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EATING AND APPETITE REGULATING HORMONES IN WOMEN WITH
POLYCYSTIC OVARY SYNDROME AND
WEIGHT-MATCHED CONTROLS

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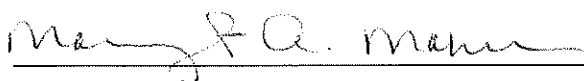
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EATING AND APPETITE REGULATING HORMONES IN WOMEN WITH
POLYCYSTIC OVARY SYNDROME AND
WEIGHT-MATCHED CONTROLS

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We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Master of Science in Biology.

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ABSTRACT

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Background: Polycystic ovarian syndrome (PCOS) is a common condition in women that affects health and fertility. It is characterized by hyperandrogenism, insulin resistance, and oligo- or anovulation. Insulin resistance is associated with excess weight and the elevated androgens in PCOS promote central (android) deposition of fat. This may lead to distress and healthy or unhealthy efforts to manage weight.

Objectives: To determine if women with PCOS exhibit disordered eating behaviors beyond weight-matched controls and if eating behaviors are associated with appetite-regulating hormones.

Study Design: Eight women with PCOS and eight weight-matched controls completed a medical and diet history questionnaire, eating attitudes test, diet frequency questionnaire, diet interview, DEXA scan, ultrasound, and fasting blood draw for hormone assays.

Main Results: Women with PCOS reported greater incidence of disordered eating, decreased daily food and micronutrient intakes, and increased fat and saturated fat intakes. There were no significant differences in fasting hormone levels between groups. Variable significance and/or contrasting correlations for some factor combinations were observed between groups.

Conclusion: Eating disorder in women with PCOS was not observed. Consistent with some other studies, trends toward subclinical disordered eating and dysregulation of appetite-regulating hormones in this study merit further study.

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INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most common endocrine (hormonal) disorders in women, affecting 5-10% of women of child-bearing age (Bethea and Nestler 2008; Ek et al. 2002; Tan et al. 2010). PCOS has been associated with multiple signs and symptoms which include insulin resistance syndrome (also known as metabolic syndrome), menstrual irregularity, infertility, obesity, acne, androgenic alopecia (male-patterned hair loss), accumulation of unruptured follicles on the periphery of the ovaries, sleep apnea, depression, dyslipidemia, high blood pressure, and elevated serum testosterone, androstenedione, and insulin (Michelmores et al. 2001; PCOS Association 2009). The diversity of signs and symptoms associated with PCOS is due to the heterogeneous nature of the syndrome (Carmina and Lobo 1999). Some of the characteristics of PCOS above may be linked to appetite, diet, and eating behaviors, which are of interest in the present study.

Obesity and PCOS

More than 35% of adult women in the United States are obese (Turner-McGrievy et al. 2015). Weight excess is even more common in women with PCOS, with approximately 50% classified as overweight or obese (Silfen et al. 2003). With the high prevalence of weight excess in PCOS patients, obesity susceptibility and insulin resistance related genes are of study interest (Tan et al. 2010). Studies have also compared endocrine and metabolic factors in lean and obese women with PCOS (Silfen et al. 2003). Insulin resistance is present in both obese and lean women with PCOS.

Obese women with PCOS have increased metabolic risk from both the syndrome itself and excess visceral fat tissue (Bethea and Nestler 2008). This metabolic risk increases the likelihood that women with PCOS will develop Type 2 Diabetes Mellitus (DM2) and associated adverse health consequences (Ehrmann et al. 1999).

Appetite Regulating Signals

Metabolism and eating behaviors are regulated by physiological signals from the nervous and endocrine (hormone) systems as well as by environmental factors. For instance, the hormone ghrelin is secreted by the stomach when the stomach is empty and functions to stimulate appetite and control energy balance and long-term regulation of body weight and composition (Schofl et al. 2002; Ursavas et al. 2010) through actions at the growth hormone secretagogue receptor (GHS-R). Ghrelin has also been shown to be involved in ovarian function (Gargari et al. 2015). Leptin, a hormone produced by fat (adipose) tissue throughout the body, as fat cells are filled, induces a feeling of fullness, known as satiety (Rask-Andersen et al. 2009; Ursavas et al. 2010). Leptin has been shown to be significantly correlated with body mass index (Baig et al. 2014). Studies have shown leptin plays a major role in interactions that take place between the body's nutritional status and the hypothalamic-pituitary-ovarian axis (Gargari et al. 2015).

Appetite Regulating Signals and PCOS

The relationships of appetite regulating hormones have been studied in obese subjects, with fasting ghrelin levels lower than lean controls and fasting leptin levels higher than lean controls (Ursavas et al. 2010). Ghrelin studies conducted in PCOS women have shown differing results, with some studies reporting decreased fasting ghrelin levels when compared to controls and others reporting no significant difference

(Daghestani et al. 2011; Gargari et al. 2015; Ma et al. 2014). The correlation between ghrelin levels and body mass index (BMI) has also been studied, where increased BMI was associated with lower ghrelin levels (Glintborg et al. 2006). Leptin has not been sufficiently studied in women with PCOS compared to controls, though a few studies have reported no significant difference in PCOS women and controls when measuring leptin levels (Glintborg et al. 2006; Baig et al. 2014). The significant differences that have been found seem paradoxical to what one would expect given the normal function of these hormones. As with the insulin resistance in DM2, these paradoxical findings may be a result of target tissue resistance to these hormones in overweight and obese subjects.

Appetite Regulating Hormones and Disordered Eating

Ghrelin and leptin have also been studied in subjects with eating disorders. Ghrelin levels and/or responsiveness are altered in anorexia nervosa (AN) but this has been interpreted as a compensatory mechanism of the body to control appetite (Rask-Andersen et al. 2009). A loss of function mutation in the ghrelin system has been hypothesized to decrease orexigenic (appetite-stimulating) hormone responsiveness and facilitate food restriction in some AN patients. A malfunction in the leptin system may also contribute to eating disorders and result in excessive food intake and obesity (Rask-Andersen et al. 2009).

Menstrual Dysfunction and Disordered Eating

The relationship between menstrual dysfunction and eating disorders has also been widely studied. Amenorrhea, the absence of a menstrual period for at least three consecutive cycles, has been a central feature in AN, while oligomenorrhea, irregular

menstrual cycles, occurs in about half of women with bulimia nervosa (BN). Nutritional status and metabolic disturbances are two factors identified that contribute to this complex relationship and can interfere in the gonadotropin and gonadal hormones essential for reproductive function. A gonadotropin deficiency caused by malnutrition and extreme weight-control behaviors is thought to be the cause of amenorrhea in AN. A disruption in the hypothalamic-pituitary-gonadal axis due to restrictive energy intake and purging is thought to be the cause of oligomenorrhea in BN (Algars et al. 2014).

Menstrual dysfunction seen in eating disorders also means ovulatory function is disrupted. The chronic fluctuations in energy balance, whether due to dieting, overeating, or overexercising, can lead to disruptions in the physiological feedback loops that contribute to fertility. Studies have shown an increased incidence of cancelled fertility treatment cycles, poorer drug response rates, lower fertilization rates, and increased incidence of early pregnancy losses in underweight and obese patients (Rodino et al. 2016). Obese women with PCOS, therefore, may experience compounding problems when it comes to menstruation and fertility.

PCOS, Diet, and Disordered Eating

Subjects with PCOS often report carbohydrate craving and may have aberrant eating behaviors associated with their metabolic dysfunction (Grassi 2007). Studies have shown women with PCOS have dietary intake high in fat and saturated fat and low in fiber, folate, iron, carbohydrates, and whole grains (Turner-McGrievy et al. 2015). There is good reason to believe that either physiological alterations in hormone levels or psychological responses to excessive weight gain may lead to disordered eating in the PCOS population.

Depression and depressive symptoms are present in 29-50% of women with PCOS, who have also been found to have a 3.8 times higher lifetime incidence of depressive episodes than age-matched women (Pastore et al. 2011). Studies have shown women with PCOS and hirsutism to have high levels of co-morbid depression and anxiety and also suffer from low self-esteem and poorer social adjustment when compared to controls (Morgan et al. 2008). Quality of life studies in women with PCOS have shown they have low satisfaction with their fertility and weight status (Turner-McGrievy et al. 2015). In a mental health study involving women with PCOS, approximately 15% of women with PCOS indicated they had psychological distress. These women differed from other women with PCOS only in BMI, a higher BMI was associated with higher psychological distress. This suggests obesity is likely a significant factor in mental health struggles in women with PCOS (Annagur et al. 2013).

Treatment of PCOS

Current treatment for PCOS focuses on clinical signs and symptoms management. Insulin resistance is commonly treated with oral antidiabetic agents. Ovulation problems are treated with specific hormone regimens, depending on women's reproductive goals. Regardless of reproductive goals, women may be helped with hormone therapy that produces a consistent ovulation pattern, which reduces dysmenorrhea and decreases the risk for endometrial cancer. Treatment of hirsutism may greatly help women's self-esteem and reduce risk for depression. Options for treating androgenic alopecia and hirsutism include oral or topical androgen-targeted therapies and hair removal procedures (Stovall, 2003). Another treatment focus is weight loss with even a slight weight reduction significantly improving insulin resistance and ovulation. Weight loss also

increases the efficacy of some infertility treatments in women with PCOS. The first-line therapy for women with PCOS and weight excess is dieting; however, dietary changes alone are not always sufficient (Ma et al. 2014). Studies have compared different diets in women with PCOS and revealed conflicting results. Certain diets may have a beneficial effect on one or more feature of PCOS, but an optimal dietary composition for women with PCOS has not been identified. Therefore, weight loss has been recommended with efforts focused on reducing caloric intake while maintaining adequate nutrient intake with healthy food choices (Moran et al. 2013).

Studies have looked at appetite regulating hormones in PCOS and eating behaviors in women with PCOS, but have not addressed correlations of appetite regulating hormones with eating-related factors. The purpose of the present study is to explore the presence of disordered eating in women with PCOS versus weight matched Controls and if there are relationships between disordered eating and appetite-regulating hormones.

METHODS

Study Design

Twenty female participants were recruited through print advertisements and signed the informed consent approved by the Institutional Review Board (IRB) for the Protection of Human Subjects at the University of Wisconsin-La Crosse (Appendix A). Eight participants with Polycystic Ovary Syndrome (PCOS) and eight age and weight-matched Control participants completed the study. Two participants withdrew due to scheduling conflicts and two relocated during the study.

Participant Dynamics

Criteria for inclusion in the study were female gender, age between 18-60 years, and preferably overweight. Participants were asked to not alter any medication or diet intake prior to blood sampling, other than following the overnight fasting instruction.

Medical and Diet History Questionnaire

All participants completed a medical and diet history questionnaire before beginning any procedures in the study (Appendix B). The questionnaire asked about medical conditions and medications currently in use that may affect the study parameters.

Eating Attitudes Test - (EAT-26)

All participants completed the EAT-26, a brief screening tool for eating disorders (Appendix C). The EAT-26 is a widely used standardized measure of symptoms and concerns characteristic of eating disorders. Though the EAT-26 does not yield a specific diagnosis of an eating disorder, it provides the basis for referral decisions.

National Institutes of Health Diet History Questionnaire II

All participants completed a 40 page National Institutes of Health (NIH) Diet History Questionnaire II that assesses typical dietary intake over the past year (Appendix D). Questions included multiple items from all food groups and corresponding portion sizes. Data was analyzed using the Diet Calc program (NIH, Bethesda, MD).

Diet Interview

All participants completed a diet interview with a registered dietitian to discuss daily eating habits (Appendix E). Answers to initial prompts and follow up questions were recorded in writing by the interviewer for later qualitative analyses. The interviewer sought subjective information about daily eating habits including timing, quantity, and selections; food cravings, restriction, and avoidance; weight concerns; dieting history; and history of diet counseling and education. All interview responses were conducted and coded for quantification by the same registered dietitian.

