EFFECT OF A LOW INSULINEMIC DIET ON CLINICAL, BIOCHEMICAL AND METABOLIC OUTCOMES IN WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

by

ALI MARIE POHLMEIER, MS

A Dissertation

In

NUTRITIONAL SCIENCES

Submitted to the Graduate Faculty of Texas Tech University in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

Approved

Mallory Boylan, PhD Committee Chair

Jennifer Phy, DO

Jamie Cooper, PhD

Julian Spallholz, PhD

Kitty Harris, PhD

Dominick Casadonte, PhD Interim Dean of the Graduate School

December 2013
ACKNOWLEDGEMENTS

I would like to thank all those that helped and supported me throughout this project. I would also like to thank my parents for their patience and support.
# TABLE OF CONTENTS

ACKNOWLEDGEMENTS .........................................................................................ii

ABSTRACT ...........................................................................................................vii

LIST OF TABLES .....................................................................................................ix

LIST OF FIGURES ..................................................................................................x

I. INTRODUCTION .................................................................................................1

  Thesis Objectives and Components.................................................................2

  Conclusion .............................................................................................................3

II. REVIEW OF LITERATURE ...................................................................................4

  Pathophysiology of PCOS ..................................................................................4

    *Hypothalamic-Pituitary-Ovarian Axis Dysfunction* .........................................6

    *Role of Insulin in Androgen Synthesis* .........................................................7

  Clinical and Biochemical Symptoms of Hyperandrogenism ............................10

    *Androidal Obesity* .......................................................................................10

    *Insulin Resistance* .......................................................................................12

    *Metabolic Dysfunction* ...............................................................................13

    *Hirsutism* .....................................................................................................14
Acne .................................................................................................................. 15
Androgenic Alopecia..........................................................................................17
Menstrual Irregularities and Reproductive Concerns......................................17
Irregular Appetite..............................................................................................19
Psychological Distress.....................................................................................21
Other Chronic Disease Risks........................................................................24
Heart Disease..................................................................................................25
Cancer...............................................................................................................26
Eating Disorders and PCOS..............................................................................27
Pharmaceutical Treatment of PCOS.................................................................30
Nutritional and Lifestyle Factors.....................................................................31
Insulinemic Foods............................................................................................32
Currently Recommended Diet and Lifestyle Modifications...........................34
Low Glycemic Index Diet................................................................................35
Low Carbohydrate Diet...................................................................................37
Low Insulinemic Diet.......................................................................................42
Conclusion.......................................................................................................48

III. METHODS..................................................................................................49
Subjects...........................................................................................................49
Inclusion Criteria

Exclusion Criteria

Intervention

Anthropometric, Biochemical, and Metabolic Outcome Measures

Anthropometric Measurements

Biochemical Measurements

Metabolic Measurements

Questionnaires

PCOS Questionnaire

Binge Eating Scale

Statistical Analysis

IV. RESULTS

Baseline Demographics

Anthropometric Outcome Measures

Biochemical Outcome Measures

Binge Eating Scale

PCOS-Specific Questionnaire

Food Logs

Metabolic Outcome Measures

Baseline Demographics
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric Data</td>
<td>71</td>
</tr>
<tr>
<td>Respiratory Exchange Ratio</td>
<td>72</td>
</tr>
<tr>
<td>Energy Expenditure</td>
<td>75</td>
</tr>
<tr>
<td>Fat Oxidation</td>
<td>78</td>
</tr>
<tr>
<td>Carbohydrate Oxidation</td>
<td>81</td>
</tr>
</tbody>
</table>

V. DISCUSSION ................................................................. 84

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthropometric Results</td>
<td>84</td>
</tr>
<tr>
<td>Biochemical Outcomes</td>
<td>85</td>
</tr>
<tr>
<td>Metabolic Outcomes</td>
<td>88</td>
</tr>
<tr>
<td>Conclusions and Implications</td>
<td>89</td>
</tr>
<tr>
<td>Limitations and Strengths</td>
<td>90</td>
</tr>
</tbody>
</table>

LITERATURE CITED ...................................................................... 91

A. SCREENING CONSENT FORM ............................................... 104

B. PARTICIPANT SCREENER .................................................. 105

C. STAGE OF CHANGE .......................................................... 107

D. INFORMED CONSENT .......................................................... 109

E. EXERCISE QUESTIONNAIRE ............................................... 117

F. BINGE EATING SCALE ...................................................... 118

G. PCOS-SPECIFIC QUESTIONNAIRE ................................. 122
ABSTRACT

The objective of this study was to determine whether a low insulinemic diet would improve anthropometric, biochemical, and metabolic outcome measures, as well as binge eating behaviors and health-related quality-of-life in women with PCOS. A low insulinemic diet was chosen versus a low glycemic diet due to emerging research indicating dissociation between glycemic and insulinemic responses to certain foods, such as starch and dairy.

Women with PCOS (*n* = 24) between 18–45 years (BMI ≥25kg/m^2^ and ≤45 kg/m^2^) participated in an 8-week dietary intervention trial. Diagnosis of PCOS was based on oligo- and/or amenorrhea, presence of hyperandrogenism, and/or presence of polycystic ovaries by ultrasound. Women were asked to discontinue insulin sensitizers, oral contraceptives, and cyclic progesterone prior to the 8-week study. Ten of the 24 participants underwent metabolic testing using a metabolic cart to analyze fasting and postprandial energy expenditure, respiratory exchange ratio (RER), and macronutrient oxidation after consuming a high-saturated fat shake.

Women experienced a significant reduction in weight (*p* < 0.0001), BMI (*p* < 0.0001), fat mass (*p* = 0.02), hip circumference (*p* < 0.0001), waist circumference (*p* < 0.0001), fasting and 2-hr insulin levels (*p* < 0.0001 and *p* < 0.03, respectively), triglycerides (*p* < 0.0001), VLDL (*p* < 0.0001), free testosterone (*p* < 0.05), total testosterone (*p* < 0.01), and vitamin D (*p* < 0.01). HDL was significantly decreased (*p* < 0.01). There was also a significant reduction in RER and carbohydrate oxidation, and a
significant increase in fat oxidation from pre- to post-diet after adjustment for body weight in kilograms. Binge eating scores and quality of life measurements measured were measured by the Binge Eating Scale (BES) and PCOS specific questionnaire (PCOSQ) and were also significantly ($p < 0.05$) improved.

