THE EFFECTS OF WHEY PROTEIN SUPPLEMENTATION ON ADIPONECTIN AND LEPTIN IN WOMEN WITH AND WITHOUT PCOS

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ABSTRACT

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The current study investigated the effects of whey protein supplementation on high molecular weight (HMW) adiponectin, leptin, and insulin concentrations in women with and without polycystic ovary syndrome (PCOS). Six PCOS women (PCO) and eight non-PCOS women (CON) were included in this study. Each subject consumed a daily 35 g whey protein pre-load 30 minutes prior to their afternoon meal for 40 days. At Day 1, Day 20, and Day 40, participants were brought to the clinic and blood samples were collected to analyze HMW adiponectin, leptin, and insulin. Statistical analyses tested within groups, between groups, and adjusted for body weight, BMI, and body fat percentage. In addition, correlations were evaluated for HMW adiponectin, leptin, and insulin. There were no significant differences between groups or within groups at Day 1, 20, and 40. Although not considered statistically significant, there was an 11% increase and 17% decrease in insulin (t = +30mins) concentrations in the CON group and PCO group, respectively, from Day 1 to Day 40. After adjusting for body weight, BMI, and body fat percentage, there was a statistically significant difference in HMW adiponectin concentration between the two groups at Day 1 ($p < .05$, partial η^2 =

.499). There was also a significant inverse correlation between HMW adiponectin and insulin at Day 40 in both groups (r = -.650). In women with PCOS, daily 35 g whey protein supplementation normalized HMW adiponectin and insulin response to concentrations similar to women without PCOS after 40 days.

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INTRODUCTION

Individuals who suffer from polycystic ovary syndrome (PCOS), a chronic endocrine disorder, are often left frustrated, hopeless, and confused.[1] It is the leading cause of infertility in women, yet it is still frequently misdiagnosed and misunderstood. Currently, there is no cure for PCOS and research is still needed to determine the best care. The most common clinical feature of PCOS is hyperandrogenism.[2] Signs of excessive androgen production in women include uncontrolled weight gain, hirsutism, acne, and ovulatory dysfunction.[1,3] In addition, having PCOS will increase the risk for obesity, type 2 diabetes, and metabolic syndrome.[1–6] In fact, one of the most prevalent secondary conditions among women with PCOS is insulin resistance. As insulin resistance and hyperandrogenism influence each other, making insulin resistance holds an important role in the persistence of PCOS.[7] Insulin resistance may not always be obvious and, sometimes, difficult to treat. However, there is postulation that normalizing adiponectin and leptin levels in women with PCOS will improve the insulin response and alleviate signs and symptoms of PCOS.

Adiponectin is a hormone that is produced by white adipose tissue.[8] Levels of this hormone are found to be higher in healthy weight individuals compared to overweight or obese individuals. Since adiponectin is known to have protective effects against insulin resistance and inflammation, it is often studied

for its relationship with insulin.[9] As adiponectin levels increase, insulin levels decrease. In women with PCOS, adiponectin levels are found to be lower than their non-PCOS counterparts.[10] One way to indirectly the improve insulin response is to increase adiponectin levels.

Leptin is another hormone synthesized within white adipose tissue.[11] It is responsible for long-term appetite regulation, glucose homeostasis, and body weight maintenance. Unlike adiponectin, an individual who is obese will produce higher levels than an individual of normal weight.[12] Oftentimes, leptin is produced at such high amounts in obesity that it leads to leptin resistance, a phenomenon that is similar to and related to insulin resistance. In non-obese women with PCOS, one study found leptin levels to be higher than non-obese women without PCOS.[13] Therefore, it is speculated that treatments that would improve leptin sensitivity would simultaneously improve insulin sensitivity.

Whey protein has shown to contain unique components that are not found in other sources of protein, leading to its purported beneficial metabolic effects on obesity and hyperglycemia.[14] In a study by Campbell and colleagues, consumption of whey protein produced a significantly greater increase in serum adiponectin when compared to a placebo.[15] Another study reported a reduction in leptin levels in overweight and obese women with PCOS following whey protein consumption when compared to a glucose beverage.[16] However, there are limited studies evaluating the direct effects of whey protein on adiponectin and leptin in humans, and not all studies have shown similar ideal effects as the trials

mentioned. Therefore, more research is needed to evaluate the metabolic effect of whey protein in women with PCOS. The purpose of this study was to evaluate plasma high molecular weight (HMW) adiponectin, serum leptin, and plasma insulin levels in women with PCOS before, during, and after 40 days of daily whey protein supplementation (35 g/day) and to determine how they differ compared to women without PCOS.

HYPOTHESIS

It is hypothesized that abnormal HMW adiponectin, leptin, and insulin levels will be found in women with PCOS. After daily supplementation of 35 g of whey protein for 40 days, HMW adiponectin and leptin levels will normalize, leading to simultaneous improvements of insulin levels.

SPECIFIC AIMS

1. Evaluate the changes in plasma HMW adiponectin levels in women with PCOS after 40 days of 35 g whey protein supplementation. This will involve assessing HMW adiponectin at baseline and comparing it to levels at Day 40.

2. Evaluate the changes in serum leptin levels in women with PCOS after 40 days of 35 g whey protein supplementation. This will involve assessing leptin at baseline and comparing it to levels at Day 40.

3. Evaluate the changes in plasma insulin levels in women with PCOS after 40 days of 35 g whey protein supplementation. This will involve assessing insulin at baseline and comparing it to levels at Day 40.

CHAPTER II

LITERATURE REVIEW

PCOS

PCOS is an endocrine disorder that affects up to 20% of reproductive-aged women.[2] It affects androgen, insulin, and progesterone. There has yet to be a specific test that can diagnose PCOS, but most professionals use the Rotterdam Criteria. This criterion requires two of the following three symptoms for an official diagnosis: elevated levels of androgen, absent or irregular periods, and ovarian cysts. Though the name of the disorder suggests that cysts in the ovaries must be present for diagnosis, the most common trait of PCOS is excess androgen levels.[2] Some synthesis of androgens, commonly known as the male sex hormone, normally occurs within the female endocrine system.[3] However, having excessive levels of androgens will cause abnormalities, including hirsutism, a male-like hair growth pattern frequently seen among women with PCOS.[4] High androgen levels may also lead to uncontrolled weight gain, acne, and ovulatory dysfunction.[4]

Other signs of PCOS include hair loss and oily skin. Having PCOS increases the risks for obesity,[2,4,5] type 2 diabetes, infertility,[2–4,6] metabolic syndrome,[2,6] cardiovascular disease,[1,3-5] depression, sleep apnea,[1,3,4] non-alcoholic fatty liver disease,[4,5] and endometrial cancer.[1,4,5] One of the most prevalent conditions related to PCOS is insulin resistance. In fact, insulin

resistance is often a key role in the persistence of PCOS, which is often associated with possible acanthosis nigricans, a darkened skin color at the folds of the body, like the neck and armpits.[7]

More than half of women with PCOS will develop diabetes or pre-diabetes.[3] Since insulin resistance and hyperandrogenism are interrelated,[2,17,18] an appropriate method to alleviate the risk for diabetes and other major symptoms of PCOS is to increase insulin sensitivity, which could simultaneously decrease free androgen levels. Drug therapy is recognized as one route to prevent or reduce insulin resistance, but taking medication can lead to unwanted side effects, [19] and there may be concerns with adherence, accessibility, or personal preferences. Another way to decrease insulin resistance is through weight loss by following a healthy diet and incorporating routine exercise,[19] but weight loss may not be suitable for women with PCOS who are normal or underweight. For both obese and non-obese women with PCOS who are also insulin resistant, another option would be to alter other hormones that are associated with insulin sensitivity, such as adiponectin and leptin.