Transabdominal or Vaginal Ultrasound

All participants with PCOS had either a transabdominal or vaginal ultrasound depending on the preference of the participant. The ultrasounds were performed using a SonoSite M-Turbo (Fujifilm, Bothell, WA) and pictures with measurements were taken of each ovary and the uterus.

Dual Energy X-ray Absorptiometry (DEXA) Scan

All participants underwent a DEXA scan to estimate body composition including fat mass, lean mass, android/gynoid fat ratio, and bone density. The DEXA scan was conducted using the Prodigy Lunar scanner (GE Healthcare, United Kingdom) and consisted of a full body scan. Participants were asked for height and weight before the

DEXA, and if unknown participants were measured or weighed. A gown was provided for the participant to change into if there was any metal on the participant's clothing.

Fasting Blood Draw

After a 12-hour fast, participants underwent a routine venipuncture to obtain a blood sample of approximately 15 mL. A 6 mL portion for serum collection was treated with the protease inhibitor 4-(2-Aminoethyl)-benzenesulfonyl fluoride (AEBSF), to stabilize the ghrelin levels, at a concentration of 1 mg/mL and allowed to clot for 30 minutes. The blood was centrifuged at 2,500 rpm for 15 minutes to separate the serum. Two milliliters of serum were aliquoted into a separate tube and acidified using HCl to a final concentration of 0.05N. A 6 mL portion for plasma collection was collected in lithium heparin tubes and spun at 2,500 rpm for 10 minutes to separate the plasma from the cells. The plasma was then aliquoted into two analysis tubes, one was frozen at -20°C for analysis of insulin, leptin, and ghrelin; the other was sent to Gundersen Health System, La Crosse, WI for analysis of estradiol, testosterone, and glucose. A 3 mL whole blood sample was collected in EDTA, refrigerated, and sent to Gundersen Health System, La Crosse, WI for analysis of glycated A1C hemoglobin.

Estradiol

Plasma samples were analyzed with the Cobas e601 (Roche Diagnostics, Indianapolis, IN) at Gundersen Health System, La Crosse, WI. The analysis was conducted by a competitive test principle using a polyclonal antibody specifically directed against 17 β -estradiol. Estradiol present in the participant sample was released by mesterolone and bound to the estradiol-specific biotinylated antibody. An estradiol derivative labeled with a ruthenium complex for the binding sites on the biotinylated

antibody was added and bound to the vacant sites of the biotinylated antibodies.

Streptavidin-coated microparticles were added to form an antibody-hapten complex. The mixture was aspirated into the measuring cell and the microparticles were magnetically captured onto the surface of the electrode. Voltage application to the electrode induced chemiluminescent emission which was measured by a photomultiplier. Results were determined with an instrument-specific two point calibration curve and master curve determined by the reagent barcode.

Testosterone

Plasma samples were analyzed with the Cobas e601 (Roche Diagnostics, Indianapolis, IN) at Gundersen Health System, La Crosse, WI. The analysis was conducted by a competitive test principle. Testosterone in the participant sample was incubated with a biotinylated monoclonal testosterone-specific antibody and bound to the binding sites. Streptavidin-coated microparticles and a testosterone derivative labeled with a ruthenium complex was added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The mixture was aspirated into the measuring cell and the microparticles were magnetically captured onto the surface of the electrode. Voltage application to the electrode induced chemiluminescent emission which was measured by a photomultiplier. Results were determined with an instrument-specific two point calibration curve and master curve determined by the reagent barcode.

Glucose

Plasma samples were analyzed with the Cobas e501 (Roche Diagnostics, Indianapolis, IN) at Gundersen Health System, La Crosse, WI. The analysis was conducted by an enzymatic reference method with hexokinase. Hexokinase

catalyzed the phosphorylation of glucose in the participant sample by ATP to form glucose-6-phosphate and ADP. A second enzyme, glucose-6-phosphate dehydrogenase was used to catalyze oxidation of glucose-6-phosphate by NADP⁺ to form NADPH. The concentration of NADPH formed was directly proportional to the glucose concentration in the sample as measured photometrically at 340 nm.

Insulin

Plasma samples were analyzed by solid phase two-site human insulin ELISA kit (Abnova KA0921 Neihu District, Taipei City, Taiwan). The analysis was based on the direct sandwich technique where two monoclonal antibodies were directed against separate antigenic determinants on the insulin molecule. Insulin in the sample reacted with enzyme (HRP)-conjugated anti-insulin antibody and anti-insulin antibody bound to the microtitration well. A chromogenic solution (TMB substrate) was added to react with the labeled antibody and caused a color change. A stop solution (1.0 N HCl) was added after the substrate incubation and the chromogenic reaction was halted. The color absorbance was measured at 450 nm on a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA) and was proportional to the insulin concentration.

Leptin

Plasma samples were analyzed by solid phase sandwich human leptin ELISA (Invitrogen Kit KAC2281, Camarillo, CA). Microtitration wells with pre-coated monoclonal anti-human leptin antibody reacted with leptin present in participant samples. A secondary biotinylated monoclonal antibody was added to bind to a second site on the leptin. Streptavidin-peroxidase enzyme was added and bound to the biotinylated antibody. A substrate solution reacted with the bound enzyme to produce a yellow color.

The reaction was halted by a stop solution and colorimetric absorbance was measured at 450 nm on a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA). The absorbance was proportional to the leptin concentration.

Ghrelin

Serum samples were analyzed by sandwich human total ghrelin ELISA (EMD Millipore Kit EZGRT-89K, St. Charles, MO). Microtitration wells with pre-coated anti-human ghrelin IgG reacted with the ghrelin (both active and des-octanoyl forms) in participant samples. A secondary biotinylated antibody was added and bound to a second site on the ghrelin. Streptavidin-horseradish peroxidase conjugate was added and bound to the biotinylated antibody. A substrate solution reacted with the bound conjugate to produce a yellow color and the reaction was halted by a stop solution. Colorimetric absorbance was measured at 450 nm and corrected from the absorbance measured at 590 nm on a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA). The measured absorbance was proportional to the ghrelin concentration.

Glycated A1C Hemoglobin

Whole blood (EDTA) samples were analyzed with the Cobas e501 (Roche Diagnostics, Indianapolis, IN) at Gundersen Health System, La Crosse, WI. The participant samples were hemolyzed using a tetradecyltrimethylammonium bromide detergent to eliminate interference from leukocytes. The glycated A1C hemoglobin (HbA1c) was determined by a turbidimetric inhibition immunoassay. The HbA1c in the participant samples reacted with the anti-HbA1c antibody to form soluble antigen-antibody complexes. A polyhapten reagent was added and reacted with the excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be

measured turbidimetrically. The liberated hemoglobin in the hemolyzed sample was converted to a derivative having a characteristic absorption spectrum which was measured bichromatically. The final result was expressed as percent HbA1c and was calculated from the HbA1c/Hb ratio according to the equation: $\text{HbA1c (\%)} = (\text{HbA1c/Hb}) \times 91.5 + 2.15$. The glycated A1C hemoglobin levels reflected the average blood sugar level for the previous two to three months.

Statistical Analyses

Statistical analyses were conducted using independent T tests, correlation studies and Fisher's Exact analysis with SPSS Version 23 (IBM Corporation, Armonk, NY). The independent variable was PCOS condition and scaled dependent variables included: ghrelin, testosterone, leptin, insulin, glucose, EAT-26 score, A1C, BMI, Fat Ratio, bone density, menstrual characteristics, daily intake of macronutrients and micronutrients, and eating behaviors. Correlations were considered significant at $p < 0.05$. Categorical dependent variables used to assess responses from the diet interview are detailed in the results section.

RESULTS

Anthropometrics

Participants were roughly weight-matched (Table 1) based on BMI. The BMI result was calculated from self-reported heights and self-reported weights unless the participant did not know her weight, so all BMI results reported herein are estimates from reported and/or actual measurements. There was a slight difference in mean age, 32.9 ± 3.5 years in participants with PCOS compared to 35.0 ± 4.5 years in Control participants. There was no difference in mean BMI, (30.8 kg/m^2) between groups.

Table 1. Participant demographics for PCOS and Control groups.

	PCOS (n=8)	Control (n=8)
Mean Age (years)	32.9 ± 3.5	35.0 ± 4.5
Age Range (years)	24 - 55	21 - 55
Mean BMI (kg/m^2)	30.8 ± 2.1	30.8 ± 3.0
BMI Range (kg/m^2)	23.4 – 36.9	21.1 – 46.9

Medical and Diet History

The medical and diet history questionnaire revealed expected differences in the medical history of participants with PCOS compared to Control participants. Participants with PCOS reported a combination of abnormal facial hair, abnormal hair loss, absent menstruation, acanthosis nigricans (darkened patches on the skin), asthma, fatigue, ovarian cysts, and Polycystic Ovary Syndrome where Control participants did not. Also, acne, depression, heavy periods, and obesity were reported more often in participants with PCOS than Control participants. Diabetes, thyroid disease, high blood pressure, and

high cholesterol were reported more often in Control versus PCOS participants (Figure 1).

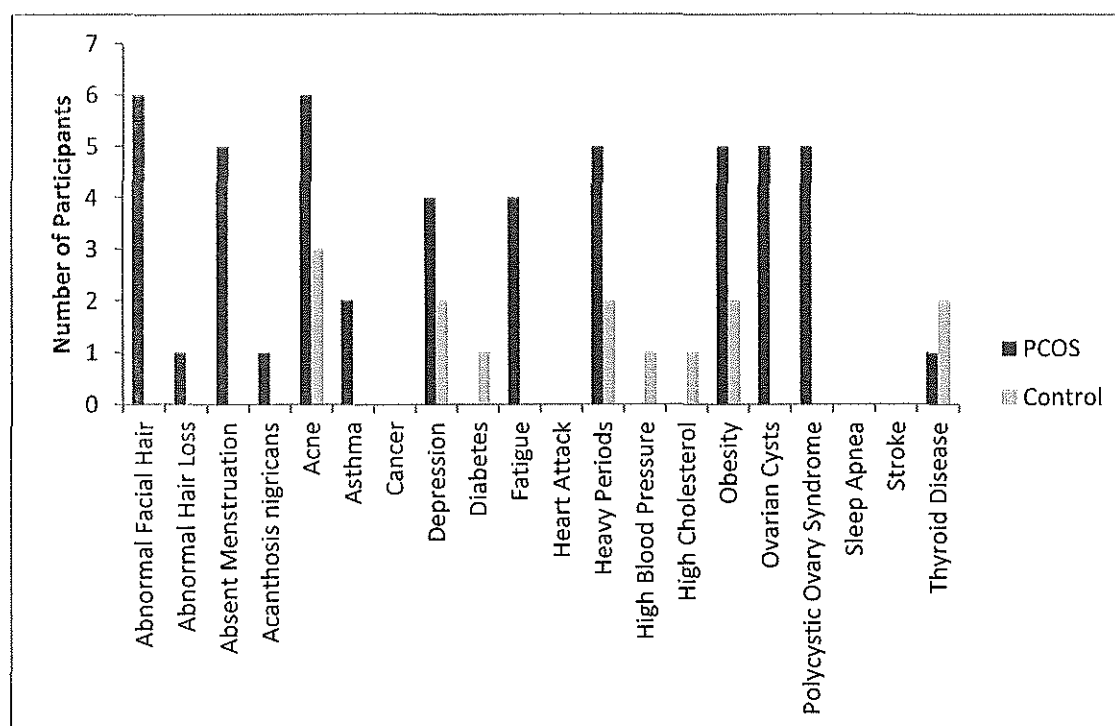


Figure 1. Medical history of study participants.