The low insulinemic diet significantly improved anthropometric, biochemical, and metabolic outcome measures in women with PCOS. Participants also had significantly improved scores on the BES and PCOSQ indicating improved eating behaviors and quality of life measures after the 8-week dietary intervention. Considering the chronic disease risks and psychological stress associated with PCOS, the reduction in weight, insulin sensitivity, blood lipids, serum testosterone, and improvement in eating behaviors and quality of life is promising.
LIST OF TABLES

1. Glycemic Loads (GL) of Commonly Eaten Carbohydrate Containing Foods………43
2. Sample Menu for a Low Insulinemic Diet.........................................................45
3. Sample Menu for a USDA Dietary Guidelines Healthy Diet..........................46
4. Nutrient Breakdown for the High Saturated Fat Liquid Meal..........................58
5. Participant Demographics........................................................................61
6. Anthropometric Data..................................................................................62
7. Laboratory Data..........................................................................................65
8. Questionnaire Scores..................................................................................69
9. Energy and Macronutrient Intake.................................................................70
10. Demographics of Subjects Completing Metabolic Testing.........................71
11. Anthropometric Data of Subjects Completing Metabolic Testing.............72
12. Respiratory Exchange Ratio......................................................................73
13. Energy Expenditure.....................................................................................76
14. Fat Oxidation..............................................................................................79
15. Carbohydrate Oxidation.............................................................................82
LIST OF FIGURES

1. Graphical Representation of Pathophysiology of PCOS .......................... 5
2. Steroid biosynthetic pathways and the role of P450c17 .......................... 9
3. Potential association between androgens and bulimia ......................... 28
4. Change in pre- and post-diet glucose, insulin, triglyceride and VLDL, and fasting and total testosterone ........................................ 66
5. Change in PCOS-Q Scores .................................................................. 69
6. Respiratory Exchange Ratio ................................................................ 74
7. Fasting Respiratory Exchange Ratio and Food Quotient ...................... 74
8. Energy Expenditure ........................................................................... 77
9. Fat Oxidation .................................................................................... 80
10. Carbohydrate Oxidation ................................................................... 83
CHAPTER I

INTRODUCTION

Polycystic ovary syndrome (PCOS), also known as Stein-Levanthal syndrome, is a condition associated with female hyperandrogenism. It affects up to 10% of American women and is the most common endocrine disorder found in women of reproductive age (Glueck, Papanna, Wang, Goldenberg, & Sieve-Smith, 2003). PCOS is a heterogenous disorder characterized by the presence of hirsutism, acne, oligo- and/or amenorrhea, ovarian cysts, and infertility. These clinical symptoms are often associated with increased rates of reactive depression and psychological stress, especially in younger women (Yildiz, Bozdag, Yapici, Esinler, & Yarali, 2012). PCOS is also associated with morbidities, including cardiovascular disease (CVD), coronary heart disease (CHD), type 2 diabetes (T2D), and the metabolic syndrome. Since this disorder poses risks to the long-term mental and physical health of these women, early screening for polycystic ovaries (Allemand, Tummon, Phy, Foong, Dumesic, & Session, 2006), as well as clinical and biochemical symptoms of PCOS can help prevent the development of co-morbidities.

PCOS is often accompanied by excessive body weight, with 40-50% of these women being obese (BMI >30 kg/m²), and increase in weight worsens the clinical and/or biochemical symptoms. Obesity seen in this population is of the android type, which is a consequence of elevated androgens and insulin resistance, and is associated with an increased waist-to-hip ratio (WHR), T2D, and CVD (Yildiz et al., 2012).

Treating obesity is one of the main goals in reducing the symptoms of PCOS. Although the signs, symptoms, and chronic disease risks are worsened by obesity, all
women with PCOS are at risk for the co-morbidities associated with the syndrome, including those who are not obese. Therefore, the abnormal hormonal environment in these women is likely the principle factor leading to clinical and/or biochemical symptoms and chronic disease risk factors, and treatment should focus on improving this hormonal balance to reduce the risk of these co-morbidities.

**Objectives and Specific Aims**

The objectives and specific aims of this study were:

- Measure changes in insulin sensitivity by comparing pre- and post-study levels of fasting serum glucose and insulin in women with PCOS.
- Measure changes in clinical and biochemical markers of elevated androgens by comparing pre- and post-study male-pattern hair growth scores and serum free and total testosterone in women with PCOS.
- Measure changes in body composition and metabolic performance by comparing pre- and post-study body mass index (BMI), waist-to-hip ratio and resting metabolic rates using established techniques in women with PCOS.
- Measure changes in quality of life scores, binge eating behaviors, and self-reported signs and symptoms of PCOS after completing an 8-week dietary intervention.
Conclusion

Women with PCOS are at increased risk of cardiovascular disease, type 2 diabetes, obesity and metabolic syndrome, as well as depression. Considering the detrimental physiological and psychological effects of hyperinsulinemia and hyperandrogenemia in women with PCOS, determining the optimal dietary treatment is paramount to improve the characteristics of the metabolic syndrome associated with PCOS.
CHAPTER II
REVIEW OF LITERATURE

Pathophysiology of PCOS

While the exact cause of PCOS is unknown, the pathophysiology of the syndrome appears to be multi-factorial (Balen, 2004). In 2003, a revised consensus was reached on the definition of PCOS, called the Rotterdam Criteria (RC) (Azziz, 2006). The RC defines PCOS as the presence of two out of the following three criteria: (1) oligo- and/or amenorrhea; (2) hyperandrogenism (clinical and/or biochemical); (3) or the presence of polycystic ovaries as determined by ultrasound. These criteria were developed due to the significant heterogeneity of signs and symptoms of this disorder. It should be noted that presence of polycystic ovaries by ultrasound can exist without clinical symptoms (i.e., hirsutism, acne, androgenic alopecia) of PCOS, although biochemical symptoms (i.e., elevated serum androgens, testosterone, or free androgen index) are often present in women with PCOS, independent of weight. The extent of these signs and symptoms may be positively correlated with body weight (Balen, 2004; Yildiz et al., 2012).

Several theories exist regarding the pathophysiology of this disorder. The most common theory suggests that PCOS is a genetic disorder and its progression is dependent on environmental factors (i.e., hyperinsulinemia). Following the consumption of a high carbohydrate meal, the β-cells of the pancreas secrete insulin to help lower blood glucose. This insulin response can often over-compensate, dropping blood glucose levels, and
leave the woman feeling hungry again for more carbohydrates, thus leading a cycle of hyperinsulinemia and hyperphagia. This hyper-secretion of insulin has even further detrimental effects on the hormonal environment in women with PCOS (Balen, 2004) (Figure 1).

Figure 1. Graphic representation of pathophysiology of PCOS. (SHBG: Sex-Hormone Binding Globulin; GnRH: Gonadotropin Releasing Hormone; LH: Luteinizing Hormone; T: Testosterone; IR: Insulin Resistance; T2D: Type 2 Diabetes; WHR: Waist-to-Hip Ratio; CHO: Carbohydrate; IGF-1: Insulin Growth Factor-1 (adapted from Balen, 2004).
Hypothalamic-Pituitary-Ovarian Axis Dysfunction

To comprehend the adverse effects of hyperinsulinemia in PCOS women, the nature of the dysfunction within the ovary and external influences that modify its hormonal balance must be fully understood. In order for ovulation to occur, a balance between luteinizing hormone (LH) and follicle stimulating hormone (FSH) must be maintained. Gonadotropin-releasing hormone (GnRH) is secreted by the hypothalamus and stimulates the release of LH and FSH from the pituitary. During a normal menstrual cycle, FSH:LH levels are higher, with a LH surge around day 14. This surge, stimulated by a GnRH pulse, triggers ovulation and results in the menstrual period 14 days later. In the polycystic ovary, LH levels are elevated during the length of the menstrual cycle, preventing the pronounced LH surge and thus ovulation (Figures 2 and 3). Hyperinsulinemia stimulates GnRH pulsatility, thus leading to the prolonged elevation in LH (Diamanti-Kandarakis, Papavassiliou, Kandarakis, & Chrousos, 2007; Balen, 2004).