It is possible to have normal blood glucose levels with insulin resistance,[20] making insulin resistance less obvious. Due to the complicated nature of measuring insulin sensitivity accurately, several tests have been developed.[7] The hyperinsulinemic euglycemic glucose clamp is the gold standard in measuring insulin sensitivity; however, it is expensive, time-consuming, and labor intensive. The homeostasis model assessment (HOMA) and quantitative insulin sensitivity

check index (QUICKI) are the most commonly used methods in clinical research, but neither test alone should be relied upon for diagnosis of insulin resistance as they each have their own limitations. This creates a need to establish other potential biochemical markers for a more reliable assessment of insulin resistance, such as adiponectin and leptin due to their close relationship with insulin.[7]

ADIPONECTIN

Adiponectin is an anti-inflammatory adipocytokine that is primarily produced by white adipocytes.[8] It has protective effects against insulin resistance, cardiovascular disease, atherogenesis, and inflammation.[9] The normal range of this adipocytokine in plasma is 3-30 μg/mL.[21] In healthy individuals, levels of adiponectin are found to be higher than in individuals who are overweight or obese. Since the major source of adiponectin is derived from adipose tissue, it may not seem plausible that obesity does not increase production of adiponectin. While a direct mechanism to explain why this occurs still needs more research, some studies have found a positive relationship between tumor necrosis factor (TNF)-alpha, a pro-inflammatory cytokine derived from adipose, and the inhibition of adiponectin expression.[22,23] It has long been established that there is chronic low-grade inflammation associated with obesity. One of the hallmarks of this inflammation is increased TNF-alpha, leading to decreased adiponectin production.

The functions of adiponectin are mediated by two receptors, AdipoR1 and AdipoR2.[21] The expression of these receptors is found in several tissues, including skeletal muscle, the liver, and structures related to reproduction. Specifically, they are found in the hypothalamic-pituitary-ovarian axis (HPG axis) and endometrium of humans. It is suggested that adiponectin may be involved in the initiation of the pre-ovulatory phase, also known as the follicular phase, and regulation of the ovarian steroidogenesis process. In support of this theory, a lower proportion of ovarian theca cells expressing AdipoR1 and AdipoR2 has been observed in polycystic ovaries compared to normal ovaries.[21,24] This explains a clinical feature associated with low adiponectin in individuals with PCOS.

ADIPONECTIN AND INSULIN

Adiponectin is often evaluated for its relationship with insulin. Shin and colleagues found that adiponectin levels were negatively associated with BMI and fasting insulin in individuals with PCOS.[25] The study also revealed adiponectin levels to be positively correlated with insulin sensitivity, measured by QUICKI, in non-PCOS controls. Panidis et al. reported adiponectin levels in PCOS subjects to be lower than non-PCOS controls.[10] Adiponectin could not be further evaluated with obesity since lean and obese were not differentiated within each group. Similarly, a study that defined lean and obese subgroups among PCOS and non-PCOS individuals found adiponectin levels to be lower in the lean PCOS group than in the lean non-PCOS group.[26] However, there was no

statistically significant difference in adiponectin levels between the obese PCOS group and obese non-PCOS group. Low adiponectin levels are not unique to women with PCOS, but it is a common feature found in women with PCOS since they have an increased risk for obesity and insulin resistance,[27] along with PCOS being an inflammatory state itself. Therefore, normalizing adiponectin while simultaneously increasing insulin sensitivity may improve the overall pathogenesis of PCOS.

LEPTIN

Leptin is the most abundant adipocytokine expressed by white adipose tissue.[11] The main functions of leptin are involvement in long-term appetite regulation, glucose homeostasis, and maintenance of body weight. It is an anorexigenic hormone that simultaneously works with ghrelin, an orexigenic hormone, to maintain energy homeostasis. When the body has sufficient energy, leptin travels to the hypothalamus as a signal to suppress hunger and decrease energy intake. When leptin levels are low, appetite is stimulated followed by increased food intake.[11]

Leptin production is positively correlated with fat mass, so an obese individual will produce significantly higher levels of leptin than someone who is of a healthy weight.[12] However, leptin is often produced at such high amounts in obese individuals that they become leptin resistant. In the case of leptin resistance, leptin receptors are desensitized to leptin causing the leptin system to be disrupted. Even though they may have high concentrations of leptin, the

hormone is unable to be recognized by the body. This causes constant feelings of hunger and increased energy intake, leading to weight gain. Additionally, increased leptin concentrations may be a biomarker specific to PCOS, independent of body weight. In support of this theory, one study found that non-obese women with PCOS had higher levels of leptin than non-obese women without PCOS.[13]

Leptin also interacts with the reproductive system impacting an effect in PCOS. It acts as a messenger between adipose tissue and the reproductive organs to indicate whether there is adequate energy to maintain optimal reproductive health.[28] Having an imbalance of leptin may cause problems with menstruation and pregnancy, major issues in PCOS.[28,29] Furthermore, in *ob/ob* male and female mice with mutated leptin gene and hormone insufficiency, food limitations and weight loss were unsuccessful in fertility restoration, but fertility was successfully restored after administration of recombinant leptin.[30] In humans, serum leptin levels were greater in women with recurrent miscarriages than in a control group. However, in another study, during Weeks 5-8 of gestation of women who later miscarried, plasma leptin concentrations were lower than women who subsequently had a term birth.[29,31]

LEPTIN AND INSULIN

Leptin and insulin have an interdependent relationship, which is why leptin resistance and insulin resistance are often found concurrently. Leptin decreases insulin secretion and increases insulin hepatic extraction while insulin stimulates

leptin production and secretion in adipose tissue.[11] In turn, leptin also decreases hepatic glucose production, decreases glucagon levels, and increases insulin sensitivity. The interaction between leptin and insulin is known as the adipoinsular feedback loop with the goal to maintain glucose homeostasis and decrease adipogenesis.[11] However, this inhibitory feedback mechanism is often disturbed in PCOS. In a study that measured insulin resistance in women with PCOS using six different methods (fasting blood glucose, fasting insulin, glucose/insulin ratio, HOMA-IR, QUICKI, and McAuley index), elevated serum leptin levels were found in the insulin resistant by all methods except fasting blood glucose and glucose/insulin ratio, which have been deemed as less sensitive indicators of insulin resistance in previous studies.[32] Treatments that focus on leptin levels in women with PCOS would not necessarily aim to decrease leptin secretion, but rather to increase leptin sensitivity. Improving leptin function would lead to beneficial effects on insulin metabolism and disease status of PCOS.

It is unclear whether abnormal adiponectin and leptin concentrations are contributors to the development of PCOS or if they are secondary outcomes of PCOS. We hypothesize that normalizing levels of these adipokines would alleviate one of the major symptoms of PCOS, insulin resistance.