The family medical history of participants did not reveal many differences between participant groups. Abnormal facial hair, absent menstruation, acne, and asthma of family were reported by one participant with PCOS and no Control participants. Cancer, depression, diabetes, fatigue, heart attack, heavy periods, high blood pressure, obesity, sleep apnea, and stroke were reported in greater numbers in participants with PCOS compared to Control participants. Thyroid disease was the only condition reported in greater numbers in Control participants (Figure 2).

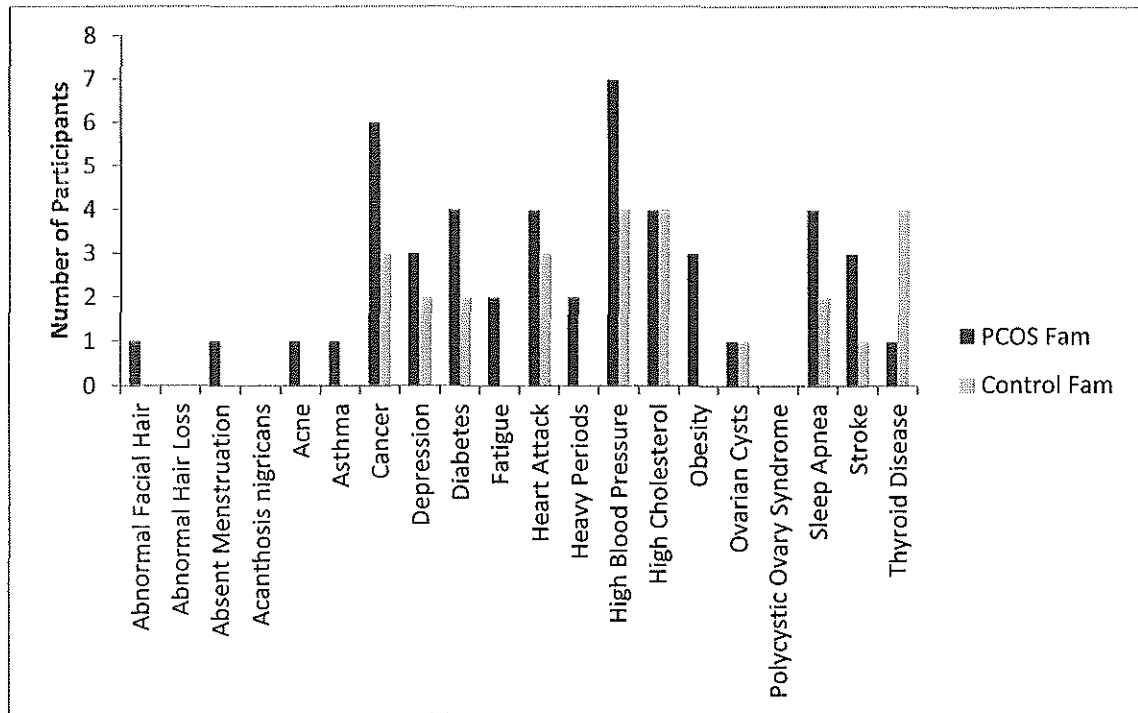


Figure 2. Family medical history of participants.

The questionnaire also asked about menstrual characteristics. Two characteristics were considered significantly greater in PCOS than in Control participants. These characteristics included absent periods and irregular periods. Only one characteristic, variable discharge, was reported in greater amounts in Control versus PCOS participants (Table 2).

Table 2. Menstrual characteristics reported by PCOS and Control participants. Data are reported as percent of participants in each group.

	PCOS (n=8)	Control (n=8)	Fisher's Exact p value
Absent	75.0%	12.5%	0.041*
Pattern			
Regular	62.5%	62.5%	1.000
Irregular	62.5%	0%	0.026*
Length			
Short	62.5%	50.0%	1.000
Long	75.0%	25.0%	0.132
Very Long	12.5%	0%	1.000
Variable	25.0%	12.5%	1.000
Discharge			
Light	50.0%	25.0%	0.608
Heavy	25.0%	0%	0.467
Variable	25.0%	37.5%	1.000
Cramps			
Minor	50.0%	37.5%	1.000
Major	37.5%	0%	0.200

*P<0.05

The questionnaire asked about weight control methods including dieting, exercise, and weight loss supplements. Participants were asked if they currently exercise regularly. There were no significant differences in response between the two participant groups (Table 3).

Table 3. Dieting and exercise characteristics reported by PCOS and Control participants. Data are reported as percent of participants in each group.

	PCOS (n=8)	Control (n=8)	Fisher's Exact p value
Dieting	87.5%	75.0%	1.000
Exercise	87.5%	87.5%	1.000
Weight Loss	12.5%	0%	1.000
Supplements			
Regular Exercise	87.5%	75.0%	1.000

Participants with PCOS, on average, began their periods earlier (12.0 ± 0.9 years) than Control participants (13.6 ± 0.6 years). Participants were also asked about pregnancy success. Four participants with PCOS and five Control participants responded that they had tried to become pregnant. Two participants with PCOS and four Control participants were successful (Fisher's Exact P value = 0.405) with pregnancy.

Participants were asked to list current medications and reason for taking (Table 4). Only one participant with PCOS was currently taking an antidiabetic. Three participants with PCOS and one Control participant were taking a selective serotonin reuptake inhibitor for the treatment of depression and/or anxiety. Three participants with PCOS and two Control participants were taking hormonal birth control. One participant in each group was taking a thyroid hormone.

Table 4. Medication list for participants with PCOS and Control participants. Data are reported as percent of participants in each group.

Medication	PCOS (n=8)	Control (n=8)
Antidiabetic	12.5%	0%
Selective Serotonin Reuptake Inhibitor (SSRI)	37.5%	12.5%
Hormonal Birth Control	37.5%	25%
Thyroid Hormone	12.5%	12.5%

Ultrasound

All participants who underwent ultrasound (PCOS participants only) had normal size and general appearance of the ovaries and uterus. One participant had a 0.7 cm cyst on her cervix. Three participants each had one ovarian cyst ranging in size from 0.4 cm to 2.4 cm. One participant had two ovarian cysts, one on each ovary, measuring 0.3 cm and 0.5 cm (Figure 3). None of the participants had multiple cysts in the ovaries.

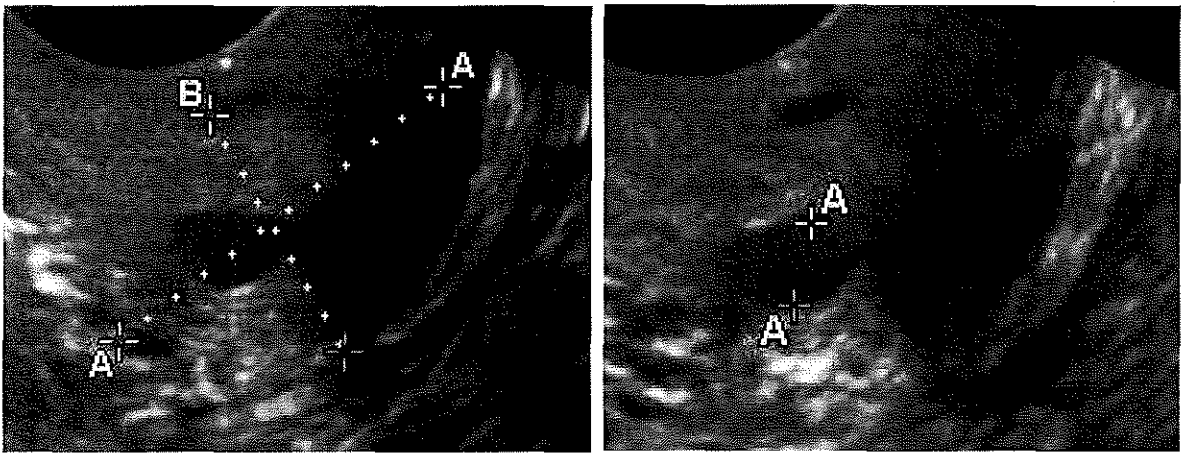


Figure 3. Example ultrasound images of an ovary and a cyst. The ovary measured 3.03 cm from points A to A x 1.99 cm from points B to B (left). The cyst measured 0.5 cm from points A to A (right).

Diet Interview

During the diet interview, participants were asked about daily eating habits, including number of eating opportunities (meals and snacks). Also discussed were any food cravings, restrictions, or avoidance. Participants were asked if they ever received nutrition advice, if they weigh themselves on a regular basis, if they have concerns about their weight, and if others are concerned about their weight. Dieting, overeating, and compensatory behaviors for overeating were discussed as well. Control participants reported self-concern about their weight and other's concern about their weight more often than participants with PCOS. Control participants also reported food avoidance

more often than participants with PCOS. PCOS participants regularly reported an average of 4.0 ± 1.2 eating opportunities, while Control participants reported 4.3 ± 0.5 eating opportunities. While there were no significant differences between PCOS and Control participants (Table 5), food intake across the eating opportunities was also lower in PCOS versus Control.

Table 5. Diet interview responses in PCOS and Control participants. Data are reported as percent of participants in each group.

	PCOS (n=8)	Control (n=8)	Fisher's Exact p value
Regular Eating Pattern	87.5%	87.5%	1.000
Meals			0.569
1	12.5%	0%	
2	25.5%	12.5%	
3	62.5%	87.5%	
Snacks			0.282
1	50.0%	75.0%	
2	50.0%	12.5%	
3	0%	12.5%	
Food Craving			1.000
Carbohydrates	50.0%	50.0%	
Savory	25.0%	37.5%	
Carbohydrates and Savory	25.0%	12.5%	
Food Avoidance			0.521
Yes	37.5%	62.5%	
Intolerance	37.5%	37.5%	
Nutrition Advice	75.0%	87.5%	1.000
Weighing	75.0%	75.0%	1.000
Self Weight Concern	62.5%	75.0%	1.000
Other's Concern about Weight	50.0%	75.0%	0.608
Dieting	50.0%	25.0%	0.608
Overeat	100.0%	100.0%	1.000
Compensation	25.0%	25.0%	1.000

EAT-26

Those who score at or above 20 on the EAT-26 are referred for potential diagnosis of eating disorder in clinical practice. None of our participants scored at or over 20. However, participants with PCOS had a higher overall EAT-26 score (6.38 ± 1.18) compared to Control participants (3.88 ± 1.60). In addition, participants with PCOS also had higher scores in dieting, bulimia and food preoccupation, and oral control subscales (Table 6), though group differences were not significant.

Table 6. EAT-26 scores with subscale differentiation. Data are reported as calculated scores $\pm$ SEM.

	PCOS (n=8)	Control (n=8)	p [*]
Mean Total Score	6.38 ± 1.18	3.88 ± 1.60	0.229
Total Score Range	2 – 13	0 – 14	
Mean Dieting Subscale	3.00 ± 0.71	2.38 ± 1.29	0.678
Dieting Subscale Range	0 – 5	0 – 11	
Mean Bulimia and Food Preoccupation Subscale	1.88 ± 0.61	0.88 ± 0.23	0.147
Bulimia and Food Preoccupation Subscale Range	0 – 5	0 – 2	
Mean Oral Control Subscale	1.50 ± 0.63	0.63 ± 0.26	0.219
Oral Control Subscale Range	0 – 5	0 – 2	

* Probability value is two-tailed

National Institutes of Health Diet History Questionnaire II

Fourteen (PCOS = 6 and Control = 8) participants completed the National Institutes of Health Diet History Questionnaire II and data were compiled using the Diet Calc program. There were no significant differences in any specific nutrient intake between PCOS and Control participants by independent T-test (Table 7), though several micronutrient differences were close to the significance cut point. Participants with PCOS reported eating less food (in grams) than Control participants ($p = 0.618$) as well as all other nutrients calculated. Participants with PCOS had a higher percent energy

from total saturated fat and total fat. The average report of participants with PCOS was less than the recommended daily intake for several micronutrients including thiamin, folate, and magnesium compared to Control participants. Both groups reported a lower daily intake of iron than recommended; however, participants with PCOS reported even less iron intake than Control participants. Participants with PCOS reported a daily intake of energy from total fat just over the recommended value.