It has been proposed that there may be a causal relationship between insulin and hyper-secretion of LH (Balen, 2004; Diamanti-Kandarakis et al., 2007). LH concentrations are significantly elevated in women with PCOS, and can be observed in 40-60% in these women. This elevated LH concentration, a member of the group of hormones called tropic hormones, has significant effects on circulating androgens. One study found that doses of LH as low as 50ng/ml have been shown to stimulate androgen synthesis in the ovary (Magoffin, 2006). Therefore, since insulin stimulates GnRH secretion and thus LH secretion, it has been proposed that the hyperinsulinemia seen in
many women with PCOS may have a causal effect on the hyperandrogenemia seen in this population.

The ovary and the adrenal cortex make equal contributions to circulating androgens, specifically androstenedione and testosterone in normal premenopausal women. However, evidence suggests that the ovary is the source of the excess androgens seen in women with PCOS (Balen, 2004). The enzymes involved in the formation of androstenedione from cholesterol are largely under the control of LH in the ovary. The rate-limiting step in androgen formation is the gene expression of cytochrome P450 (P450), which is completely dependent on tropic hormones (i.e., LH) (Magoffin, 2006). This steroidogenic response to tropic hormones has been shown to be modulated by various peptides, namely insulin.

**Role of Insulin in Androgen Synthesis**

Insulin acts through multiple avenues to increase levels of endogenous androgens. Insulin increases insulin growth factor-1 (IGF-1) expression, which is known to cause gonadotropin dysregulation (Delafontaine, Song, & Li, 2003). Hyperinsulinemia also inhibits insulin growth factor binding protein-1 (IGFBP-1), leading to an increased bioavailability of IGF-1. Insulin is thought to increase P450 enzymatic activity, which is important for ovarian and adrenal steroid hormone biosynthesis. Hyperinsulinemia also decreases the synthesis of sex hormone binding globulin (SHBG) in the liver. SHBG binds to circulating testosterone in the bloodstream, which leaves only a small amount of the testosterone “free” and biologically active. Therefore, low levels of circulating
SHBG lead to an increase in the amount of “free” testosterone in the blood (Balen, 2004). This “free” testosterone is primarily responsible for many of the symptoms that we associate with PCOS (Carmina & Lobo, 1999), namely acne, hirsutism, and alopecia.

The hyperandrogenism of PCOS seems to be caused by the dysregulation of steroidogenesis within the ovaries and adrenal glands. However, what is unknown is why some women with hyperinsulinemia do not experience the signs and symptoms associated with PCOS. Obese women with PCOS may have a genetic disadvantage that promotes steroidogenic dysregulation in the presence of insulin. This proposed genetic disadvantage is thought to be due to over-expression P450c17. This gene expression appears to be the rate-limiting step in the formation of androgens in the ovaries and is stimulated by insulin (Magoffin, 2006; Balen, 2004). P450c17 encodes the enzymes 17-hydroxylase and 17,20-lyase, and catalyzes many reactions involved in steroid biosynthesis, specifically in the Δ⁴ pathway (Figure 2).
Figure 2. Steroid biosynthetic pathways and the role of P450c17. (adapted from Qin & Rosenfield, 1998).

P450c17 expression is under developmental, species-specific, and tissue-specific control (Magoffin, 2006), and its activity in the ovary is regulated by tropic hormones, specifically LH. Therefore, PCOS could potentially be the result of gene mutations that specifically increase P450c17 expression and environmental factors (i.e., insulin, IGF-1) that stimulate its activity.
Clinical and Biochemical Symptoms of Hyperandrogenism

The clinical signs of hyperandrogenism are heterogeneous and differ among women with PCOS, whereas similar biochemical signs are shared by most of these women (Teede, Deeks & Moran, 2010). Up to 70% of women with PCOS have signs of hyperandrogenism (Hunter & Carek, 2003), which is often measured by testosterone (>3nmol/L) and free androgen index (FAI; 100 x total testosterone/SHBG). These measurable biochemical signs of hyperandrogenism are positively correlated, but not absolutely predictive of the clinical symptoms seen in women with PCOS (Michelmore, Balen & Dunger, 2001; Azziz, 2004). The measurement of testosterone and FAI are thought to be sensitive methods for assessing hyperandrogenemia, but the varied response to testosterone and other androgens seen in women with PCOS makes diagnosis difficult (Azziz, 2004). However, medical professionals continue to assess testosterone status by simply monitoring the appearance of clinical symptoms among their patients. The clinical symptoms seen in hyperandrogenic women include android obesity, hirsutism, acne, and androgenic alopecia (Azziz, 2004). As previously discussed, excess plasma androgens lead to the deposition of fat in the abdominal area (android), leading to a greater WHR. The excess androgens can also lead to male pattern hair growth (hirsutism), hair loss (androgenic alopecia), and acne in some hyperandrogenic women.
Androidal Obesity

More than 60% of women with PCOS are overweight or obese, two conditions leading to an increased incidence of insulin resistance (IR) and hyperinsulinemia (Liepa, Sengupta, & Karsies, 2008). The type of obesity associated with PCOS is that of excessive accumulation of fat on the trunk, specifically in the abdominal area. The risk factors associated with androidal obesity in women differ greatly from women displaying lower or gynoidal obesity. Androidal obesity has been clearly associated with IR and T2D in the literature (Balen, 2004; Liepa et al., 2008; Yildiz et al., 2012), therefore, waist:hip ratio (WHR; e.g., android > .8, gynoid < .8) may be more helpful than body mass index (BMI) as an indicator for IR and PCOS.

Evidence suggests that the relationship between obesity and PCOS begins as early as puberty, and the onset of obesity during this period may represent an important factor for the development of the disorder. Elevated testosterone and LH may lead to a disruption in follicular maturation, and consequently, result in anovulation. In overweight adolescents with irregular menses, high LH and testosterone levels were found, suggesting this period represented the initial stage for altered androgen synthesis (Coviello, Legro, & Dunaif, 2006). Therefore, early screening in adolescence is important to impede the development of PCOS and co-morbidities.

While obese women with PCOS may or may not show signs of excess androgens, weight is highly correlated with the presence of these symptoms, as well as IR. However, data suggests that there may be factors in women with PCOS that promote IR that are independent of obesity. Some studies (Tsilchorozidou, Overton, & Conway, 2004;
Balen, 2004) suggest that this defect in pancreatic function remains despite weight loss, albeit improved glucose tolerance.