WHEY PROTEIN

Whole milk protein consists of two types of proteins, casein and whey. Whey makes up roughly 20% of whole milk protein and the remaining 80% is

casein.[33] Whey protein is of interest due to its beneficial metabolic effects on obesity and hyperglycemia attributable to its unique components not found in other sources of protein.[14] Pal and Ellis evaluated the effects of various types of protein in healthy males where whey protein led to a significant decrease in glucose, increased insulin response, and reduced appetite when compared to tuna, turkey, and egg.[34] Whey protein can be broken down into several peptides, some of which may contribute to its insulinotropic properties. They include β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, bovine lactoferrin, lactoperoxidase, and glycomacropeptide (GMP).[33]

Digestion of whey protein results in bioactive peptides and amino acids that promote the release of anorexigenic gastrointestinal peptides, such as glucagon-like peptide 1 (GLP-1),[14,35,36] glucose-dependent insulinotropic polypeptide (GIP),[14] cholecystokinin (CCK),[14,35] and peptide YY (PYY).[14,36] The secretion of these hormones suppresses hunger cues, thus decreasing calorie intake. Albutensin A is a bioactive peptide released from the hydrolysis of whey protein via tryptic digestion.[33] It exerts an ileum-contracting effect and, in mice, causes delayed gastric emptying, affecting glucose levels after meals. GLP-1 also slows gastric emptying and stimulates insulin production, but individuals with type 2 diabetes have been shown to experience hyposcecretion of GLP-1.[37,38] When Jakubowicz et al. assessed the postprandial effects of a 50 g whey protein beverage before a high glycemic breakfast, levels of intact GLP-1 (iGLP-1) and total GLP-1 (tGLP-1) after breakfast were significantly higher with the whey

beverage compared to the control water beverage.[39] Moreover, the bioactive peptides from whey protein have a role in inhibiting dipeptidyl peptidase-4 (DPP-4), a glycoprotein that degrades GIP and GLP-1.[14]

When King and colleagues evaluated consumption of a 15 g whey protein beverage before mixed macronutrient meals, a significant reduction in peak postprandial hyperglycemia and an increase in plasma insulin levels were observed after meals with consumption of the whey protein beverage when compared to the control beverage (calorie-free flavored water).[40] Similarly, in the study by Jakubowicz et al*.* that assessed a higher consumption (50 g) of a whey protein pre-load before a high-glycemic breakfast, reduced postprandial hyperglycemia and increased plasma insulin levels after breakfast were observed when compared to the control beverage.[39] It is worth mentioning that the subjects of these studies were individuals with type 2 diabetes, some managed by metformin, and outcomes were based on acute, single visit interventions. Nonetheless, partial contributions of this glycemic response may be from the presence of albutensin A and incretins, such as GLP-1, when whey protein is consumed as a pre-load, or to a lesser extent, co-ingested with meals.

Studies suggest that the benefits of whey protein on glucose metabolism is a dose-dependent effect.[41] In acute trials, a whey protein dose of 20 g per serving or more resulted in markedly decreased blood glucose levels and increased insulin levels compared to consumption of 5-10 g of whey protein.[41] In addition, research has also shown whey protein to be more effective in

stimulating insulin and incretin hormone secretion and slowing gastric emptying when consumed as a pre-load 30 minutes before meals compared to whey consumption during meals.[42]

WHEY PROTEIN AND ADIPONECTIN

In a study using diet-induced obese rats, whey protein intake resulted in the highest levels of plasma adiponectin compared to the control group and three other sources of dietary protein: soy protein, milk protein, and red meat.[43] Currently, there is limited research about the direct effects of whey protein on adiponectin in humans. In a human trial with a small sample size (*n* = 9) of healthy individuals, Campbell and colleagues demonstrated that consumption of whey protein resulted in a significantly greater increase in serum adiponectin when compared to a placebo.[15] However, when a whey protein supplement was given to overweight or obese women with PCOS for 2 months while consuming a caloric deficient diet, adiponectin concentrations decreased compared to a glucose beverage.[16] Though not statistically significant, a decrease in fasting insulin was also observed.

Other studies show a link between major components of whey protein, such as α-lactalbumin and leucine, with adiponectin. Followed by β-lactoglobulin, α-lactalbumin is the second most abundant protein in whey protein and has been shown to increase liver glutathione in rodent models, partly contributing to its effects of improved glucose tolerance and insulin sensitivity.[44] Yamaguchi and Takai demonstrated in a 10-week rat study that α-lactalbumin supplementation

led to significantly increased levels of high molecular weight adiponectin in normal-weight rats with type 2 diabetes (Goto-Kakizaki) when compared to control Goto-Kakizaki rats (given distilled water) and rats without diabetes (Wistar).[45] Additionally, blood glucose levels from an oral glucose tolerance tests (OGTT) were significantly lower in the α-lactalbumin supplemented Goto-Kakizaki rats compared to control Goto-Kakizaki rats. The results were somewhat similar when Gregersen et al. evaluated a 13-week administration of whey protein isolate, whey protein hydrolysate, α-lactalbumin, casein, and standard chow to Zucker Diabetic Fatty (ZDF) rats and Wistar rats.[46] Comparing the various diet interventions among the Wistar rats, α-lactalbumin led to the highest level of adiponectin gene expression in adipose tissue. In ZDF rats, the consumption of whey isolate and α-lactalbumin led to slightly increased levels of adiponectin gene expression compared to standard chow, but differences were insignificant. However, levels of adiponectin receptor gene expression in muscle tissue were significantly increased in ZDF rats given whey isolate compared to the remaining four experimental diets.[46] Furthermore, leucine is a branched chain amino acid (BCAA) known to regulate glucose homeostasis via mammalian target of rapamycin (mTOR) signaling.[47] In a study using previously obese rats, 6 weeks of leucine supplementation alone increased serum adiponectin when compared to a control group, with even higher levels when leucine supplementation was combined with exercise training.[48]

WHEY PROTEIN AND LEPTIN

Since women with PCOS have an increased risk for hyperleptinemia and leptin resistance, the goal is to improve leptin sensitivity. However, most research on leptin is conducted in rodents, demonstrating the need for human studies. In mice fed a high-fat diet (HFD) with leucine supplementation at varying concentrations, serum leptin levels were significantly lower in the HFD + leucine groups than the control group (HFD with no leucine supplementation).[49] This study also evaluated leptin receptor expression, which was significantly higher in the HFD + leucine groups than the control group, indicating improved leptin sensitivity when leucine is consumed. In another study using mice fed a high-fat/cholesterol diet (HFCD), serum leptin was also found to be significantly lower in the HFCD + leucine groups compared to the HFCD group with no leucine consumption.[50] Additional findings in this study also identified lower HOMA-IR values and insulin levels in the HFCD + leucine groups than the HFCD group with no leucine supplementation. Even though all groups consumed a similar number of calories per day, the HFCD + leucine groups gained significantly less weight than the HFCD group. In a rat study that focused on whole whey protein and other components of whey besides leucine, consumption of whey protein consumption significantly lowered levels of plasma leptin and insulin when compared to the control group.[51] Similarly, when overweight and obese women with PCOS consumed whey protein for 2 months while under a simultaneous caloric deficit, leptin levels decreased compared to a glucose beverage.[16]

A human crossover study performed in overweight men with type 2 diabetes showed opposite effects. Though not statistically significant, whey protein co-ingestion with a mixed-macronutrient meal increased plasma leptin levels compared with the control group.[40] Another human trial that evaluated the effects of a daily 40 g whey protein supplementation for 8 weeks in overweight women observed a similar effect of insignificant increased levels of leptin when compared to the control group (38 g hydrolyzed collagen supplementation).[52] In addition, a mouse study comparing the effects of a high-fat, whey protein, and high-calcium diet to a high-fat, casein isolate, and low-calcium diet, leptin mRNA expression was more than twice as high in the high-calcium whey group when compared to the low-calcium casein group, suggesting calcium or whey protein to be the cause of the effect.[53]

Since the effects of whey protein on adiponectin and leptin are unclear, there is a need for more research, especially in women with PCOS, who may be more susceptible to hypoadiponectinemia, hyperleptinemia, and leptin resistance. Whey protein does not require a medical prescription and holds potential to be a simple complementary intervention to incorporate into the lifestyle of women with PCOS.