Table 7. Daily nutrient intake of PCOS and Control participants. Data are presented as mean $\pm$ SEM. Averages different than recommendations are underlined.

	PCOS (n=6)	Control (n=8)	p [*]	Recommended Intake
Food Weight (g)	3759 $\pm$ 825	4514 $\pm$ 1111	0.618	---
Energy kCal	1589 $\pm$ 205	1660 $\pm$ 134	0.766	---
Carbohydrate (g)	191.2 $\pm$ 26.0	212.5 $\pm$ 20.1	0.474	130
Protein (g)	62.9 $\pm$ 7.7	67.7 $\pm$ 7.6	0.922	46
Alcohol (g)	3.0 $\pm$ 1.2	4.1 $\pm$ 1.3	0.803	---
Cholesterol (mg)	210.0 $\pm$ 41.8	295.7 $\pm$ 64.6	0.523	<300
Retinol (μ g)	386.7 $\pm$ 66.1	557.5 $\pm$ 51.8	0.672	---
Vitamin K (μ g)	98.8 $\pm$ 37.2	168.0 $\pm$ 61.4	0.570	---
Vitamin C (mg)	104.1 $\pm$ 27.5	110.9 $\pm$ 22.0	0.325	75
Thiamin (mg)	<u>1.07 $\pm$ 0.11</u>	1.43 $\pm$ 0.14	0.061	1.1
Riboflavin (mg)	2.03 $\pm$ 0.35	2.56 $\pm$ 0.25	0.395	1.1
Niacin (mg)	16.8 $\pm$ 2.2	19.8 $\pm$ 1.6	0.848	14
Total Folate (μ g)	<u>304.5 $\pm$ 48.0</u>	449.8 $\pm$ 52.2	0.080	400
Vitamin B6 (mg)	1.48 $\pm$ 0.20	2.08 $\pm$ 0.25	0.233	1.3 (19-50); 1.5 (50+)
Vitamin B12 (μ g)	3.88 $\pm$ 0.58	5.90 $\pm$ 0.85	0.275	2.4
Calcium (mg)	<u>998.0 $\pm$ 205.1</u>	1209.8 $\pm$ 225.5	0.071	1000 (19-50); 1200 (50+)
Iron (mg)	<u>10.2 $\pm$ 1.7</u>	15.1 $\pm$ 1.7	0.105	18 (19-50); 8 (50+)
Magnesium (mg)	<u>284.3 $\pm$ 39.4</u>	361.1 $\pm$ 48.1	0.093	310 (19-30); 320 (30+)
Phosphorus (mg)	1195.7 $\pm$ 160.8	1303.4 $\pm$ 127.7	0.516	700
Zinc (mg)	8.98 $\pm$ 1.0	11.2 $\pm$ 0.7	0.065	8
Sodium (mg)	2314.8 $\pm$ 297.1	2461.6 $\pm$ 328.5	0.263	1500 (19-50); 1300 (50+)
Selenium (μ g)	75.2 $\pm$ 9.8	92.8 $\pm$ 12.0	0.605	---
% Energy from Total SFA	<u>12.8 $\pm$ 2.7</u>	10.5 $\pm$ 0.9	0.096	$\leq$ 10
% Energy from Total Fat	<u>35.7 $\pm$ 4.0</u>	33.5 $\pm$ 2.4	0.755	25 – 35, though recently revised
Total Grain Servings	3.5 $\pm$ 0.7	4.0 $\pm$ 0.5	0.302	3 – 10

* Probability value is two-tailed

Fat, Glucose, and Hormone Levels

Fat indices, including total fat and android:gynoid fat ratio, were measured using the DEXA scan. There were no significant differences between participants with PCOS and Control participants (Figure 4). Glucose indices, including fasting glucose, glucose to insulin ratio and hemoglobin A1C, were measured using fasting blood samples. There were no significant differences between participants with PCOS and Control participants (Figure 5). However, the glucose to insulin ratio was 12.8 ± 2.0 in participants with PCOS and 11.8 ± 1.6 in Control participants and the hemoglobin A1C was $5.2 \pm 0.08\%$ in participants with PCOS and $5.5 \pm 0.09\%$ in Control participants. Hormone indices, including leptin, ghrelin, insulin, testosterone and estradiol, were also measured from fasting blood samples. There were no significant differences between participants with PCOS and Control participants for leptin, ghrelin, and estradiol. However, testosterone was 38.1 ± 7.2 ng/dl in participants with PCOS and 22.7 ± 4.5 ng/dl in Control participants which was significantly different ($p = 0.045$, one tailed) (Figure 6). Bone density was measured during the DEXA scan and there was no significant difference between participants with PCOS and Control participants (Figure 7; Table 8).

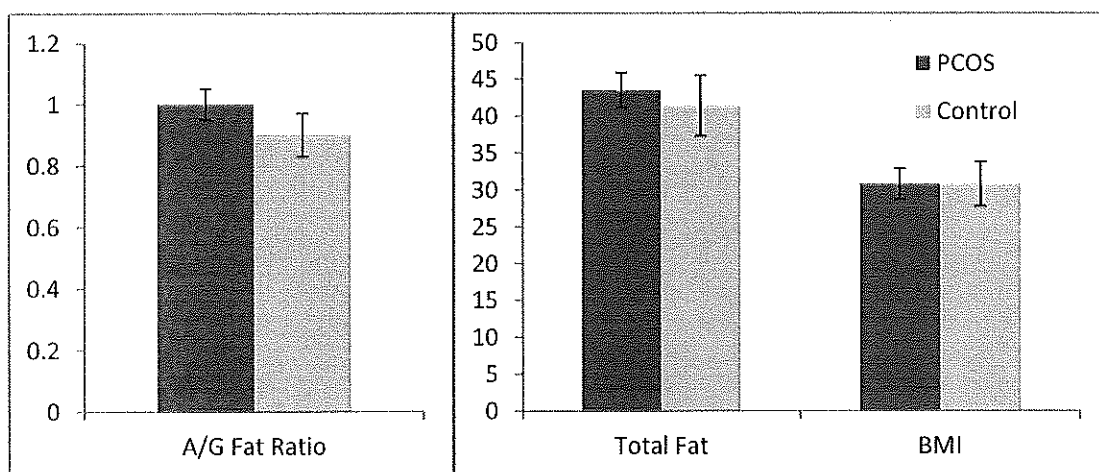


Figure 4. Fat indices for participants with PCOS and Control participants. Data presented as mean $\pm$ SEM.

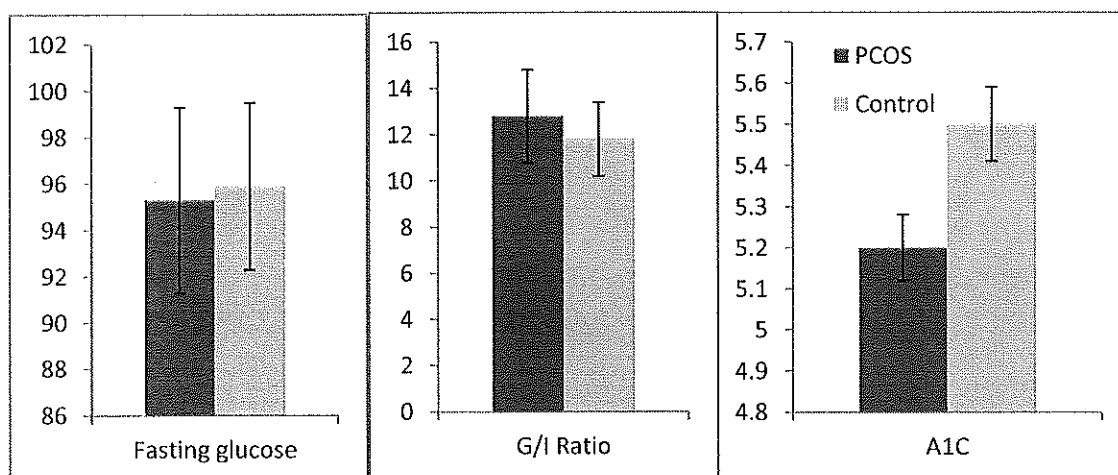


Figure 5. Glucose indices for participants with PCOS and Control participants. Data presented as mean $\pm$ SEM.

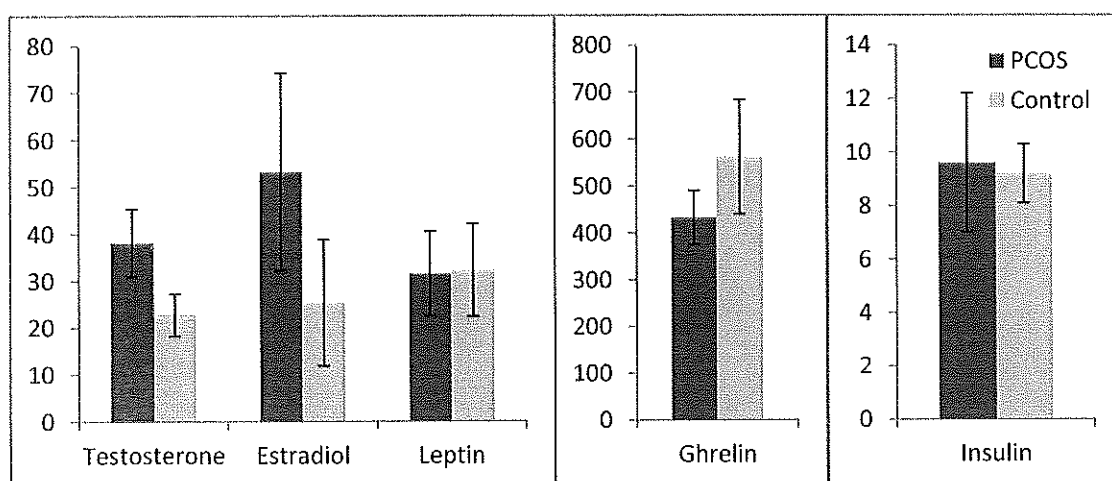


Figure 6. Hormone indices for participants with PCOS and Control participants. Data presented as mean $\pm$ SEM.

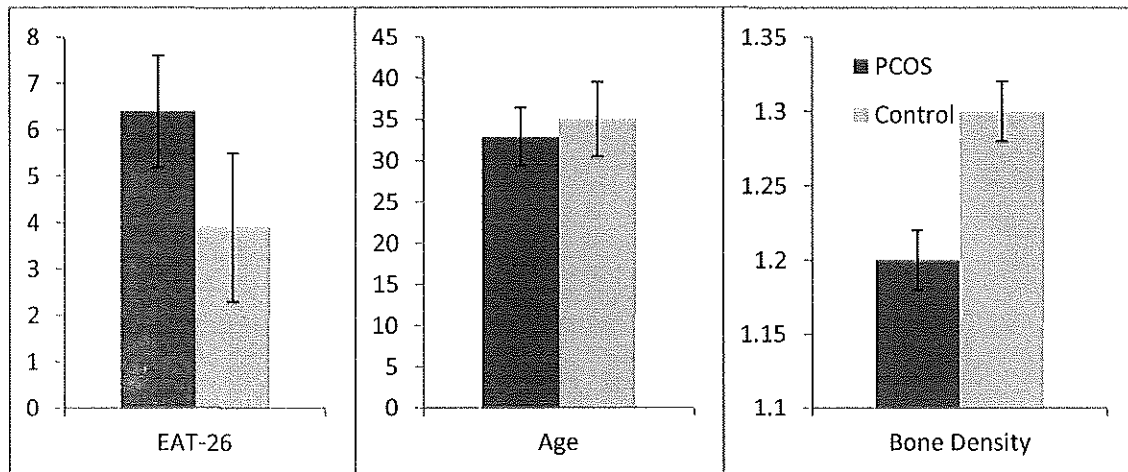


Figure 7. Other characteristics in participants with PCOS and Control participants. Data presented as mean $\pm$ SEM.