While studies on whether weight loss improves insulin sensitivity in women with PCOS are conflicting, weight loss has been shown to improve hyperandrogenism, decrease LH concentrations, and restore fertility in obese women with PCOS (Escobar-Morreale, Botella-Carretero, Alvarez-Blasco, Sancho, & San Millan, 2005). Unfortunately, not all women respond positively to weight loss, and there are no differences in the clinical or hormonal features between responders and non-responders. Thus, it is important to understand the mechanisms that underlie the potential impact of diet on hyperinsulinemia, irrespective of weight loss. Researchers are beginning to place a greater emphasis on the role that nutrition plays in the hormonal dysregulation seen in both lean and obese women with PCOS.

**Insulin Resistance**

The defect in insulin action in PCOS may be explained by a dysfunction in insulin receptor-mediated signal transduction, causing maximal responses to insulin to be significantly decreased in women with PCOS. *In vitro* studies have shown abnormal autophosphorylation of these receptors, which is the first step in insulin signal transduction (Dunaif, Wu, Lee, & Diamanti-Kandarakis, 2001; Sykiotis & Papavassiliou, 2001). This abnormality is the increase in serine phosphorylation, as opposed to tyrosine phosphorylation, which appears in at least 50% of women with PCOS. Phosphorylation causes insulin receptor substrates to promote glucose uptake; tyrosine phosphorylation
increases activity of the insulin receptor substrates and serine phosphorylation inhibits glucose assimilation (Balen, 2004; Gerasimos et al., 2001). Excess serine phosphorylation is a common finding among men and women with IR and T2D. Serine phosphorylation also increases P450c17 activity, thus working synergistically to promote IR and androgen synthesis. However, the exact molecular mechanism of insulin signaling inhibition by serine phosphorylation is presently unknown.

Metabolic Dysfunction

Women with PCOS often demonstrate metabolic abnormalities associated with insulin resistance and compensatory hyperinsulinemia. Research on metabolic flexibility, or the capacity to switch back and forth from lipid oxidation to glucose oxidation under insulin-stimulated conditions, has demonstrated the reduced capacity of insulin-resistant individuals to effectively oxidize fatty acids, leading to tissue accumulation of lipids as triglycerides and further impaired insulin signaling (Galgani, Moro, & Ravussin, 2008). An impaired drop in overnight respiratory exchange rate (RER) may be associated with metabolic inflexibility to lipid and is often seen in obese and insulin resistant individuals (Galgani et al., 2008). RER is the ratio of carbon dioxide expired to oxygen inspired, which provides data regarding substrate oxidation.

Research shows that insulin-mediated suppression of fatty acid oxidation can be improved with weight loss and lifestyle change in insulin resistant individuals (Corpeleijn, Saris, & Blaak, 2009). Considering the profound insulin resistance in women with PCOS, one study aimed at measuring whether metabolic inflexibility was a
feature in hyperandrogenic women with PCOS (Di Sarra, Tosi, Bonin, Fiers, Kaufman, Signori, et al, 2013). The researchers found that 76% of their 68 participants were insulin resistant, 73% were hyperandrogenic, and 70% had impaired metabolic flexibility, however, these results were not found in another study (Straczkowski, Kowalska, Adamska, Karczewska-Kupczewska, Nikolajuk, Lebkowska, et al., 2009). Di Sarra et al., (2013) proposed that both insulin resistance and androgen excess may be contributing to this metabolic abnormality. Due to conflicting results, more research is needed on determining the existence of metabolic inflexibility in women with PCOS, as well as defining whether an association exists between metabolic flexibility and both the hyperinsulinemic and hyperandrogenic state. Methods on improving hyperinsulinemia and hyperandrogenemia may have important implications in improving fatty acid oxidation in women with PCOS.

**Hirsutism**

Hirsutism is defined as the presence of course (terminal) hairs in females that follow a male-type pattern (Azziz, 2003). While not every women with PCOS experiences hirsutism, approximately 75-80% of hirsute women suffer from PCOS. This abnormal hair growth is extremely distressing and has a negative impact on psychological well-being of these patients. The degree of hirsutism depends on the method used to determine its presence, and the most common method of scoring hirsutism is the Ferriman and Gallwey method, developed in 1961 (Ferriman & Gallwey, 1961). This method utilizes subjective scoring at eleven different body sites (upper lip, chin, chest,
upper-back, lower-back, upper-abdomen, lower-abdomen, arm, forearm, thigh, and lower leg). In each of these areas, a score of 0 (absence of abnormal hairs) to 4 (extensive abnormal hair growth) is given to describe the degree of hirsutism.

Androgens are the most important determinant of the type and distribution of hairs throughout the body. Androgens influence not only the rate of growth, but also the transformation of soft and short (vellus) hairs to terminal hairs in areas that are androgen sensitive (Azziz, 2003). Testosterone, specifically, stimulates dermal papilla to produce terminal hair where a vellus hair previously grew. Thus, there is a strong positive correlation between bioavailable testosterone concentration in the blood and the presence of hirsutism (Hunter et al., 2003). The presence of hirsutism may be used as a diagnostic tool to represent the degree of underlying hormonal abnormalities in women complaining of this issue.

Acne

Acne vulgaris is another clinical symptom associated with PCOS that causes great distress in hyperandrogenic women. Approximately 50% of women with hyperandrogenism will have acne lesions on the neck, chest, or upper back (Archer & Chang, 2004). Sebaceous gland activity, dependent on androgens, is positively correlated with the presence of acne (Makrantonaki, Ganceviciene, & Zouboulis, 2011). Therefore, while this is often a cosmetic concern for women with PCOS, it often signifies underlying hormonal abnormality. Sebum, which correlates positively with the severity of the acne, is increased by elevated androgen concentrations.
According to Shaw (2002), acne vulgaris generally does not develop in the absence of androgens. Yet, while the role of androgens is essential for the development of acne, the mechanism is complex. This may explain why some hyperandrogenic women may not develop acne lesions. It is proposed that the varied response to androgens by these women suggest other factors may also influence acne development (Shaw, 2002; Archer et al., 2004). These factors include keratinocyte adhesion, bacterial overgrowth of \textit{Propionibacterium acnes}, and several inflammatory mediators. Nevertheless, without the presence of androgens, these subsequent steps in acne development do not occur.

Extensive research has been conducted on the subject of diet and acne, and researchers who have denied the possibility of a link between the two lack convincing evidence to support their arguments (Danby, 2008). More recent research is beginning to show evidence of biochemical and endocrine parameters associated with acne. Insulin and IGF-1, in addition to testosterone, and the dietary factors that cause their elevation are being examined as the culprits for acne development.

While diets high in carbohydrate may contribute to this elevated insulin, IGF-1, and testosterone, other dietary factors are beginning to present acnegenic effects as well (Danby, 2008; Cordain, 2005). One such dietary component is dairy protein, which has unexpectedly shown to produce a hyperglycemic and hyperinsulinemic response independent of fat content. This response is approximately triple what would be predicted by the carbohydrate content of milk (Hoyt et al., 2004; Östman et al., 2001; Danby, 2008). Dairy has a primary function of facilitating growth and contains a broad
array of growth factors, specifically IGF-1 (Östman et al., 2001). Considering the association between insulin, IGF-1, and androgen secretion, dairy intake may increase the presentation of acne and other androgen dependent disorders, but further research is needed.