Though exercising and having a healthy eating pattern will have additional advantages, whey protein alone does show some promise to normalize adiponectin, leptin, and insulin. This study aimed to evaluate the effects of whey

protein supplementation on adiponectin, leptin, and insulin in women with PCOS compared to women without PCOS.

CHAPTER III

METHODOLOGY

SUBJECT RECRUITMENT

The study was approved by the Institutional Review Board of Texas Woman's University, Denton campus (see Appendix A). This research is part of a larger study. The primary method of recruitment was mass emails sent to the student body of Texas Woman's University. In addition, flyers were posted around the Denton campus of Texas Woman's University. Flyers were also distributed to Denton's local shops and women's clinics. As shown in Figure 1, 51 women were interviewed for eligibility. Of those 51 women, 37 women were excluded from the study due to ineligibility or withdrawal. Six women with PCOS and eight women without PCOS completed the study.

EXCLUSION AND INCLUSION CRITERIA

Inclusion criteria included women between 18 and 45 years old. The PCO group included women who had an existing medical diagnosis of PCOS or presence of two of the three diagnostic characteristics based on the Rotterdam Criteria. Exclusion criteria eliminated potential women who smoked, drank frequently (more than two drinks per week), had other metabolic disorders, such as diabetes, use antidiabetic medications, or engaged in frequent routine exercise (more than 30 minutes of exercise per day for more than 3 days per week). Pregnant and lactating women were also excluded from the study.

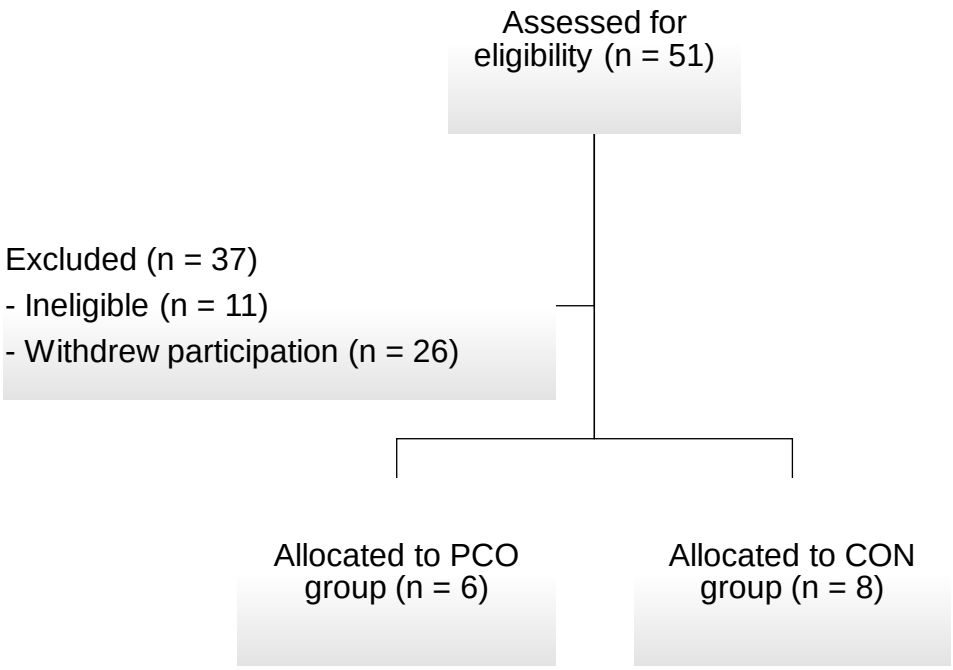

Figure 1. CONSORT Flow Diagram**.** PCO = PCOS group, CON = control group.

STUDY VISIT AND INTERVENTION

Participants who responded to any of the recruitment methods were initially contacted by telephone for a screening interview. If the women met the inclusion criteria for the study, they were scheduled for their Day 1 appointment at the Pioneer Performance Clinic. If the participant experienced regular menstrual cycles, their Day 1 appointment was scheduled within the first 7 days of the participant's menstrual cycle (early follicular phase). If the subject experienced oligomenorrhea or amenorrhea, the date of the Day 1 appointment was scheduled freely.

When subjects arrived at their Day 1 appointment, they were required to sign an informed consent to continue in the study. They were also notified that they could withdraw from the study at any point. Each subject who signed the

informed consent was assigned an identification number that indicated whether they were a part of the PCOS (PCO) group or control (CON) group. Upon completion of the entire study, the participants would receive $40. Participants were instructed to come to all clinic visits following a minimum 10 hour fast. The first venous blood sample (t = 0 min) of Day 1 was collected prior to consumption of a 35 g whey protein supplement (35 g whey protein, 76.58 mg sucralose and 975.09 mg vanilla flavor in 250mL water) provided by Glanbia Foods (Twin Falls, ID). Thirty minutes following whey protein ingestion, the second blood sample (t = +30 mins) was collected. The blood samples collected on Day 1 were considered baseline measurements. Participants were given a 20-day supply of whey protein powder on Day 1 to take home and were directed to consume 35 g daily as a whey protein pre-load 30 minutes before their afternoon meal daily. On Day 20, participants returned to the clinic for a midpoint single fasted blood draw and to receive a replenishment of their whey protein supply. On Day 40, they returned for their final two blood draws, collected in the same manner as Day 1. Height and weight were collected at Day 1 and Day 40. Body composition was also measured at Day 1 and Day 40 with an FDA-approved dual energy X-ray absorptiometer (DXA) scan by a trained DXA operator. Subjects received their monetary incentive ($40) on Day 40 (see Table 1).

Table 1. Summary of Study Visits

Screening	***Day 1 BASELINE***	***Day 20 MIDPOINT***	***Day 40 FINAL***
• *Phone interview to ensure subject fits criteria*	• Sign informed consent • Fasted blood draw (t = 0min) • Whey protein ingestion • Blood draw (t = +30mins) 30 mins after whey protein ingestion • Body composition measured with DXA • Subject received 20-day whey protein powder supply	• Fasted blood draw (t = 0min) • Subject replenished with 20 additional servings of whey protein powder	• Fasted blood draw (t = 0min) • Whey protein ingestion • Blood draw (t = +30mins) 30 mins after whey protein ingestion • Body composition measured with DXA • Subject received monetary incentive ($40)

BLOOD COLLECTION AND ANALYSIS

Blood samples from the participants on Days 1, 20, and 40 were collected using BD Vacutainer 10.0 mL Lavender K2-EDTA and 3.5 mL Gray Mottled SST blood collection tubes. Within 30 minutes, the samples were centrifuged at 1000xG to isolated platelet poor plasma and serum and were stored in color-coded aliquots in a -80°C freezer for subsequent analysis. For analysis, plasma for insulin and adiponectin and serum for leptin were removed from the freezer and thawed. Adiponectin, leptin, and insulin were analyzed by ELISA (ALPCO, Windham, NH, USA) where adequate sample was obtained using a BioTek Synergy Microplate Reader following the manufacturer's protocol. All analytes were run in duplicate samples.

NULL HYPOTHESIS

The hypothesis was that HMW adiponectin, leptin, and insulin concentrations would be abnormal in women with PCOS and normalized to levels similar to the control group with supplemental whey protein ingestion. The independent variables were presence of PCOS and time. The dependent variables were HMW adiponectin, leptin, and insulin concentration. The null hypotheses of the study include:

1. There was no significant difference in HMW adiponectin, leptin, and insulin concentrations between women with PCOS and the control group.
2. There was no significant within-group difference in HMW adiponectin, leptin, and insulin concentrations among women with PCOS supplemented with whey protein.
3. There was no significant correlation between HMW adiponectin, leptin, and insulin concentrations.