Table 8. Fat, glucose, and hormone characteristics in PCOS and Control participants. Data presented as geometric mean $\pm$ SEM.

	PCOS (n=8)	Control (n=8)	p [*]
Fat indices			
Total fat	43.5 $\pm$ 2.4	41.4 $\pm$ 4.1	0.663
A/G Fat ratio	1.0 $\pm$ 0.05	0.9 $\pm$ 0.07	0.291
BMI (kg/m ²)	30.8 $\pm$ 2.1	30.8 $\pm$ 3.0	0.995
Glucose indices			
Fasting glucose (mg/dl)	95.3 $\pm$ 4.0	95.9 $\pm$ 3.6	0.909
Glucose:Insulin ratio	12.8 $\pm$ 2.0	11.8 $\pm$ 1.6	0.709
A1C	5.2 $\pm$ 0.08	5.5 $\pm$ 0.09	0.059
Hormone indices			
Leptin (ng/ml)	31.5 $\pm$ 9.0	32.2 $\pm$ 9.9	0.960
Ghrelin (pg/ml)	432.8 $\pm$ 57.9	562.3 $\pm$ 121.6	0.352
Insulin (uIU/ml)	9.6 $\pm$ 2.4	9.2 $\pm$ 1.1	0.898
Testosterone (ng/dl)	38.1 $\pm$ 7.2	22.7 $\pm$ 4.5	0.091
Estradiol (pg/ml) ¹	53.2 $\pm$ 21.0	25.3 $\pm$ 13.5	0.281
Other			
EAT-26 score	6.4 $\pm$ 1.2	3.9 $\pm$ 1.6	0.229
Age (years)	32.9 $\pm$ 3.5	35.0 $\pm$ 4.5	0.715
Bone density	1.2 $\pm$ 0.02	1.3 $\pm$ 0.02	0.059

A/G, android:gynoid; BMI, body mass index, A1C, glycohemoglobin. ^{*} Probability value is two-tailed. ¹ Estradiol levels measuring <5.0 pg/ml were treated as 5.0 pg/ml.

Correlation Studies

Correlation studies were done using fat, glucose, nutrient, and hormone characteristics and compared to ghrelin, leptin, and EAT-26 score. There were a number of significant correlations between scaled values seen in participants with PCOS and Control participants (Table 9).

Table 9. Pearson correlation coefficients (r) between fat, glucose, nutrient, and hormone characteristics and ghrelin, leptin, and EAT-26 scores in PCOS and Control participants.

	PCOS			Control		
	Ghrelin	Leptin	EAT-26	Ghrelin	Leptin	EAT-26
Age	0.775*	-0.096	0.291	-0.175	-0.070	-0.263
BMI	0.055	0.757*	0.685	-0.833*	0.705*	0.160
Total Fat	-0.050	0.893**	0.400	-0.730*	0.783*	0.414
A/G Fat Ratio	0.266	0.642	0.464	-0.624	0.448	0.031
EAT-26	-0.140	0.299	---	-0.207	0.229	---
Bone Density	-0.495	0.122	0.006	0.121	0.241	-0.492
Fasting Glucose	0.535	0.262	0.344	-0.534	0.301	0.053
A1C	0.007	0.693	0.716*	-0.423	0.136	-0.168
Testosterone	0.058	0.033	0.414	0.723*	-0.099	0.056
Estradiol	0.250	-0.288	-0.512	-0.093	-0.329	-0.124
Insulin	0.245	0.081	0.180	-0.431	0.261	-0.547
Fasting G:I Ratio	0.148	-0.260	-0.090	0.232	-0.143	0.685
Ghrelin	---	-0.292	-0.140	---	-0.570	-0.207
Leptin	-0.292	---	0.299	-0.570	---	0.229
kCal	0.282	-0.805*	---	-0.176	0.296	---
Carbohydrates	0.616	-0.479	---	0.085	0.044	---
Protein	0.503	-0.948**	---	-0.220	0.258	---

BMI, body mass index; A/G, android:gynoid; A1C, glycohemoglobin; G:I, glucose to insulin. *P<0.05, **P<0.01

Two strong correlations were significant in both groups: total anthropometric fat and leptin (PCOS $r(8) = 0.893$, $p = 0.003$; Control $r(8) = 0.783$, $p = 0.022$) and BMI and leptin (PCOS $r(8) = 0.757$, $p = 0.030$; Control $r(8) = 0.705$, $p = 0.051$). There were four strong correlations significant in participants with PCOS and not Control participants.

These included: age and ghrelin (PCOS $r(8) = 0.775$, $p = 0.024$; Control $r(8) = -0.175$, p

= 0.679), kCal and leptin (PCOS $r(6) = -0.805$, $p = 0.054$; Control $r(8) = 0.296$, $p = 0.476$), protein and leptin (PCOS $r(6) = -0.948$, $p = 0.004$; Control $r(8) = 0.258$, $p = 0.537$) and hemoglobin A1C and EAT-26 score (PCOS $r(8) = 0.716$, $p = 0.046$; Control $r(8) = -0.168$, $p = 0.691$). There were three strong correlations significant in Control participants and not participants with PCOS. These included: BMI and ghrelin (PCOS $r(8) = 0.055$, $p = 0.897$; Control $r(8) = -0.833$, $p = 0.010$), total anthropometric fat and ghrelin (PCOS $r(8) = -0.050$, $p = 0.906$; Control $r(8) = -0.730$, $p = 0.040$) and testosterone and ghrelin (PCOS $r(8) = 0.058$, $p = 0.891$, Control $r(8) = 0.723$, $p = 0.043$).

While not considered significant, there were fourteen cases where trendlines were either positive in participants with PCOS and negative in Control participants or negative in participants with PCOS and positive in Control participants. These included:

android:gynoid fat ratio and ghrelin (PCOS $r(8) = 0.266$, $p = 0.524$; Control $r(8) = -0.624$, $p = 0.098$), bone density and ghrelin (PCOS $r(8) = -0.495$, $p = 0.212$; Control $r(8) = -0.121$, $p = 0.776$), fasting glucose and ghrelin (PCOS $r(8) = 0.172$; Control $r(8) = -0.534$, $p = 0.172$), hemoglobin A1C and ghrelin (PCOS $r(8) = 0.007$, $p = 0.987$; Control $r(8) = -0.423$, $p = 0.297$), estradiol and ghrelin (PCOS $r(8) = 0.250$, $p = 0.551$; Control $r(8) = -0.093$, $p = 0.827$), insulin and ghrelin (PCOS $r(8) = 0.245$, $p = 0.558$; Control $r(8) = -0.431$, $p = 0.286$), kCal and ghrelin (PCOS $r(6) = 0.282$, $p = 0.588$; Control $r(8) = -0.176$, $p = 0.677$), protein and ghrelin (PCOS $r(6) = 0.503$, $p = 0.309$; Control $r(8) = -0.220$, $p = 0.601$), testosterone and leptin (PCOS $r(8) = 0.033$, $p = 0.937$; Control $r(8) = -0.099$, $p = 0.815$), carbohydrates and leptin (PCOS $r(6) = -0.479$, $p = 0.336$; Control $r(8) = 0.044$, $p = 0.917$), age and EAT-26 score (PCOS $r(8) = 0.291$, $p = 0.484$; Control $r(8) = -0.263$, $p = 0.529$), bone density and EAT-26 score (PCOS $r(8) = 0.006$, $p = 0.990$; Control $r(8) = -$

0.492, $p = 0.215$), insulin and EAT-26 score (PCOS $r(8) = 0.180$, $p = 0.670$; Control $r(8) = -0.547$, $p = 0.161$), and fasting glucose:insulin ratio and EAT-26 score (PCOS $r(8) = -0.090$, $p = 0.832$; Control $r(8) = 0.685$, $p = 0.061$).

In both groups, leptin and BMI were positively correlated (PCOS $r(8) = 0.757$, $p = 0.030$; Control $r(8) = 0.705$, $p = 0.051$), total anthropometric fat and leptin was also positively correlated in both groups (PCOS $r(8) = 0.893$, $p = 0.003$; Control $r(8) = 0.783$, $p = 0.022$). However, kCal and leptin had a positive trendline in Control participants ($r(8) = 0.296$, $p = 0.476$) and was negatively correlated in participants with PCOS ($r(6) = -0.805$, $p = 0.054$). The relationship between kCal and BMI also differed between the two groups with a positive trendline in Control participants ($r(8) = 0.302$, $p = 0.467$) and a negative trendline in participants with PCOS ($r(6) = -0.432$, $p = 0.392$) (Figure 8).

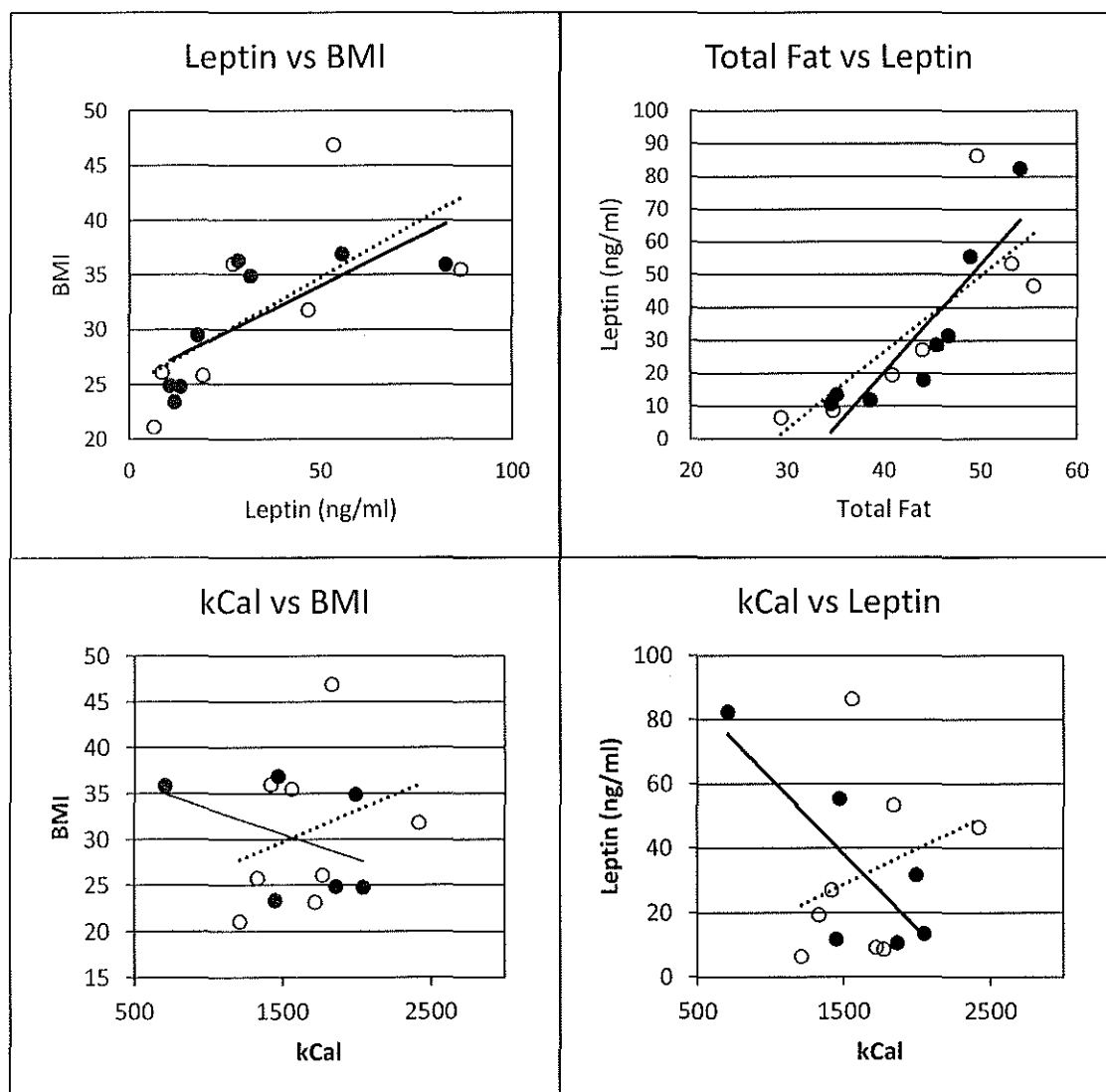


Figure 8. Correlation studies for leptin, BMI, total fat, and kilocalories (kCal). Values for participants with PCOS (● and solid lines) and Control participants (○ and dashed lines).

