulin AUC showed no significant difference: some of the
effects before and after 3 h canceled each other out. Yet, when only the early part of the curve is included in the AUC analysis, the result was highly significant ($P = 0.009$, paired t test).

**Changes in serum cortisol, DHEA, and androstenedione**

During the protein challenge test, serum cortisol and DHEA declined during 5 h (Figure 2). Cortisol decreased from a baseline of 10.1 ± 0.6 to 6.2 ± 0.4 μg/dL, and DHEA decreased from a baseline of 15.6 ± 1.6 to 10.7 ± 1.0 ng/mL ($P < 0.0001$). During the OGTT, serum cortisol and DHEA declined until the third hour but did not decrease further after that time. Cortisol decreased from a baseline of 10.2 ± 0.6 to 7.6 ± 0.5 μg/dL at the third hour and then increased to 8.9 ± 0.5 μg/dL at 5 h; DHEA decreased from a baseline of 15.4 ± 1.5 to 11.7 ± 1.1 ng/mL at the third hour and then increased to 15.7 ± 1.4 ng/mL. Timepoint model–based analysis showed a highly significant difference by treatment at or after $t = 240$ min for cortisol ($P < 0.01$) and DHEA ($P < 0.001$). This observation is confirmed by a paired t test comparing AUC by treatment for cortisol ($P = 0.090$) and DHEA ($P = 0.004$). The differences in the AUC were smaller because the values responsible for these differences were concentrated at the late portion of the experiment, which explained the borderline result for cortisol AUC.

Changes in androstenedione also showed similar trends. During the OGTT, the average androstenedione values were 1.77 ± 0.20, 1.46 ± 0.10, 1.39 ± 0.13, 1.56 ± 0.11, 1.58 ± 0.12, and 1.64 ± 0.10 ng/mL at 0 min and 1, 2, 3, 4, and 5 h, respectively. During the protein challenge test, these values were 1.53 ± 0.16, 1.23 ± 0.10, 1.18 ± 0.14, 1.24 ± 0.13, 1.32 ± 0.15, and 1.40 ± 0.13 ng/mL, respectively. The protein challenge showed consistently lower concentrations of androstenedione than did OGTT. However, the overall treatment effect was not significant ($P = 0.221$, model-based).

Next, we examined whether the increases in adrenal steroids were related to the changes in plasma glucose. Before data analysis, we defined the hypoglycemic group as subjects with a glucose nadir of <69 mg/dL during OGTT. We contrasted baseline and peak measurements against the nadir and introduced an interaction term to allow testing for the peak response. The hypoglycemic group ($n = 18$) had larger increases in cortisol and DHEA than did the nonhypoglycemic group ($n = 10$). Cortisol concentrations were 10.1 ± 0.5 μg/dL at baseline, 5.9 ± 0.1 μg/dL at nadir, and 12.7 ± 0.9 μg/dL at peak in the hypoglycemic group and 10.4 ± 0.4 μg/dL at baseline, 6.1 ± 0.1 μg/dL at nadir, and 7.8 ± 1.0 μg/dL at peak in the nonhypoglycemic group ($P < 0.01$). The DHEA concentrations were 15.6 ± 1.3 ng/mL at baseline, 11.2 ± 1.0 ng/mL at nadir, and 24.6 ± 1.6 ng/mL at peak in the hypoglycemic group and 13.2 ± 3.3 ng/mL at baseline, 9.1 ± 1.2 ng/mL at nadir, and 11.9 ± 1.9 ng/mL at peak in the nonhypoglycemic group ($P < 0.01$). The hypoglycemic group also had a greater increase in androstenedione from the nadir to the peak than did the nonhypoglycemic group ($P = 0.06$).

Characteristics of the hypoglycemic group were that these 18 subjects were significantly less obese (94.1 ± 5.0 kg) and had significantly lower fasting plasma glucose (91.5 ± 2.2 mg/dL) than did the remaining 10 subjects (weight: 106.8 ± 4.1 kg, $P = 0.07$; fasting glucose 106 ± 4 mg/dL, $P < 0.001$). No difference was observed between fasting insulin concentrations in the 2 groups.

**Changes in plasma ghrelin**

Both protein and glucose ingestions suppressed ghrelin (from 935 ± 57 to 777 ± 51 pg/mL and from 948 ± 60 to 816 ± 61 pg/mL, respectively). After glucose ingestion, ghrelin returned to baseline by 4 h and increased to 1094 ± 135 pg/mL at 5 h. After the protein challenge, ghrelin remained below the baseline even at 5 h (872 ± 60 pg/mL) (Figure 3). Significant treatment differences were found during the 3–5-h period, and the overall treatment effect was highly significant ($P < 0.0001$). The ghrelin response during OGTT was not related to the presence or absence of hypoglycemia (data not shown).