**Androgenic Alopecia**

Androgenic alopecia (AA), or male pattern hair loss, is one of the most common causes of hair loss in women. (Cela, Robertson, Rush, Kousta, White, Wilson, Lyons, et al., 2003). While AA is associated with androgen excess, the correlation between indices of abnormal androgen production and AA is poor, most likely due to individual variations in androgen sensitivity of the skin and hair follicles (Cela et al., 2003). Nevertheless, one study that set out to determine the strength of the association between AA and PCOS confirmed that women with AA had higher prevalence of polycystic ovaries and hirsutism and than controls ($p<.00001$) (Cela et al., 2003). Therefore, the presence of AA may be used as a diagnostic tool for determining the degree of abnormality in the hormonal environment of women complaining of hair loss. Further research is needed on the correlation between AA and androgen concentration in women with PCOS.

**Menstrual Irregularities and Reproductive Concerns**

The majority of women suffering from PCOS have oligo- or anovulation, often leading to reproductive concerns. Infertility due to anovulation is often treated with medication, instead of treating the underlying causes of hormonal imbalance leading to
anovulation. Another concern for women with PCOS is pregnancy loss. The rate of recurrent spontaneous abortion in this population ranges from 25-40% (Pandey, Rani, & Agrawal, 2005), at least double that of normal women. Reasons for this are unclear, but several researchers suggest it is due to elevated LH levels in the ovary leading to an elevation in levels of testosterone and estrogen. As previously discussed, women with PCOS often have elevated LH throughout their cycle, preventing the LH surge on day 14, and thus ovulation. Furthermore, once pregnancy is established, perinatal mortality is increased 1.5 times, especially if the woman is obese. In a large scale meta-analysis conducted to assess pregnancy outcomes in women with PCOS, results showed that women with PCOS demonstrated significantly higher chances of developing gestational diabetes, OR 2.94 (95% CI: 1.70-5.08); pregnancy-induced hypertension, OR 3.64 (95% CI: 1.98-6.81); premature delivery, OR 1.75 (95% CI: 1.16-2.62); lower neonatal birth-weight; higher rate of admission to a neonatal intensive care unit (NICU), OR 2.31 (95% CI: 1.25-4.26); and a significantly increased chance of delivering by Cesarean section, OR 1.56 (95% CI: 1.20-2.02) (Boomsma, Eijkemans, Hughes, Visser, Fauser & Macklon, 2006).

Methods to reduce weight and circulating insulin are important for restoring ovulation and preventing negative pregnancy outcomes in this population (Phy, Conover, Abbott, Zschunke, Walker, Session, et al., 2004). Several researchers are looking towards dietary modifications that reduce total dietary carbohydrate or specific dietary carbohydrates in an effort to improve insulin sensitivity. As discussed previously, results from one prospective study showed that both total and type of dietary carbohydrate intake
were positively related to ovulatory infertility (Chavarro, Rich-Edwards, Rosner, & Willett, 2009). Consumption of dairy, specifically those low in fat, may also impair fertility by affecting ovulation. Results from analysis of 18,555 women found that the relative risk of anovulatory infertility in women consuming ≥2 servings of low fat dairy products per day compared to women consuming ≤1 per week was 1.85 (1.24-2.77; \( p = .002 \)). There was also a significant inverse relationship between intake of dairy fat and anovulatory infertility (\( p = .05 \)). Intakes of lactose, calcium, phosphorous, and vitamin D were unrelated to anovulatory infertility (Chavarro et al., 2007). Further research is needed to determine whether total dietary carbohydrate or specific types of carbohydrates affect anovulatory infertility in women with PCOS.

Irregular Appetite

The dysregulation of appetite hormones in women with PCOS is also being extensively studied, with conflicting results. It has been widely suggested (Pagotto, Gambineri, Vicennati, Heiman, Tschop & Pasquali, 2002; Moran, Noakes, Clifton, Wittert, Le Roux & Ghatei, 2007; Hirshberg, Naessén, Stridsberg, Byström & Holte, 2004; Wright, Zborowski, Talbott, McHugh-Pemu, & Youk, 2004) that women with PCOS have impaired appetite regulation, however, the mechanisms which promote this abnormality are yet to be understood.

Ghrelin, an appetite-stimulating hormone, has been shown to be negatively correlated with insulin and obesity. While abnormal ghrelin activity is seen in obese patients with PCOS, studies show it does not seem to be associated with the increased
appetite described by this population (Koliaki, Kokkinos, Tentolouris & Katsilambros, 2010; Pagotto et al., 2002). Leptin has also been disproven as an answer to the increased appetite in women with PCOS. Leptin is highly synchronous with LH, androstenedione, and testosterone and has a positive correlation with all three, with high levels signaling appetite suppression. According to this, women with high levels of testosterone, LH, and androstenedione should have reduced appetites, but the reverse is often seen.

Cholecystokinin (CCK) is one potential candidate for the irregular appetite in women with PCOS. CCK plays an important role in regulating appetite and is released from the small intestine in response to a meal. Low levels correspond with greater appetite, whereas high levels correspond with satiety. Sex steroids are known to influence the release and action of CCK, and testosterone is a known appetite stimulant (Hirshberg et al., 2004). One study aimed to compare whether women with PCOS have abnormal CCK secretion or glucose regulation in relation to disturbed appetite (Hirshberg et al., 2004). The results of this study showed that the ‘satiety peptide’ CCK was negatively correlated with testosterone and women with greater levels of plasma testosterone experienced a reduced CCK response following a meal. These results may explain the insatiable appetite described by women with PCOS, but further research is needed to determine the extent of CCK-impaired appetite regulation in the presence of hyperandrogenemia.

Adiponectin is another candidate for the irregular appetite experienced in this population. Adiponectin is produced exclusively in the fat tissue and is involved in several metabolic processes, such as glucose regulation and fat metabolism (Glintborg,
Levels of adiponectin are inversely correlated with BMI and insulin, with low levels blunting appetite and increasing energy expenditure, and obese individuals typically have low levels of adiponectin which should theoretically help to reduce appetite, increase caloric expenditure, and prevent weight gain. However, studies assessing the adiponectin status of women with PCOS have shown that while adiponectin may be negatively correlated with BMI and insulin, it is positively correlated with testosterone (Glintborg et al., 2006; Ardawi & Rouzi, 2005; Vrbikova, Dvorakova, Hill, Vcelak, Stanicka, Vankova, et al., 2005). According to Vrbikova (2005), this association is due to complex interrelations between testosterone, estradiol, and adiponectin. Understanding these interrelations is important to understanding the irregular appetite in hyperandrogenic women with PCOS. Further studies are needed to clarify the relationship between testosterone, insulin, and adiponectin in PCOS.