The relationship of testosterone and android:gynoid fat ratio was also different in participants with PCOS and Control participants. In participants with PCOS the trendline was positive ($r(8) = 0.398$, $p = 0.329$), while there was a negative correlation in Control participants ($r(8) = -0.823$, $p = 0.012$). Opposite relationships were also seen in ghrelin and BMI. In participants with PCOS, the trendline was slightly positive ($r(8) = 0.055$, $p = 0.897$), while there was a negative correlation in Control participants ($r(8) = -0.833$, $p = 0.010$). The relationship between testosterone and ghrelin showed a slight positive

trendline in participants with PCOS ($r(8) = 0.058$, $p = 0.891$) and a positive correlation in Control participants ($r(8) = 0.723$, $p = 0.043$). The relationship observed in android:gynoid fat ratio and ghrelin trended positive in participants with PCOS ($r(8) = 0.266$, $p = 0.524$) and trended negative in Control participants ($r(8) = -0.624$, $p = 0.098$) (Figure 9).

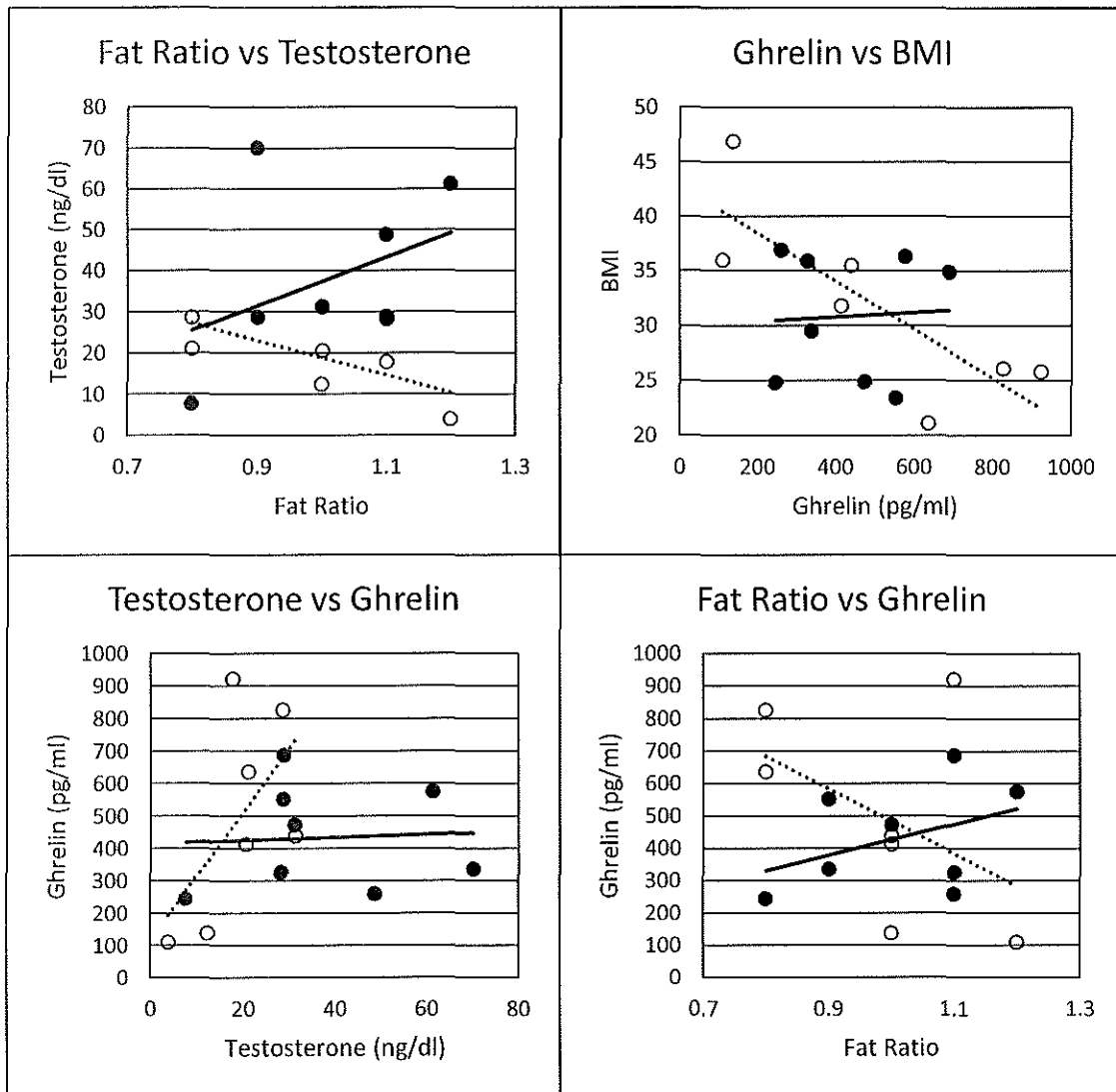


Figure 9. Correlation studies for fat ratio, testosterone, ghrelin, and BMI. Values for participants with PCOS (● and solid line) and Control participants (○ and dashed line).

DISCUSSION

Unlike studies in hirsute women with PCOS (Morgan et al. 2008), and similar to eating disorder studies in women with PCOS and women undergoing fertility treatments (Michelmores et al. 2001; Rodino et al. 2016), this study demonstrated no significant association between PCOS and eating disorder. However, several observed variables suggested trends toward disordered eating and dysregulation of appetite-regulating hormones that may be more detectable in larger samples of the PCOS population, as discussed below. In addition, interesting observations not directly related to the intended purpose of the study are also discussed.

Medical and Diet History

The medical and diet history questionnaire was geared toward the signs and symptoms involved in PCOS. Therefore, the expectations were that participants with PCOS would have a higher rate of reporting for those signs and symptoms. As expected, participants with PCOS reported numerous signs and symptoms related to PCOS (abnormal facial hair, abnormal hair loss, absent menstruation, acanthosis nigricans, and ovarian cysts) more often than Control participants. However, Control participants reported some signs and symptoms of metabolic syndrome (diabetes, high blood pressure, and high cholesterol) more than participants with PCOS. This is of interest because studies have shown women with PCOS to have a higher incidence of metabolic syndrome (Bethea and Nestler 2008).

The family history of the participants with PCOS was similar to that of the Control participants. Diabetes and high blood pressure, two of the markers involved in metabolic syndrome, were reported more in the family history of participants with PCOS compared to Control participants. This is in contrast to the participant medical history where the Control participants reported these markers more than the participants with PCOS.

An interesting observation in this study was the reporting of thyroid disease. Control participants reported thyroid disease more often in their own medical history and family history compared to participants with PCOS. This is interesting because studies have shown a high prevalence of thyroid disorders in women with PCOS. In these cases, multiple and confounding hormone disturbances may initiate, maintain, or worsen both conditions and greatly affect fertility (Sinha et al. 2013). However, thyroid comorbidity with PCOS was not observed in this small sample size study.

Oligo- and anovulation are part of the diagnostic criteria for PCOS and therefore, absent or irregular periods would be expected to be reported in women with PCOS as was observed in this study. These characteristics were reported significantly more in participants with PCOS compared to Controls. Long menstrual bleeding, light and heavy discharge, and major and minor cramps were also reported more in participants with PCOS. Collectively the reported menstrual characteristics show more dysmenorrhea in participants with PCOS, which is consistent with previous studies.

Ultrasound

The possibility for cysts in the ovaries is greater with the diagnosis of PCOS, though presence of ovarian cysts is not a requirement for diagnosis. Ultrasound

visualization of polycystic ovaries does not necessarily reflect anovulation and is therefore, not one of the diagnostic criteria for PCOS. Moreover, women who meet the NIH criteria for PCOS may not have polycystic ovaries upon ultrasound (Stovall 2003). In fact, many women with ovulatory disorders other than PCOS may also demonstrate polycystic ovaries (Azziz, 2006). Polycystic-appearing ovaries also occur in the normal population with 16-25% of women displaying this ovarian morphology (Carmina and Lobo 1999). Thus, it is not unusual that only half (four of eight) of our participants with PCOS had at least one ovarian cyst and three of the eight participants were on some type of hormonal birth control, which can reduce ovarian cysts. The presence of a cervical cyst in one participant is of unknown origin or consequence.

Diet Interview

Control participants expressed concern about their weights and reported avoiding certain foods more than participants with PCOS. Since women with PCOS are informed and thus expect that overweight may be associated with their diagnosis, this may reflect a perceptual normalization for persons who have a condition (PCOS in this case) often associated with overweight and obesity. When looking at eating opportunities, women with PCOS had slightly fewer than Control participants. This could indicate a conscious and habitual attempt to limit food intake in the PCOS group to lose weight for management of their condition. It was encouraging to find that nearly all of the women in this study were not routinely skipping meals, engaging in destructive compensatory behaviors after self-recognized overeating, or allowing their overweight or obese status to interfere with engaging in physical activity.

EAT-26

The EAT-26 scores were expected to show more disordered eating in participants with PCOS. The results of this study trended to support this hypothesis but were not statistically significant. Participants with PCOS in this small study scored higher in all subscales of the EAT-26 questionnaire, but none scored high enough to warrant a referral to a medical professional for potential diagnosis of an eating disorder. In a study using the eating disorder examination (EDE), women with PCOS on average scored higher for features of dieting and overall eating disorder symptomatology, but the differences were not significant (Michelmores et al. 2001), as observed in our study. However, a different study using the EDE observed a significantly higher score in all subsets compared to controls (Morgan et al. 2008). Yet another recent study utilizing a different quality of life and eating and exercise behaviors questionnaire reported significantly poorer eating disorder scores and eating behaviors in women with PCOS (Coker, 2016). These conflicting results outline the need for further study of the prevalence and severity of disordered eating with PCOS.

National Institutes of Health Diet History Questionnaire II

The Diet History Questionnaire showed participants with PCOS have a daily intake of less food (in grams) than Control participants (PCOS 3759 ± 825 g/day; Control 4514 ± 1111 g/day) and not surprisingly, also have a lower estimated energy intake (PCOS 1589 ± 205 kCal/day; Control 1660 ± 134 kCal/day). This is consistent with the reported lower number of eating opportunities for women with PCOS reported in the interview in this study (PCOS 4.0 ± 1.2 ; Control 4.3 ± 0.5). However, participants with PCOS reported a higher percent of energy from total saturated fat (PCOS $12.8 \pm 2.7\%$;

Control $10.5 \pm 0.9\%$) and total fat (PCOS $35.7 \pm 4.0\%$; $33.5 \pm 2.4\%$). This is consistent with a previous study looking at women with PCOS interested in losing weight and achieving pregnancy (Turner-McGrievy et al. 2016). Moreover, higher fat diets have been shown to amplify leptin resistance and obesity in animal studies (Prior et al. 2010). Therefore, a higher fat diet could potentially amplify any present leptin resistance in women with PCOS.