**DISCUSSION**

This study showed that glucose ingestion caused larger fluctuations in blood glucose and more hyperinsulinemia than did the intact whey protein. Hyperinsulinemia contributes to obesity by stimulating lipoprotein lipase and fatty acid synthase. The lipoprotein lipase enzyme releases the fatty acids from the triacylglycerol-rich lipoproteins, and fatty acid synthase facilitates the storage of fatty acids as triacylglycerol in the adipose tissue. Simultaneously, insulin inhibits the hormone-sensitive lipase, thus interfering with the mobilization of the triacylglycerols stored in the adipose tissue. Hyperinsulinemia also contributes to the other endocrine abnormalities seen in PCOS: insulin
concentrations and DHEA occurred in all the subjects who had blood glucose.

The novel finding of our study was that the prompt increase in cortisol with plasma glucose or stimulate adrenal steroids. It is well established that hypoglycemia stimulates secretion of pituitary ACTH, which, in turn, stimulates adrenal steroids (31). In fact, cortisol is known to suppress ghrelin independent of the changes in glucose concentrations (59, 60). Brogli (26) showed that both oral glucose intake and intravenous insulin administration suppressed ghrelin, although they had opposite effects on plasma glucose. It is interesting that the same study showed that intravenous arginine did not suppress ghrelin, despite increasing insulin. Therefore, the oral route may be necessary for protein-induced suppression of ghrelin. Alternatively, the intrinsic properties of proteins may be important. The protein used in this study, whey protein, does not coagulate in the stomach, increases plasma concentrations of amino acids rapidly, and may suppress hunger more effectively (29).

Differences between responses to the 2 treatments are expressed through treatment-by-time interaction effect. Overall, the interaction effect is highly significant ($P < 0.001$). Key timepoint $P$ values are shown on the graph. All tests are based on the Wald test performed by using a linear mixed model applied to all available data.

Our observations related to ghrelin can be important to the nutritional management of PCOS. The findings that protein ingestion suppressed ghrelin for a longer time than glucose and that it did not cause a rebound increase are consistent with the recent report of Blom et al (58) and suggest that protein intake may prolong satiety. Previous reports indicated that protein intake neither suppressed nor increased ghrelin concentrations (32–34). Those studies compared the ingestion of glucose with that of solid mixed meals or determined the ghrelin response during 2-3 h. We compared glucose and protein in equal weights, volumes, and calories over a longer period. The protein-induced suppression of ghrelin was probably due to the increase in insulin. Insulin is known to suppress ghrelin independent of the changes in glucose concentrations (59, 60). Brogli (26) showed that both oral glucose intake and intravenous insulin administration suppressed ghrelin, although they had opposite effects on plasma glucose. It is interesting that the same study showed that intravenous arginine did not suppress ghrelin, despite increasing insulin. Therefore, the oral route may be necessary for protein-induced suppression of ghrelin. Alternatively, the intrinsic properties of proteins may be important. The protein used in this study, whey protein, does not coagulate in the stomach, increases plasma concentrations of amino acids rapidly, and may suppress hunger more effectively (29).

DFigure 3. Mean ± SE changes in total plasma ghrelin during oral-glucose-tolerance test (−−; $n = 28$) and protein challenge (−; $n = 23$). Differences between responses to the 2 treatments are expressed through treatment-by-time interaction effect. Overall, the interaction effect is highly significant ($P < 0.001$). Key timepoint $P$ values are shown on the graph. All tests are based on the Wald test performed by using a linear mixed model applied to all available data.
In summary, oral glucose intake caused larger fluctuations in plasma glucose, increased hyperinsulinemia, and stimulated adrenal steroid secretion in patients with PCOS. In addition, glucose intake suppressed the hunger signal ghrelin for a shorter period of time than did protein intake. These acute challenge studies showed that nutrients have significantly different endocrine effects and that protein may be a preferred nutrient over glucose for patients with PCOS. The findings of these acute studies need to be validated with the use of natural foods and carbohydrate-enriched rather than protein-enriched diets. Further research is necessary to determine whether foods with high glycemic load contribute to the progression of obesity, insulin resistance, and hirsutism in PCOS patients by stimulating counterregulatory hormones and increasing hunger. In addition, these studies need to be extended to both nonobese and obese women without PCOS to determine whether postprandial hypoglycemia has significant adverse consequences in these populations.

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