Psychological Distress

As to be expected, the devastating cosmetic and metabolic problems these women experience lead to an increase in psychological disturbances. Among these include decreased quality of life, depressed mood, depression, decreased sexual satisfaction, and problems with feminine identity (Hahn, Janssen, Tan, Pleger, Mann, Schedlowski, Kimmig, Benson, Balamitsa, & Elsenbruch, 2005; Barnard, Ferriday, Guenther, Strauss, Balen, & Dye, 2007). Very little research has been conducted on the effects of the
clinical symptoms on psychological functioning in these women, and several studies support only the hypothesis of obesity as being the underlying cause of any mental disturbances. Due to the varied array of symptoms of PCOS, several factors could conceivably cause psychological distress. These include changes in appearance (i.e., hirsutism, AA, acne), mood disturbances (i.e., hormonal irregularity), infertility (i.e., oligo- or anovulation), and CVD, T2D, cancer (i.e., chronic disease risk factors) (Hahn et al., 2005; Ragson, Rao, Hwang, Altshuler, Elman, Zuckerbrow-Miller, & Korenman, 2003).

Another cause for distress is the lack of information that women with PCOS typically receive from their primary care physicians. An alarming number of women are not diagnosed unless fertility is their main concern, nor are they properly informed of the syndrome and its underlying hormonal and metabolic factors (Ching, Burke & Stuckey, 2007). One study sought to assess the quality of life (QOL) in 443 diagnosed women and found that women with PCOS not only have lower QOL than normal women ($p < .01$), they also have increased prevalence of psychological morbidity ($p < 0.0001$) (Ching et al., 2007). In addition, psychological morbidity was significantly related to a lack of- or poor information received on the disorder ($p < 0.01$). The results of this study showed that 47% of participants reported that they had gained most of the information about PCOS from a specialist physician, 39% gained their information from the internet, 27% from books, 16% from their family doctor, 15% from magazines, 9% from medical journals, 6% from a dietitian, 6% from a friend, and 2% from a nurse (Ching et al., 2007). These are alarming findings considering the prevalence of the disorder, especially when
looking at the low percentage of women gaining information from a dietitian, in view of the strong diet-disorder relationship.

The psychological morbidities associated with this disorder are especially important when assessing the time of life that the disorder presents itself. The majority of women with PCOS develop symptoms at or around menarche. This is a vulnerable time for the adolescent female, and the clinical symptoms of this disorder can have major negative effects on self-esteem, self-image, and QOL. In obese adolescent females with PCOS, the relationship between obesity and psychological health may be bi-directional, with each perpetuating the other. According to a survey of 198 diagnosed young women, Lim, Norman, Clifton, & Noakes (2009) found that this increased stress leads to intense cravings for chocolate, candy, chips, bread, and pasta, in order of intensity. A significant association was also found between FAI and increased impulsivity in food intake. The foods attributed to cause intense cravings are high starch containing foods. The consumption of these foods results in large amounts of insulin being secreted, which perpetuates the cycle of obesity, hyperandrogenemia, and psychological stress (Lim et al., 2009).

The relationship between the cycle of hyperinsulinemia and hyperphagia, specifically for more carbohydrate rich food, has been widely established (Rodin, Wack, Ferrannini, & DeFronzo, 1985; Rodin, 1985; Rodin, 1991). Therefore, the effects of low insulinemic diets on hunger and carbohydrate cravings in women with PCOS should be explored. Previous studies have demonstrated benefits in psychological measures when protein and carbohydrate intake was manipulated (Latner & Schwartz, 1999). One study
sought to determine whether a particular diet composition was psychologically advantageous to women with PCOS (Galletly, Moran, Noakes, Clifton, Tomlinson, & Norman, 2007). Twenty eight women with PCOS were randomly assigned to either a low-protein/high-carbohydrate diet (LPHC) or a high-protein/low-carbohydrate diet (HPLC) for 16 weeks. The major finding of the study showed that only the HPLC diet was associated with significant improvements in depression and self-esteem, which could play a major role in addressing the psychological concerns, hyperandrogenemia, and obesity of this population.

There has been little public discussion of PCOS outside the medical community, which often leaves women suffering from the disorder with feelings of shame and confusion surrounding their perceived lack of feminine identity. One study utilized qualitative interviews to explore the experiences and identity perceptions of this population (Kitzinger & Willmott, 2002). Results of this analysis showed that women with PCOS are challenged in their perceptions of themselves as women and possessing femininity. The main theme that emerged from the interview was that these women view themselves as “freakish” and “not normal.” In light of these findings, steps should be made to inform adolescent and adult females with PCOS of the commonality of the disorder, and the underlying metabolic and hormonal causes of their experiences.

**Other Chronic Disease Risk**

Chronic disease risk factors in this population have been well documented and include higher levels of total cholesterol, LDL-C, triglycerides, IGF-1, and insulin and
lower levels of HDL-C (Teede et al., 2010). Impaired glucose tolerance is also increased in women with PCOS and large longitudinal cohort studies have also reported that up to 65% of cardiovascular disease (CVD) deaths occur in individuals with impaired glucose metabolism. These risk factors suggest women with PCOS are at risk for developing T2D, CVD, and certain types of cancer (Teede et al., 2010).

**Heart Disease**

Heart disease is the number one killer in women and studies show that women with PCOS have increased CVD risk factors (Dokras, 2013). In one retrospective cohort of 1028 diagnosed women, researchers found that women with PCOS had higher rates of diabetes ($p = 0.002$), hypertension ($p = 0.04$), hypercholesterolemia ($p < 0.01$), hypertriglyceridemia ($p = 0.02$), and increased WHR ($p = 0.004$) (Wild, Pierpoint, McKeigue, & Jacobs, 2000; Teede et al., 2010). They also found that after adjustment for BMI, the odds ratios were 2.2 (0.9-5.2) for diabetes, 1.4 (0.9-2.0) for hypertension, 3.2 (1.7-6.0) for hypercholesterolemia, and 2.8 (1.1-7.1) for cerebrovascular disease. This suggests that BMI is not the sole cause for the increased risk for CVD in women with PCOS. Women with PCOS also have significantly elevated C-reactive protein (CRP), a marker of low grade chronic inflammation, compared to controls (Kelly, Lyall, Petrie, Gould, Connell, & Sattar, 2001). Low grade inflammation is known as an independent predictor of coronary heart disease and has recently been linked to insulin resistance. There is sufficient evidence to confirm the presence of subclinical atherosclerosis in women with PCOS compared to age matched controls, yet few
prospective studies have been conducted examining non-fatal and fatal cardiac events in women with PCOS (Dokras, 2013). Longitudinal research studies are needed to better estimate the risk of cardiac morbidity and mortality in this population.