DATA AND STATISTICAL ANALYSIS

IBM Statistical Packages for the Social Sciences (SPSS) version 25.0 (SPSS Inc., Chicago, IL) was used to analyze all data identified. Significance of the results was considered if $p \leq 0.05$. The tests identified below were applied:

1. Independent *t*-test was used to compare baseline anthropometric characteristics (age, body weight, BMI, and body fat percentage) between the two groups.

2. Paired-samples *t*-tests were used to determine if significant differences of anthropometric characteristics exist between Day 1 and Day 40.
3. Two-way mixed ANOVA were used to compare fasted HMW adiponectin, leptin, and insulin concentrations at Days 1, 20, and 40 between and within groups.
4. One-way ANCOVA were used to compare the adjusted HMW adiponectin and leptin concentration means between groups at Day 1 and Day 40 after controlling for body weight, BMI, and body fat percentage. Analyses were separated by day.
5. Two-way mixed ANCOVA were used to compare the adjusted insulin concentration means between and within groups on insulin concentrations after controlling for body weight, BMI, and body fat percentage. Fasted (*t* = 0 min) and post-whey (*t* = +30 mins) concentrations were included in each analysis and analyses were separated by day.
6. Pearson's correlations were run to assess the relationships among HMW adiponectin, leptin, and insulin (*t* = 0 min) concentrations. Analyses were separated by day.

CHAPTER IV

RESULTS

The purpose of the study was to evaluate the effect of daily whey protein supplementation (35 g) for 40 days on HMW adiponectin, leptin, and insulin concentrations in women with PCOS. A total of 14 subjects, eight control and six PCOS women, completed the study.

ANTHROPOMETRICS

An independent-samples *t*-test was run to compare baseline anthropometrics between the two groups. Data were expressed as mean ± standard deviation. There was homogeneity of variances, as assessed by Levene's test for equality of variances, for weight, BMI, and body fat percentage, but homogeneity was violated for age ($p = 0.016$). Weight (208.62 ± 61.90 vs. 172.09 ± 25.74), BMI (36.11 ± 10.12 vs. 29.54 ± 4.07), and body fat percentage (42.83 ±10.07 vs. 40.537 ± 6.76) were higher in the PCO group than the CON group at baseline. The average age of participants was 24.03 ± 5.11 and 22.54 ± 2.86 in the CON group and PCO group, respectively (see Table 2). A paired-samples *t*-test was used to determine whether there were statistically significant mean differences of anthropometric characteristics between Day 1 and Day 40. One outlier was found in the CON group for weight, but was kept in the analysis. The assumption of normality was not violated, as assessed by Shapiro-Wilke's

test (p > .05). There were no statistically significant differences in body weight, BMI, or body fat between Day 1 and Day 40 for each group (see Table 3).

Table 2. Baseline Anthropometric Characteristics

	Control (N = 8)	*PCOS (N = 6)*	*p*
Age (yrs)	24.0 ± 5.1	22.5 ± 2.9	.016*
Weight (lbs)	172.1 ± 25.7	208.6 ± 61.9	.212
BMI (kg/m^2)	29.5 ± 4.1	36.1 ± 10.1	.114
Body Fat (%)	40.5 ± 6.8	42.8 ± 10.1	.557

Data are mean ± standard deviation. * = statistically significant at p <.05.

Table 3. Anthropometric Characteristics at Day 1 and Day 40

	Group	*Day 1*	*Day 40*	*Mean Difference*	*p*
Weight (lbs)	Control	172.1± 25.7	169.1 ± 26.8	-2.9	.327
	PCOS	208.6 ± 61.9	216 ± 64.3	7.7	.106
BMI (kg/m^2)	Control	29.5 ± 4.1	30.0 ± 5.3	0.5	.558
	PCOS	36.1 ± 10.1	37.5 ± 10.2	1.4	.077
Body Fat (%)	Control	40.5 ± 6.8	40.7 ± 6.8	0.2	.608
	PCOS	42.8 ± 9.8	43.1. ± 9.8	0.3	.645

Data are mean ± standard deviation.

BETWEEN AND WITHIN GROUPS

Two-way mixed ANOVA were conducted to compare adiponectin, leptin, and insulin concentrations at Days 1, 20, and 40 between and within groups. One extreme outlier was found by inspection of boxplots and removed. Normal distribution was assessed by Shapiro-Wilk's test (p > .05). Data from HMW adiponectin at Day 1 for both groups, leptin at Day 1 for the CON group, and insulin (t = 0 min) at Day 20 for the CON group were not normally distributed. There was homogeneity of variances (p > .05), as assessed by Levene's test of homogeneity of variances, except for data from HMW adiponectin at Day 20. Homogeneity of covariances was confirmed by Box's M test. Mauchly's test of sphericity indicated that the assumption of sphericity was violated for the two-way

interaction of HMW adiponectin, $X^2 = 6.25$, $p = .044$. Therefore, Greenhouse-Geisser was used to interpret statistical significance. There was no statistically significant interaction between day and group on HMW adiponectin concentration, $F(1.3,13.3) = 1.56$, $p = .241$, partial $\eta^2 = .135$. The main effect of day did not show a statistically significant difference in mean HMW adiponectin concentration at Day 1, Day 20, or Day 40, $F(1.3,13.3) = 2.36$, $p = .144$, partial $\eta^2 = .191$ (see Table 4). The main effect of group did not show a statistically significant difference in mean HMW adiponectin concentration between the CON and PCO groups, $F(1,10) = .00$, $p = .952$, partial $\eta^2 = .000$ (see Figure 2).

Table 4. Unadjusted Mean Hormone Concentrations at Days 1, 20, and 40

Hormone	***Group (N)***	***Day 1***	***Day 20***	***Day 40***	***p***	***Partial η^2***
Fasted HMW Adiponectin (mcg/mL)	Control (7)	1.0 ± 0.9	1.0 ± 0.4	1.3 ± 0.7	.14	.19
	PCOS (5)	0.6 ± 0.3	1.4 ± 1.3	1.3 ± 1.1		
Fasted Leptin (ng/mL)	Control (7)	20.5 ± 6.9	21.1 ± 2.7	24.1 ± 5.4	.86	.02
	PCOS (5)	20.1 ± 9.5	21.4 ± 11.1	16.7 ± 8.6		
Insulin (t=0min) (mIU/mL)	Control (7)	14.1 ± 11.5	24.6 ± 25.5	19.9 ± 15.8	.20	.14
	PCOS (6)	16.6 ± 13.5	19.2 ± 11.9	22.4 ± 20.8		
Insulin (t=+30mins) (mIU/mL)	Control (6)	52.8 ± 39.4	NC	58.7 ± 23.4	.73	.01
	PCOS (5)	72.6 ± 41.1	NC	59.9 ± 25.5		

Data are mean ± standard deviation. Extreme outliers were removed. P-value and partial η^2 represent within-subjects effect. NC = data was not collected in the study.