Participants with PCOS also reported a daily intake less than Control participants for all other nutrients calculated from the questionnaire. Furthermore, several micronutrients (folate, iron, and calcium) were reported in lower quantities than the recommended daily intake by participants with PCOS. These results are also consistent with a previous study looking at the dietary intake of women with PCOS (Turner-McGrievy et al. 2016).

The average dietary intake of a woman with PCOS observed in this study is low in some recommended nutrients and high in total fat and saturated fat. While on average the total grain servings for women with PCOS in this study was within the recommended daily intake value, two of the six participants who completed the Diet History Questionnaire had a total grain serving less than the recommended value. This could indicate a low-carbohydrate diet. Overall, the dietary intake results seen in this study are consistent with previous studies (Turner-McGrievy et al. 2016). Furthermore, a study conducted on the effect of dietary composition on anthropometric, reproductive, metabolic, and psychological outcomes in PCOS reported that a diet aimed at reducing weight was of clinical benefit in PCOS regardless of the diet composition (Moran et al. 2013).

Fat, Glucose, and Hormone Levels

In PCOS, an android fat distribution is commonly observed and is associated with insulin resistance and an increased risk of DM2 and cardiovascular disease (Glintborg et al. 2006). Consistent with this, the present study observed a more android fat distribution in participants with PCOS compared to Control participants (PCOS 1.0 ± 0.05 ; Control 0.9 ± 0.07). Also, the DEXA scan showed a greater total fat mass in the participants with PCOS (PCOS 43.5 ± 2.4 ; Control 41.4 ± 4.1). These results are as expected due to the high prevalence of obesity in women with PCOS.

The increased risk of insulin resistance and DM2 makes fasting glucose, glucose:insulin ratio and hemoglobin A1C of interest. While no significant differences were seen in this study, the glucose:insulin ratio and hemoglobin A1C results were interesting. Participants with PCOS had a slightly lower fasting glucose level compared to Control participants which was unexpected (PCOS 95.3 ± 4.0 mg/dl; Control 95.9 ± 3.6 mg/dl). However, the glucose:insulin ratio was higher in participants with PCOS (PCOS 12.8 ± 2.0 ; Control 11.8 ± 1.6). This was unexpected because previous studies have seen increased fasting insulin levels which combined with similar fasting glucose levels should result in a decreased glucose:insulin ratio. Studies also have shown the fasting glucose:insulin ratio to be a good measure of insulin sensitivity in obese women with PCOS (Legro et al. 1998). Participants with PCOS had a lower hemoglobin A1C result compared to controls (PCOS 5.2 ± 0.08 ; Control 5.5 ± 0.09). This result was unexpected because women with PCOS are thought to have glucose intolerance and increased risk for development of DM2 and one would expect glucose levels to be higher as well as hemoglobin A1C. These results may be consistent with the observation that

with lower food and energy intake and higher fat intake as % of total calories, our PCOS participants are successfully managing their blood glucose levels, despite any insulin resistance that may be present.

Studies have shown inconsistent results when looking at the appetite regulating hormones leptin and ghrelin. Some studies have shown ghrelin levels similar to Controls (Daghestani et al. 2010; Gargari et al. 2013) and some have shown decreased ghrelin levels compared to Controls (Glintborg et al. 2006; Schofl et al. 2002) in women with PCOS. This study observed no significant difference in fasting ghrelin levels (PCOS 432.8 ± 57.9 pg/ml; Control 562.3 ± 121.6 pg/ml), although, participants with PCOS did have a lower fasting ghrelin level. Studies of leptin in women with PCOS have also been inconsistent. Some studies have observed an increase in leptin levels with increasing BMI (Glintborg et al. 2006), others have seen a higher leptin level in women with PCOS (Gargari et al. 2013), and yet others have found no significant difference in leptin levels between groups (Baig et al. 2014). The present study reports no significant difference in fasting leptin levels (PCOS 31.5 ± 9.0 ng/ml; Control 32.2 ± 9.9 ng/ml).

Sex hormone levels have been of particular interest in PCOS research, specifically androgens. One of the NIH criteria for diagnosis of PCOS is hyperandrogenism. Participants with PCOS had a significantly higher testosterone level ($p = 0.045$, one tailed) compared to Control participants (PCOS 38.1 ± 7.2 ng/dl; Control 22.7 ± 4.5 ng/dl). This result was expected as testosterone has been widely studied in women with PCOS. Estradiol was not significantly different between participant groups but was higher in participants with PCOS (PCOS 53.2 ± 21.0 pg/ml; Control 25.3 ± 13.5 pg/ml). This result was consistent with a previous study in women with PCOS (Daghestani et al.

2011) and may be related to the increased levels of the precursors, namely testosterone, for estradiol production.

An interesting observation in this study was the difference in bone density. While not significant, participants with PCOS had a slightly lower bone density compared to Control participants (PCOS 1.2 ± 0.02 ; Control 1.3 ± 0.02). A previous study looking at bone density and PCOS found a significantly lower bone density in women with PCOS compared to Control women. The difference in bone density was hypothesized to be a result of abnormal sex hormone levels and insulin levels (Katulski et al. 2014). Average calcium intake was slightly lower than recommended in our PCOS group, while Control intake was above recommended levels.

Correlation Studies

Strong positive correlations were observed in this study between total anthropometric fat and leptin (PCOS $r(8) = 0.893$, $p = 0.003$; Control $r(8) = 0.783$, $p = 0.022$) and BMI and leptin (PCOS $r(8) = 0.757$, $p = 0.030$; Control $r(8) = 0.705$, $p = 0.051$) in both participant groups. As total anthropometric fat or BMI increased, leptin levels also increased. These results are consistent with other studies (Baig et al. 2014). Leptin is produced in fat (adipose) tissue and therefore, would be expected to be correlated to anthropometric fat and BMI.

Four strong correlations were observed in participants with PCOS but not in Control participants. Two of which, age and ghrelin ($r(8) = 0.775$, $p = 0.024$); hemoglobin A1C and EAT-26 score ($r(8) = 0.716$, $p = 0.046$), were positive correlations and could be random occurrences seen in this study. The other two, kCal and leptin ($r(6) = -0.805$, $p = 0.054$); protein and leptin ($r(6) = -0.948$, $p = 0.004$), were negative

correlations and were unexpected. Leptin is a satiety hormone and therefore would be expected to have a positive correlation with energy intake (kCal). As energy intake increases, satiety and thus, leptin increases. The same is true for protein and leptin.

Three strong correlations were observed in Control participants but not participants with PCOS. A negative correlation between BMI and ghrelin was observed ($r(8) = -0.730$, $p = 0.040$) and total anthropometric fat and ghrelin ($r(8) = -0.730$, $p = 0.040$). Ghrelin is an appetite stimulating hormone and therefore, ghrelin should increase as BMI and total anthropometric fat decreases. A positive correlation was observed between testosterone and ghrelin ($r(8) = 0.723$, $p = 0.043$). Previous studies have shown a strong positive correlation between ghrelin and testosterone in men and postmenopausal women (Greenman et al. 2009).

Eight additional trends for ghrelin, while not significant or strong, provoked interest in this study and of these eight trends, only one correlation was negative in participants with PCOS while positive in Control participants.

Leptin

Consistent with our recruitment goal to achieve weight-matched control for the study, there were no significant differences in BMI or adiposity between participant groups. There were also no significant differences between participant groups in average fasting leptin (PCOS 31.5 ± 9.0 ng/ml versus Control 32.2 ± 10.0 ng/ml) or average reported daily caloric consumption (PCOS 1589 ± 205 kcal/d versus Control 1660 ± 134 kcal/day).

Given these results, some interesting differences were found when triangulating correlations among BMI/adiposity, fasting leptin, and reported average daily caloric

intake as follows. Fasting leptin was directly related to BMI and Total Fat as expected in both participant groups, given that leptin is released by fat and more adiposity provides a greater source for leptin output. However, one would expect kcal intake to be directly related to BMI, as was true in our Control participants, because higher kcal intake would shift energy balance to weight gain and result in increases in BMI. An unexpected negative relationship between kcal intake and BMI was observed in the participants with PCOS, which could be due to under or inaccurate reporting of food intake or restrictive eating behavior in this group at higher weights.

Moreover, one would expect kcal intake to be directly related to fasting leptin levels, as was true in our Control participants, because leptin, as a satiety hormone, would be secreted in conditions of more positive energy balance. An unexpected negative relationship between kcal intake and fasting leptin was observed in participants with PCOS, which would be especially unexpected if leptin resistance (like insulin resistance) is at play in PCOS. However, if the adipostat sensing of adipose tissue is impaired in PCOS, that could explain such a result and increased appetite resulting in increased food intake.

Ghrelin

Consistent with the criteria for PCOS, androgen levels (testosterone in this study) were significantly elevated in participants with PCOS compared to Controls (PCOS 38.1 ± 7.2 ng/dl; Control 22.7 ± 4.5 ng/dl). Given this result, one would expect anthropometric fat distribution to reflect an android pattern in women with PCOS, as was true in this study. Interestingly, as testosterone levels increased in Control participants, their anthropometric fat distribution became more gynoid in pattern.

Ghrelin is released from the stomach when empty to stimulate appetite, food intake, and body weight gain. Therefore, one would not expect lower ghrelin levels with higher BMIs, as was true in our Control participants. However, a weak positive correlation was observed in participants with PCOS. More ghrelin could promote appetite and higher food intake and BMI. This result is in contrast with previous studies (Daghestani et al. 2011), and higher food intake was not observed here, but this could be associated with food cravings that reportedly accompany PCOS.

Previous studies have shown testosterone to have a strong positive correlation with ghrelin in men and postmenopausal women (Greenman et al. 2009). However, in the present study, a strong positive correlation was observed between testosterone and ghrelin in Control participants. This correlation was weakly positive in participants with PCOS.

In this study, the android:gynoid fat distribution ratio was negatively correlated with testosterone in Control participants and testosterone was positively correlated with ghrelin. From these results, one would expect ghrelin levels to be lower as the fat distribution is more in the gynoid pattern, as was true in our Control participants. In participants with PCOS, as the fat distribution reflected a more android pattern, testosterone levels increased, as one would expect. Moreover, at higher testosterone levels in participants with PCOS, ghrelin levels were slightly higher. Therefore, one would suggest ghrelin levels increase as the fat distribution reflects a more android pattern, as was true in our participants with PCOS. These correlation studies, therefore, show ghrelin levels to be highest at each end of the fat distribution spectrum.

Limitations of the Study

The sample sizes, duration of this study, and compliance with participation expectations were limiting in this study. The PCOS study population was based on volunteers from the community willing to fill out questionnaires, meet with a dietitian, undergo an ultrasound and DEXA scan, and provide a fasting blood sample. Due to limited response in this smaller community, only twelve participants with PCOS were initially recruited for the study. Of these twelve, two withdrew due to scheduling conflicts and two relocated during the study. Therefore, eight BMI-matched Control participants were recruited. Of the eight participants completing most aspects of the study, two participants with PCOS did not complete the Diet History Questionnaire. The study was conducted over a span of three and a half years due to obligations associated with the study leader's educational program, which led to the loss of PCOS participants. The Control participants were recruited later in the study timeline and were all retained.