Cancer

The etiology of female reproductive cancer is still poorly understood, but the relationship between sex hormones and cancer is receiving increased attention (Folkerd & Dowsett, 2010). Women with PCOS have a 2.7-fold increased risk of endometrial cancer and some studies suggest an increased risk of breast and ovarian cancer (Berrino, Pasanisi, Bellati, Venturelli, Krogh, Mastrianni, Berselli, Muti, & Secreto, 2005; Dumesic & Lobo, 2013), but the evidence is inconclusive. Two interrelated possibilities for this increased risk of cancer are thought to be IGF-1 and estrogens (Muti, 2004). First, obesity accelerates the peripheral conversion of androgens to estrogens, and bioavailable estrogens have been shown to contribute to increased cancer risk (Dumesic et al., 2013). Second, IGF-1 has been detected as a tumorogenic growth factor because of its potent mitogenic and anti-apoptotic characteristics, and is positively correlated with estrogen, insulin, and WHR. According to this, hyperinsulinemia leads to hyperandrogenemia in women with PCOS and these excess androgens are then converted to estrogens and stimulate IGF-1 secretion, both increasing risk of gynecological cancer (Dumesic at al., 2013; Muti, 2004). Methods for improving insulin resistance and hyperinsulinemia may prove to be an important factor in reducing the risk of reproductive cancers in women with PCOS.
Eating Disorders and PCOS

The association between eating disorders and sex hormones is beginning to receive attention (Hirschberg, 2012). Research also suggests bulimic or binge eating behaviors is common in women with PCOS (Resch, Szendei & Haász, 2004; Morgan, Scholtz, Lacy, & Conway, 2008; Michelmore et al., 2001; Cotrufo, Monteleone, d’Istria, Fuschino, Serino & Maj, 2000). Disturbance of menstruation is a common characteristic in women with bulimia nervosa, as well as polycystic ovaries, acne, hirsutism, and elevated testosterone (Naessén, Carlström, Garoff, Glant & Hirschberg, 2006). This abnormal hormonal environment mimics that seen in women with PCOS, which led researchers to believe that the two disorders may be interrelated. Studies on women with PCOS have not only found appetite dysregulation, but also increased scores on the bulimia investigation test (BITE) (Naessén et al., 2006). It is still unknown whether bulimia promotes the development of the symptoms of PCOS, or whether PCOS exacerbates or promotes the development of bulimia, or even if the two are related. Thus, the question remains, is bulimia solely a psychological disorder, or is there also a potentially underlying hormonal component promoting its debilitating cycle?

As previously discussed, women with PCOS often have extreme cravings for high carbohydrate foods, and the compulsive overeating of these foods leads to hyperinsulinemia and hyperandrogenemia (Lim et al., 2009; Wylie, Barr, & Jeanes, 2009). The hyperinsulinemia that ensues following a very high carbohydrate meal leads to excess androgen synthesis and hormonal dysfunction. These elevated androgens, in addition to negatively affecting appetite hormones, can promote bulimic behavior by
influencing food cravings, specifically for carbohydrate-rich foods, and impulse control (Naessén et al., 2006; Cotrufo et al., 2000). Therefore, it seems that there could potentially be more to bulimia than psychological distress alone (figure 3).

Figure 3. Potential association between androgens and bulimia. Psychological factors associated with bulimia are outside the scope of this paper, but may be linked to the hormonal environment in some individuals. T: testosterone; CHO: carbohydrate; CCK: cholecystokinin.

Due to the complex interactions involved in the cycle of bulimia, it is unknown at what point the cycle could potentially be broken. The consumption of a low insulinemic diet may prevent the excess production of androgens in women with PCOS, and thus reduce the compulsive urge to eat, specifically carbohydrate-rich food. A low insulinemic
diet may also help to regulate appetite hormones, leaving women with PCOS suffering from bulimia with a normalized appetite. While outside the scope of this paper, studies have also shown that high protein/low carbohydrate diets also have psychological benefits in women with PCOS, resulting in feelings of well-being and reduced depression (Galletly et al., 2007). This may be due to improved hormonal balance due to increased ratio of protein to carbohydrate, or the satiating effect of protein resulting in fewer feelings of deprivation.

In support of this theoretical framework of bulimia and its associated factors, one study looked at the effects of treatment with androgen receptor antagonists (ARA) on symptoms of bulimia (Naessén, Carlström, Byström, Pierre, & Hirschberg, 2007). Results showed that ARA treatment significantly reduced the hunger response and craving for sweets \( (p = 0.05) \), as well as in increased CCK response \( (p < 0.001) \). There was also a decrease in free and total testosterone, as well as improved acne and hirsutism scores. Three of the twenty-one bulimics no longer fulfilled the criteria for bulimia nervosa diagnosis following ARA treatment, and six of the twenty-one bulimics displayed reduced symptoms of bulimia following ARA treatment. According to this data, methods of reducing serum insulin concentrations may be successful in the treatment of bulimia by reducing testosterone levels, and thus impulsive behavior, carbohydrate cravings, and hunger. This hormonal improvement may also help to alleviate some of the psychological issues associated with the disorder as well. Further research involving methods of reducing the hyperandrogenic state is needed to improve the cycle of compulsive overeating and PCOS.
Pharmaceutical Treatment of PCOS

Untreated PCOS can lead to a variety of health disorders, including CHD, metabolic syndrome, and several forms of cancer. The metabolic syndrome is characterized by the presence of IR, obesity, and three of the following conditions: hypertension, hypertriglyceridemia, low HDL cholesterol, fasting glucose $\geq 110$ mg/dL, or a waist circumference $\geq 35$ inches. Therefore, young women should be screened early and followed closely (Liepa et al., 2008) to prevent the development of chronic disease. The optimal treatment of PCOS has yet to be established, and some researchers suggest a multi-factorial approach. Pharmaceuticals, diet and lifestyle change, and surgery are all interventions that are currently used either alone or in combination to treat this disorder.

The majority of research aimed at reducing these symptoms and risks has been focused on pharmaceutical approaches (Sharma, Walker, & Atiomo, 2010). Pharmaceutical interventions are primarily aimed at improving hyperandrogenism, anovulation, and insulin resistance through the use of oral contraceptives, antiandrogens, and insulin sensitizers (Diamanti-Kandarakis, Kandaraki, Christakou & Panidis, 2009). While most endocrinologists and gynecologists agree that pharmaceutical treatment should be second to diet and lifestyle change (Liepa et al., 2008), physicians and their patients often take the “band-aid” approach to treating the individual symptoms of PCOS instead of improving the underlying hormonal environment.

Metformin is the most common drug administered to women with PCOS because of its established beneficial effects on insulin sensitivity and hyperandrogenism (Israni & Goyal, 2010). However, it can lead to unfavorable side effects including diarrhea,
nausea, abdominal discomfort, anorexia, and metallic taste in the mouth. Oral contraceptives (OCs) are another treatment commonly prescribed to women with PCOS in order to improve insulin sensitivity, reduce hyperandrogenism, and regulate menstrual periods (Cibula, Fanta, Hill, Sindelka, Skrha & Zivny, 2002). While OCs have also shown to have beneficial effects, they are not advantageous to the large proportion of women with PCOS that are trying to conceive. Alternative methods for balancing the hormonal environment are needed for treating the host of symptoms and metabolic issues associated with PCOS.