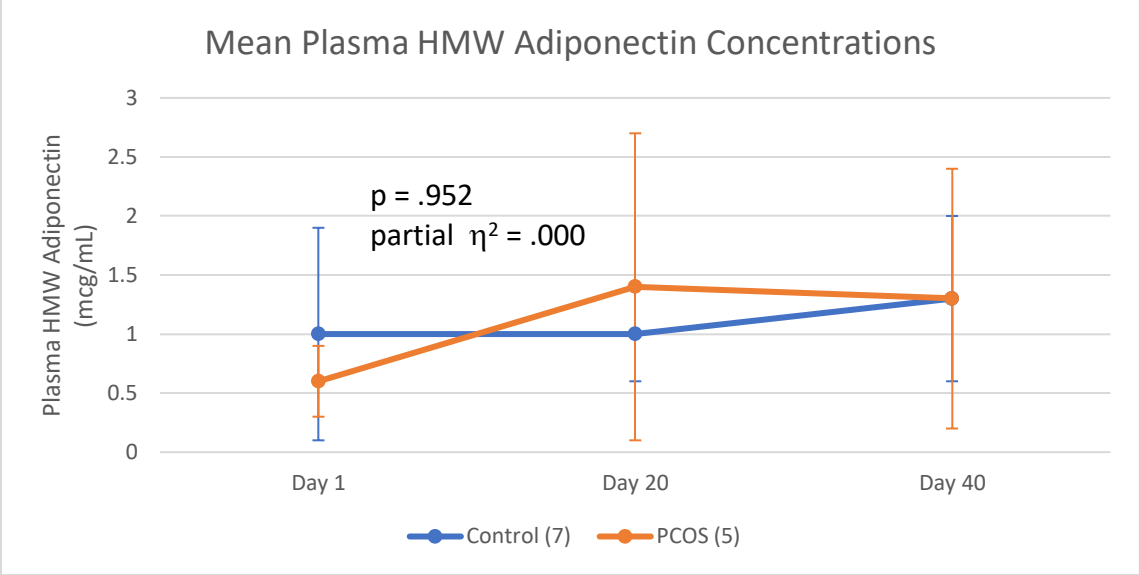

Figure 2. Mean plasma HMW adiponectin concentrations between groups. P-value and partial η^2 were calculated between control and PCOS groups.

Mauchly's test of sphericity indicated that the assumption of sphericity was met for leptin concentrations for the two-way interaction, $X^2 = 2.68$, $p = .261$. There was no statistically significant interaction between day and group on leptin concentration, $F(2,20) = 2.70$, $p = .091$, partial $\eta^2 = .213$. The main effect of day did not show a statistically significant difference in mean leptin concentration at Day 1, Day 20, or Day 40, $F(2,20) = .16$, $p = .855$, partial $\eta^2 = .016$ (see Table 4). The main effect of group did not show a statistically significant difference in mean leptin concentrations between the CON and PCO groups, $F(1,10) = .43$, $p = .526$, partial $\eta^2 = .041$ (see Figure 3).

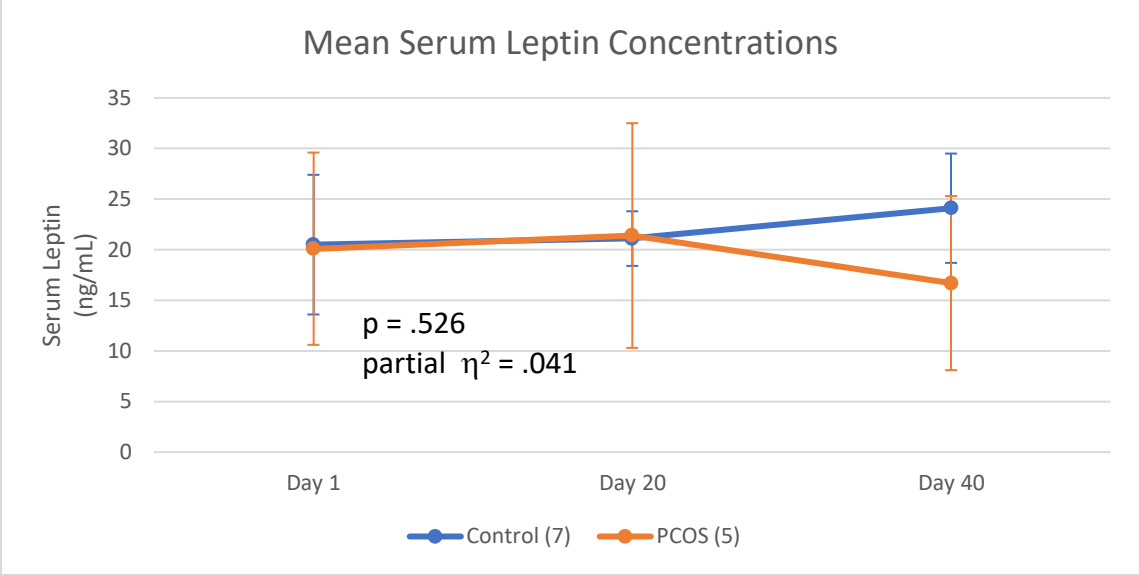

Figure 3. Mean serum leptin concentrations between groups. P-value and partial η^2 were calculated between control and PCOS groups.

Analysis by two-way mixed ANOVA for insulin concentrations were separated by time points, fasted (*t* = 0 min) and post-whey consumption (*t* = +30 mins). Mauchly's test of sphericity indicated that the assumption of sphericity was met for fasted insulin concentrations, $X^2 = 1.87$, $p = .392$. There was no statistically significant interaction between day and group on fasted insulin concentration, $F(2,22) = .69$, $p = .512$, partial $\eta^2 = .059$. The main effect of day did not show a statistically significant difference in mean fasted insulin concentration at Day 1, Day 20, or Day 40, $F(2,22) = 1.73$, $p = .200$, partial $\eta^2 = .136$ (see Table 4). The main effect of group did not show a statistically significant difference in mean fasted insulin concentration between the CON and PCO groups, $F(1,11) = .00$, $p = .990$, partial $\eta^2 = .000$ (see Figure 4). Evaluating the effect of post-whey consumption on insulin concentrations, although there

was no statistically significant interaction between day and group on post-whey insulin concentration, $F(1,9) = .92$, $p = .362$, partial $\eta^2 = .093$, there was an 11% increase and 17% decrease in the CON group and PCO group, respectively, from Day 1 to Day 40. The main effect of day did not show a statistically significant difference in post-whey insulin concentration at Day 1 or Day 40, $F(1,9) = .13$, $p = .731$, partial $\eta^2 = .014$ (see Table 4). The main effect of group did not show a statistically significant difference in mean post-whey insulin concentration between the CON and PCO groups, $F(1,9) = .35$, $p = .567$, partial $\eta^2 = .038$ (see Figure 4).

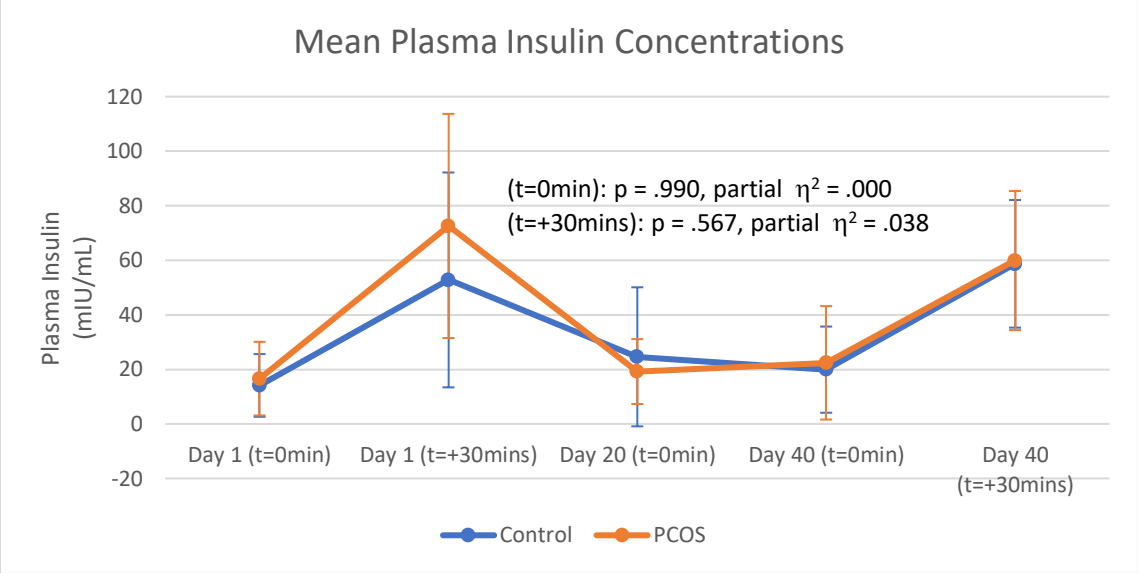

Figure 4. Mean plasma insulin concentrations between groups**.** P-value and partial η^2 were calculated between control and PCOS groups.