Technical limitations included questionable BMI values and unscaled estradiol results. BMI was calculated from self-reported heights and self-reported weights in many cases. This resulted from misunderstanding among the study leaders and technician conducting the DEXA scans, with each thinking the other was taking the actual measurements. There was no way to verify the accuracy of the self-reported results and so BMI results are dependent on the memory of participants and the accuracy of the measuring devices they used. The estradiol assay used had a higher than anticipated detection limit, such that many participant samples were unquantifiable below 5.0 pg/ml.

CONCLUSION

Eating disorder in women with PCOS was not observed in this study. However, several observed variables suggested trends toward subclinical disordered eating and dysregulation of appetite-regulating hormones. Promotion of healthy diet and exercise lifestyle modifications should remain first line interventions for this population based on the results of our study and others reviewed during the preparation of this manuscript. Because of the comorbid conditions associated with PCOS, especially metabolic syndrome features, as well as the trend towards reported higher fat and lower micronutrient intake in our PCOS participants, more attention to promoting intake of more “nutrient dense” foods may be warranted in this population. Finally, routine assessment for disordered eating and support to prevent disordered eating is recommended for the PCOS population and others with conditions that make weight management even more difficult than usual in the present obesigenic environment.

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APPENDIX A

INFORMED CONSENT WAIVER PRESENTED TO

AND SIGNED BY ALL PARTICIPANTS

Informed Consent

Hormones and Appetite in Polycystic Ovary Syndrome

I have been informed that the purpose of this research is to study the associations of appetite-regulating and metabolic hormones in women with polycystic ovary syndrome compared to control women. My participation in this study will involve: 1) completion of medical and diet history questionnaires and an interview with a registered dietitian, 2) a transabdominal or vaginal ultrasound to detect the presence or absence of polycystic ovaries or I can provide a previously recorded sonogram, 3) a dual energy X-ray absorptiometry (DEXA) test to assess my body composition and bone density, and 4) a fasting blood draw (as described in detail below). My participation will take approximately six hours over three days and will be conducted at the Health Science Center on the UW-L campus.

Medical and Diet History Questionnaires

I will be asked to complete a medical history form before beginning any procedures in the study. All questionnaires and all other pieces of my study information will be coded so they are not identifiable for anyone other than the researchers and kept in a locked file cabinet within the Health Sciences Center. I may contact the investigator if I want access to my study results.

I will be asked to complete three eating behavior-related forms and meet with a registered dietitian to ensure I understand all questions and the dietitian understands my answers.

Transabdominal or Vaginal Ultrasound

An ultrasound is an instrument that uses sound waves to determine the location of structures and cavities in the body. Ultrasound measurements will be conducted in a private exam room after I have removed my clothing from the waist down and placed a drape across my abdomen for privacy, with only a physician and another female study investigator present. I will have the option of either a transabdominal ultrasound OR vaginal ultrasound. A transabdominal ultrasound involves the physician moving the ultrasound probe with warmed gel over structures of interest on my abdomen and will require a full bladder. The probe may cause some discomfort when pressed down on my full bladder. A vaginal ultrasound involves insertion of a sheathed probe with gel into my vaginal canal. Probe insertion may cause minimal discomfort similar to speculum insertion during routine pelvic exams. After the probe is removed from my vaginal canal, I will be provided with a sanitary napkin to wear for any vaginal/gel discharge. If I choose to have the vaginal ultrasound, I will be given the option of inserting the transducer myself or having the physician gently insert it for me. If I have been previously diagnosed with polycystic ovary syndrome and received a sonogram of my

ovaries, I may obtain a copy and provide it to the investigators instead of having another ultrasound.

Dual Energy X-ray Absorptiometry (DEXA)

If there is any chance of me being pregnant I should not undergo a DEXA scan. The DEXA scanner is a very low level x-ray machine that does a short (no more than 5 minutes) scan of the entire length of the body while I lay on a flat table. The amount of radiation emitted from the DEXA scan is within acceptable range as determined by the Wisconsin Department of Health and Family Services and is estimated to be less radiation than you would receive if you flew on a plane to Denver. The DEXA scan provides information about % fat mass, % lean mass, and bone density. The DEXA scan will be conducted at the Health Science Center and subjects may access the results and discuss them with the physician if they request to do so.

Fasting Blood Draw

I will be asked to refrain from consuming anything other than water 12 hours prior to arriving at an early morning scheduled appointment at the Health Science Center. A needle will be placed in my arm or hand vein to draw approximately 12 milliliters of blood in three separate tubes. I may experience minor bruising or swelling at the site of the venipuncture and there is a remote chance of developing an infection from any venipuncture procedure. After the test is over, I will need to eat a provided snack before leaving the Health Science Center.

I have been informed that my participation in this study may benefit me by providing me with increased awareness of my body composition, important hormone levels and eating habits. This information may help me to improve my quality of life and manage my polycystic ovary syndrome if it is present. I may also benefit from nutritional information discussed with the dietitian involved in the study.

The results of this study may be published in scientific journals or presented at professional meetings. However, all of my data will not be linked to any personal identifier information and will be presented in group form only. My participation in this study is voluntary and I may interrupt any procedure during this study at any time, for any reason, without penalty.

Questions regarding study procedures may be directed to Amanda Sanders (Principal Investigator), sanders.aman@uwlax.edu (920) 585-3861 or Dr. Peg Maher (Major Advisor) mmaher@uwlax.edu, Department of Biology, 1725 State St, La Crosse, WI 54601, (608) 785-6967. Questions regarding the protection of human subjects may be addressed to irb@uwlax.edu.

Participant's Signature: _____ Date: _____
Researcher's Signature: _____ Date: _____

APPENDIX B

MEDICAL AND DIET HISTORY QUESTIONNAIRE PRESENTED TO AND
COMPLETED BY ALL PARTICIPANTS AT THEIR FIRST APPOINTMENT

Medical and Diet History Questionnaire

1. Demographics

Age _____ Weight _____ Height _____ Race/Ethnicity _____

2. Do you use any tobacco products? YES NO

If YES, how often? _____

3. Have you or anyone in your family ever been diagnosed with or experienced any of the following conditions? (CHECK ALL THAT APPLY)

Medical Condition	You	Family	Relation
Abnormal Facial Hair			
Abnormal Hair Loss			
Absent Menstruation			
Acanthosis nigricans (dark skin spots)			
Acne			
Asthma			
Cancer			
Depression			
Diabetes			
Fatigue			
Heart Attack			
Heavy Periods			
High Blood Pressure			
High Cholesterol			
Obesity			
Ovarian Cysts			
Polycystic Ovary Syndrome			
Sleep Apnea			
Stroke			
Thyroid Disease			

4. Reproductive Related Questions:

- a. Age of first period _____
- b. Is there any chance you could be pregnant at this time? YES
NO
- c. If you tried to get pregnant, were you successful? YES NO
N/A
- d. Have you ever taken hormonal contraceptives? YES
NO
 - i. If YES, please circle which:
ORAL PATCH INJECTION

e. Are you currently taking any hormonal contraceptives?

YES NO

f. Describe periods since (CHECK ALL THAT APPLY):

Features	Check	Description
Absent		
Light Discharge		
Heavy Discharge		
Variable in Discharge		
Regular (approximate 28 day cycle)		
Irregular (please describe)		
Short (1-4 days)		
Long (5-7 days)		
Very Long (more than 7 days)		
Variable in Length of Bleeding		
Minor Cramps		
Major Cramps		

5. Are you currently taking any medications?

YES

NO

If YES, please list them:

Medication Name	Reason Taking

6. Have you ever tried any of the following methods to control your weight?

Method	Yes	No	Please Specify (if possible)
Diet			
Exercise			
Weight Loss Drugs or Supplements			

7. Do you regularly exercise?

YES

NO

If YES, please describe activity, frequency, and duration

APPENDIX C

EATING ATTITUDES TEST – (EAT-26) PRESENTED TO AND
COMPLETED BY ALL PARTICIPANTS AT THEIR FIRST APPOINTMENT

Age: Sex: F ☐ M ☐ Height: Current Weight:
 Highest Weight: Lowest Adult Weight:

Please Circle a Response for Each of the Following Statements:

Question: I...	Always	Usually	Often	Sometimes	Rarely	Never
1. Am terrified about being overweight	3	2	1	0	0	0
2. Avoid eating when I am hungry.	3	2	1	0	0	0
3. Find myself preoccupied with food.	3	2	1	0	0	0
4. Have gone on eating binges where I feel I may not be able to stop.	3	2	1	0	0	0
5. Cut my food into small pieces.	3	2	1	0	0	0
6. Aware of the calorie content of foods I eat.	3	2	1	0	0	0
7. Particularly avoid food with a high carbohydrate content (bread, rice, potatoes, etc.)	3	2	1	0	0	0
8. Feel that others would prefer if I ate more.	3	2	1	0	0	0
9. Vomit after I have eaten.	3	2	1	0	0	0
10. Feel extremely guilty after eating	3	2	1	0	0	0
11. Am preoccupied with a desire to be thinner.	3	2	1	0	0	0
12. Think about burning up calories	3	2	1	0	0	0

when I exercise.						
13. Other people think I'm too thin.	3	2	1	0	0	0
14. Am preoccupied with the thought of having fat on my body.	3	2	1	0	0	0
15. Take longer than others to eat my meals.	3	2	1	0	0	0
16. Avoid foods with sugar in them.	3	2	1	0	0	0
17. Eat diet foods.	3	2	1	0	0	0
18. Feel that food controls my life.	3	2	1	0	0	0
19. Display self-control around food.	3	2	1	0	0	0
20. Feel that other pressure me to eat.	3	2	1	0	0	0
21. Give too much time and thought to food.	3	2	1	0	0	0
22. Feel uncomfortable after eating sweets.	3	2	1	0	0	0
23. Engage in dieting behavior.	3	2	1	0	0	0
24. Like my stomach to be empty.	3	2	1	0	0	0
25. Have the impulse to vomit after meals.	3	2	1	0	0	0
26. Enjoy trying new rich foods.	0	0	0	1	2	3

Please respond to each of the following questions:

1. Have you gone on eating binges where you feel that you may not be able to stop?
(Eating much more than most people would eat under the circumstances). No ☐ YES ☐

☐ , If YES, on average, how many times per month in the last 6 months?

2. Have you ever made yourself sick (vomited) to control your weight or shape? No ☐

YES ☐ , If YES, on average, how many times per month in the last 6 months?

3. Have you ever used laxatives, diet pills or diuretics (water pills) to control your weight or shape? No ☐ YES ☐ If YES, on average, how many times per month in the last 6 months?

4. Have you ever been treated for an eating disorder? No ☐ YES ☐ If YES, when?

5. Have you recently thought of or attempted suicide? No ☐ YES ☐ , If YES, when?

APPENDIX D

NATIONAL INSTITUTES OF HEALTH DIET HISTORY QUESTIONNAIRE II

GIVEN TO ALL PARTICIPANTS TO COMPLETE AND RETURN

TO THE RESEARCHER

The National Institutes of Health Diet History Questionnaire II can be found at <http://epi.grants.cancer.gov/dhq2/>. Last accessed 6/28/2016.

APPENDIX E

DIET INTERVIEW QUESTIONNAIRE COMPLETED DURING
AN APPOINTMENT WITH A REGISTERED DIETITIAN

Participant: _____ **Date:** _____

Have you ever received advice about what or how to eat to manage your PCOS (or diet for Controls)?

If so, from who? When? Where?

How often and under what circumstances do you weigh yourself?

Are you concerned about your weight?

Are others concerned about your weight? If so, who?

Do you crave particular foods?

Do you avoid particular foods?

Do you feel like you ever "overeat"? If so, what and how often? Do you ever "compensate" for overeating in any way (explain)? If so, how often?

Describe a typical day of eating:

Do you have questions or concerns about your diet (and PCOS)?