ADJUSTING FOR BODY WEIGHT, BMI, AND BODY FAT

One-way ANCOVA were conducted to compare the effects between groups on HMW adiponectin and leptin concentration at Day 1 or Day 40 after controlling for body weight, BMI, and body fat percentage. Hormone

concentrations were run with anthropometrics from their respective day. There were linear relationships between body weight, BMI, and body fat percentage with adiponectin and leptin at each day for each group, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant ($p > .05$). After adjustment for Day 1 anthropometrics, there was a statistically significant difference in HMW adiponectin concentrations between the CON and PCO groups at Day 1, $F(1,7) = 6.963$, $p < .05$, partial $\eta^2 = .499$. Post hoc analysis was performed with a Bonferroni adjustment. HMW adiponectin concentrations were statistically significantly greater in the CON group than the PCO group (mean difference of 1.306 (95% CI, 0.136 to 2.476) mcg/mL, $p < .05$). There were no statistically significant differences between the two groups in HMW adiponectin concentrations at Day 40, $F(1,8) = 0.017$, $p = .898$, partial $\eta^2 = .002$, leptin concentrations at Day 1, $F(1,9) = 0.034$, $p = .858$, partial $\eta^2 = .004$, and leptin concentrations at Day 40, $F(1,8) = 0.204$, $p = .663$, partial $\eta^2 = .025$, whilst controlling for anthropometrics from their respective day (see Table 5).

Table 5. Mean Hormone Concentrations at Day 1 and Day 40 After Adjusting for Covariates

Hormone	*Day*	*Control*	*PCOS*	*p*	*Partial η^2*
Fasted HMW Adiponectin (mcg/mL)	Day 1	1.4 ± 0.3	0.1 ± 0.3	.03*	.499
	Day 40	1.3 ± 0.4	1.4 ± 0.5	.90	.002
Fasted Leptin (ng/mL)	Day 1	19.5 ± 2.7	18.7 ± 3.2	.86	.004
	Day 40	20.7 ± 2.8	18.3 ± 3.8	.66	.025
Insulin (mIU/mL)	Day 1	44.5 ± 16.1	48.9 ± 17.7	.87	.004
	Day 40	39.7 ± 8.6	35.6 ± 9.6	.78	.014

Data are mean ± standard error. Insulin values for each group represent an average of t=0min and t=+30mins concentrations of that day. P-value and partial η^2 were calculated between control and PCOS groups. * = statistically significant at p <.05.

Two-way mixed ANCOVA were conducted to compare the adjusted mean insulin concentrations between groups and within each group after controlling for body weight, BMI, and body fat percentage. Each analysis included fasted (*t* = 0 min) and post-whey (*t* = +30 mins) insulin concentrations and were separated by day. There were linear relationships between body weight, BMI, and body fat percentage with insulin concentrations at each time point for each group, as assessed by visual inspection of a scatterplot. There was homogeneity of regression slopes as the interaction term was not statistically significant ($p > .05$). There was no statistically significant two-way interaction between time and group on insulin concentration at Day 1, $F(1,8) = 0.134$, $p = .724$, partial $\eta^2 = .016$, whilst controlling for body weight, BMI, and body fat percentage at Day 1. The main effect of time did not show a statistically significant difference in adjusted mean insulin concentration at fasted or post-whey consumption, $F(1,8) = .668$, $p = .437$, partial $\eta^2 = .077$. The main effect of group did not show a statistically

significant difference in adjusted mean insulin concentration between the CON and PCO groups, $F(1,8) = .0.029$, $p = .870$, partial $\eta^2 = .004$ (see Table 5).

With respect to insulin concentrations at Day 40, there was no statistically significant two-way interaction between time and group on insulin concentration at Day 40, $F(1,6) = 0.286$, $p = .612$, partial $\eta^2 = .046$, whilst controlling for body weight, BMI, and body fat percentage at Day 40. The main effect of time did not show a statistically significant difference in adjusted mean insulin concentration at fasted or post-whey consumption, $F(1,6) = 1.867$, $p = .221$, partial $\eta^2 = .237$. The main effect of group did not show a statistically significant difference in adjusted mean insulin concentration between the CON and PCO groups, $F(1,6) = .083$, $p = .783$, partial $\eta^2 = .014$ (see Table 5).

CORRELATIONS BETWEEN HMW ADIPONECTIN, LEPTIN, AND INSULIN

Pearson's correlations were run to assess the relationships between HMW adiponectin, leptin, and insulin ($t = 0$ min) concentrations. Analyses were separated by day. HMW adiponectin concentration on Day 1 had a small negative correlation with leptin at Day 1, $r(10) = -.296$, $p = .351$, and a small negative correlation with insulin at Day 1, $r(10) = -.320$, $p = .311$. There was a moderate positive correlation between leptin and insulin at Day 1, $r(11) = .388$, $p = .190$. HMW adiponectin concentration at Day 40 had a moderate negative correlation with leptin at Day 40, $r(10) = -.467$, $p = .126$ and a statistically significant, strong negative correlation with insulin on Day 40, $r(11) = -.650$, $p <$

.05. There was a moderate positive correlation between leptin with insulin on Day 40, $r(10) = .515$, $p = .087$ (see Table 6).

Table 6. Correlations Between Hormone Concentrations at Day 1 and Day 40

Day 1	**Leptin**	**Insulin**
HMW Adiponectin	-.296	-.320
Leptin		.388
Day 40	**Leptin**	**Insulin**
HMW Adiponectin	-.467	-.650*
Leptin		.515

* = statistically significant at $p < .05$.

CHAPTER V

IMPLICATIONS, RECOMMENDATIONS, AND CONCLUSIONS

SUMMARY

A total of 14 subjects completed the study. There were no significant differences in body weight, BMI, and body fat percentage between the CON and PCO group at baseline. There was a statistically significant difference in age between the groups at baseline, with the CON group being older than the PCO group. There were no statistically significant differences in body weight, BMI, and body fat percentage within each group from Day 1 to Day 40.

When evaluating HMW adiponectin, leptin and insulin concentrations at Day 1, Day 20, and Day 40, there were no statistically significant differences found between or within each group. After adjusting for weight, BMI, and body fat percentage, a statistically significant difference in HMW adiponectin concentration was found between the CON and PCO groups at Day 1. Additionally, a statistically significant correlation was found between fasted HMW adiponectin and insulin concentrations at Day 40.

DISCUSSION

At Day 1, after adjusting for body weight, BMI, and body fat mass, a statistically significant difference in HMW adiponectin concentration was found between the PCO and CON groups (see Table 5). Throughout the trial, adiponectin concentrations of the CON group slightly decrease while the PCO

group increases, resulting in no significant difference between the two groups at Day 40. Therefore, the HMW adiponectin concentration of the PCO group, which was lower than the CON group at baseline, normalized to a level similar to the CON group by Day 40. This contrasts with a study conducted by Kasim-Karakas et al[16] that found a statistically significant decrease in adiponectin after women with PCOS consumed a daily dose of whey protein for 60 days. There are, however, differences between the current study and the study completed by Kasim-Karakas and colleagues. In the current study, the PCO group gained weight from Day 1 to Day 40 (mean difference of 7.7 lbs), while the CON group lost weight (mean difference of -2.9 lbs). Part of the intervention in the study by Kasim-Karakas and colleagues was a 450 kcal/day caloric deficit, ultimately leading to weight loss (mean difference of -3.4 kg [7.5 lbs] from Day 1 to Day 60). The results of these two studies are interesting because current research suggests that weight loss in overweight or obese individuals is associated with increased levels of adiponectin,[23,45] yet the current study and the study by Kasim-Karakas and colleagues do not align with that theory. Despite weight gain in the PCO group, although not considered statistically significant, HMW adiponectin was found at an increased concentration at Day 40 compared to Day 1. However, when evaluating unadjusted means of the PCO group (see Table 4 and Figure 2), HMW adiponectin concentration begins to trend slightly downwards from Day 20 to 40, which could potentially lead to the decreased level seen 60 days into whey protein supplementation, as seen in the study by

Kasim-Karakas and colleagues. It is possible that only a short-term intervention (20 days) of whey protein supplementation is required to increase HMW adiponectin concentrations.

A statistically significant inverse correlation was found between HMW adiponectin concentration and insulin concentration at Day 40 (see Table 6). By visual inspection of the unadjusted means of insulin concentration throughout the trial (see Figure 4), the insulin concentration, specifically at Day 1 (t = +30 mins), of the PCO group eventually normalize to levels of the CON group at Day 40 (t = +30 mins). Insulin (t = +30 mins) concentrations increase by 11% from Day 1 to Day 40 in the CON group while there is 17% decrease found in the PCO group. While insulin concentrations were similar at Day 1 (t = 0 min) for the CON and PCO groups, the groups showed a difference in insulin response to whey ingestion (t = +30 mins). However, by Day 40, the insulin response to whey ingestion in the PCO group was similar to the CON group. These data imply that whey ingestion in PCOS may lead to normalization of the insulin response. This response may be attributable to the increase in HMW adiponectin concentration of the PCO group from Day 1 to Day 40.

Although there were no statistically significant differences in leptin concentration between the two groups throughout the trial, there is an increase in the CON group and a decrease in the PCO group of unadjusted means at Day 40 (see Table 4 and Figure 3). Despite the weight gain, the PCO group displayed ideal effects in leptin concentration. However, considering that an increase in

leptin levels is typically associated with weight gain,[12] it is surprising that the CON group experienced increased leptin concentration with simultaneous weight loss. It is worth mentioning that in a study by Wyskida et al[54] that measured leptin levels throughout different phases of the menstrual cycle in normal-weight women who experienced regular menstruation, leptin levels have been shown to be higher in the midcycle and luteal phase than the follicular phase, having a significant association with 17-OH progesterone. Since the CON group experienced regular menstrual cycles, this may have partly contributed to the increase in leptin concentration seen at Day 20 and Day 40 as this may reflect the luteal phase and midcycle phase of the menstrual cycle, respectively.

STRENGTHS AND LIMITATIONS

Strengths of this study include the use of HMW adiponectin for analysis rather than total adiponectin. There are three different forms of adiponectin: trimer, hexamer, and HMW multimer.[8,55] The HMW complex has been shown to be a more sensitive indicator of insulin resistance and type 2 diabetes compared to total adiponectin levels. This is likely because the HMW isoform is the most active in binding to receptors and stimulating AMP-activated protein kinase, a molecule with a critical role in regulating adiponectin's metabolic actions.[55]

The main limitation of this study was the small sample size. If a larger sample size would have been evaluated, stronger conclusions and, potentially, more statistically significant relationships could have been identified. Along with the inclusion of more participants, having lean PCOS women and control

subjects of healthy and underweight BMI would represent the population more accurately. In this study, there was one control subject with a BMI of 24.9 and one PCOS subject with a BMI of 17.0 at Day 1. With a larger range of BMIs, it would have been possible to categorize the groups into subsets of BMI (underweight, healthy weight, overweight, obese, etc.), which would likely offer more details about the role of BMI on the hormone response. Including a wider age range of participants would also reflect a realistic population. The inclusion criteria allowed individuals between 18-45 years of age, but as most participants were college students, all subjects included in the study were 18-30 years of age. Additionally, the duration of the trial was held during the COVID-19 pandemic and the February 2021 North American winter storm, each of which delayed clinic appointments or led participants to drop from the study altogether.

Another limitation of the current study occurred during sample testing. The concentrations of the standards and controls from the plasma HMW adiponectin ELISA were slightly lower than the ideal range stated by the manufacturer. Therefore, the values of all the subject samples included in the HMW adiponectin ELISA were likely lower than their true value.

CONCLUSIONS

In conclusion, there were no significant differences in HMW adiponectin, leptin, and insulin concentrations between the CON group and PCO group or within each group at Day 1 or following whey protein ingestion for 20 and 40 days. After adjusting for body weight, BMI, and body fat, there was a statistically

significant difference in HMW adiponectin concentrations between the two groups at Day 1 and no significant difference at Day 40. In addition, there was a significant inverse correlation between HMW adiponectin and insulin concentrations at Day 40.

RECOMMENDATIONS FOR FUTURE STUDIES

Future studies should include a larger sample size. An acute trial (20 days) should be studied to confirm whether that is all that is necessary to increase adiponectin levels, along with further evaluation if changes that occur extend beyond the completion of protein. Should a trial be longer than 28 days, the average length of a complete menstrual cycle, collection of blood samples from women who experience regular menstruation should be consistent in menstrual cycle phase. For example, collecting on Day 1, Day 14, Day 28, and Day 42 may be more meaningful as Day 14 and Day 42 fall on the same day within the menstrual cycle. This would account for the hormonal fluctuations that occur throughout the menstrual cycle. Future studies should consider evaluating comparisons in adiponectin and leptin levels between an experimental group taking an antidiabetic medication and a group taking daily whey protein supplementation. A second study could investigate the relationship between adiponectin and leptin levels with common physical traits of PCOS, such as hirsutism, acne, and acanthosis nigricans.

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APPENDIX A

Institutional Review Board Letter

TWU
TEXAS WOMAN'S UNIVERSITY
DENTON DALLAS HOUSTON

Institutional Review Board
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https://www.twu.edu/institutional-review-board-irb/

DATE: December 4, 2020

TO: Dr. Monique LeMieux
Nutrition and Food Sciences

FROM: Institutional Review Board (IRB) - Denton

Re: Extension for Examination of Whey Protein Benefits in PCOS vs. Non-PCOS Women (Protocol #: 19799)

The request for an extension of the IRB approval for the above referenced study has been reviewed by the TWU IRB (operating under FWA00000178). This study was originally approved on November 30, 2017 and has been renewed. Approval for this study expires on November 29, 2021.

If applicable, agency approval letters must be submitted to the IRB upon receipt prior to any data collection at that agency. If subject recruitment is on-going, a copy of the approved consent form with the IRB approval stamp is enclosed. Please use the consent form with the most recent approval date stamp when obtaining consent from your participants. A copy of the signed consent forms must be submitted with the request to close the study file at the completion of the study.

Any modifications to this study must be submitted for review to the IRB using the Modification Request Form. Additionally, the IRB must be notified immediately of any unanticipated incidents. All forms are located on the IRB website. If you have any questions, please contact the TWU IRB.

cc. Dr. Shane Broughton, Nutrition and Food Sciences