**Nutritional and Lifestyle Factors**

While impressive efforts have been made in the pharmaceutical management of the disorder via improving sensitivity to insulin, diet-induced reduction of serum insulin is beginning to gain attention. One study by Slabber, Barnard, & Kuyl (1994) compared the effects of two iso-hypocaloric diets on serum insulin concentrations and weight loss in 30 obese hyperinsulinemic females over a 12 week period. One diet was a low insulinemic diet (LD) and the other was a conventionally balanced diet (BD), with both diets supplying approximately 1200 kcal per day and a macronutrient breakdown of 50% carbohydrate, 20% protein, and 30% fat. The main principle of the LD was consumption of carbohydrate sources known to evoke a low insulin response. While both diets resulted in weight loss, fasting insulin concentrations decreased significantly more after LD than with BD ($p = 0.01$) despite the same level of energy restriction and weight loss. This
study shows that the quality of the diet must be taken into account to interpret the results of studies showing no effect of weight loss on hormonal parameters.

It is obvious that a low insulinemic diet would be one which reduces dietary carbohydrate. Reduced carbohydrate diets have been utilized for many years as successful methods of weight reduction. These diets are often higher in fat and protein, which increase serum insulin far less than carbohydrate, thus becoming of interest in the treatment of disorders of insulin resistance. In one prospective study, 18,555 premenopausal women were followed over eight years as they attempted pregnancy (Chavarro, Rich-Edwards, Rosner & Willett, 2009). The results showed that the amount as well as the type of carbohydrate in the diet may be important determinants of ovulation and fertility. Any improvement in ovulation can be expected to be a result of improving the hormonal environment in the ovary, and thus, the amount and type of carbohydrate in the diet may be important determinants of other PCOS-related symptoms as well. It is unknown the degree- or type of carbohydrate restriction necessary to promote improvements in the hormonal environment or clinical symptoms of PCOS (Sheard, Clark, Brand-Miller, Franz, Pi-Sunyer, Mayer-Davis, Kulkarni, & Geil, 2004), but various approaches to carbohydrate restriction and their effects will be described in detail later.

*Insulinemic Foods*

Studies measuring the glycemic and insulinemic effects of different foods have provided surprising results, which may be useful in the treatment of PCOS and its related
conditions (Layman, Clifton, Gannon, Krauss, & Nuttall, 2008). One study found that meals high in carbohydrates from starch resulted in higher glucose area responses and insulin area responses than meals containing non-starchy carbohydrate sources (Gannon, Nuttall, Westphal, Fang & Ercan-Fang, 1998; Nuttall & Gannon, 2007).

One explanation for the insulinotropic properties of foods high in starch is that postprandial blood glucose and insulin responses are greatly affected by food structure. Any process that disrupts the physical structure of a food increases the insulin response, including cooking (Björck, Granfeldt, Liljeberg, Tovar & Asp, 1994). This structural transformation of starch when exposed to heat and water is called gelatinization. The degree of gelatinization is an important factor in the metabolic response to starchy foods, and the glucose and insulin response is significantly greater after ingestion of cooked versus raw starches (Holm, Lundquist, Björk, Eliasson & Asp, 1988). According to these results, the consumption of starchy foods in their raw state would be preferable to reducing the insulin response in women with PCOS. However, raw starch is not palatable and the consumption of some legumes in their raw state can be poisonous and should not be encouraged (Hove & King, 1979).

The dissociation between the glycemic and insulnemic responses to milk and milk products is also being extensively studied. Several studies (Makrantonaki et al., 2011; Hoyt, Hickey & Cordain, 2005; Östman, Elmståhl & Björk, 2001; Melnik & Schimiz, 2009) have found that the consumption of milk results in significant increases in insulin and IGF-1 concentrations comparable with high glycemic foods. In most carbohydrate containing foods, the insulin response is predictable and closely tied to the
glucose response of the food. According to Hoyt et al., (2005), some foods demonstrate dissociation between the insulin response and the glucose response, such as oatmeal, kidney beans, lentils, and milk. Unexpectedly, milk has been shown to be a potent insulin secretagogue. The observed insulin response after consumption of milk and milk-products is often triple that predicted by the actual carbohydrate content of milk (Danby, 2008). Östman et al. (2001) found that insulinemia was greater after the consumption of milk products than after an equivalent amount of lactose and water. Therefore, it has been proposed that a specific component of milk is responsible for this exaggerated insulin response, and research points to whey protein. The insulin response to a whey only meal has been reported to be higher than that of a milk meal, suggesting that whey may be the component of milk that causes its insulinotropic properties (Melnik & Schmitz, 2009). Casein, the other protein in milk and milk products has been shown to be a potent stimulator in IGF-1, which was previously explained as an analogue of insulin and promoter of reproductive cancers (Hoppe, Mølgaard, Dalum, Vaag, & Michaelson, 2009; Muti, 2004). Although further research is needed regarding the adverse effects of milk on insulin metabolism and IGF-1, data suggests that caution is warranted in recommending milk consumption to women with PCOS.

**Currently Recommended Diet and Lifestyle Modifications**

Diet modification is effective in improving the signs and symptoms of PCOS (Mavropoulos, Yancy, Hepburn & Westman, 2005; Galletly et al., 2007). Weight loss alone may lead to significant reductions in the symptoms of PCOS, but some researchers
suggest that further improvement is seen with strict carbohydrate restriction and insulin control (Chavarro et al., 2009; Norman, Davies, Lord, & Moran, 2002). The current National Institutes of Health (NIH) recommendations for the dietary treatment of PCOS propose a diet low in fat (~30% calories from fat and ~10% calories from saturated fat), moderate in protein (~15%), and high in carbohydrate (~55%), as well as increased consumption of whole grains, fiber, beans, and cereals (Norman et al., 2002). However, research does not support these recommendations for this specific population (Douglas, Gower, Darnell, Ovalle, Oster & Azziz, 2006; Mavropoulos et al., 2005; Chavarro et al., 2009; Hellerstein, 2002; Hays, DiSabatino, Gorman, Vincent & Stillabower, 2003).

Researchers are beginning to establish that beneficial effects are seen when dietary protein is increased at the expense of carbohydrate (Hellerstein, 2002). These beneficial effects are thought to be due to the satiating effect of diets high in protein, as well as the concomitant lowering of insulin from the reduction of dietary carbohydrates. Despite these findings, there is still an inconsistency among primary care providers regarding dietary recommendations to reduce symptoms and risks in these patients (Chavarro et al., 2009; Jones, Balen & Ledger, 2008). While some physicians and dietitians recommend lifestyle changes that include simply reducing refined sugar and starch, others recommend a more dramatic approach.

Low Glycemic Index Diet

Low glycemic index (GI) diets are often recommended for this population in order to help them lose weight, control blood glucose, improve insulin sensitivity,
normalize menses, and improve hirsutism and acne, but their effectiveness has yet to be established. In one study by Marsh, Steinbeck, Atkinson, Petocz & Brand-Miller (2010), ninety-six obese, premenopausal women with PCOS were assigned to consume either an ad libitum low GI diet or a macronutrient-matched healthy diet (based on Dietary Guidelines for Americans). The women were followed for 12 months or until they achieved a 7% weight loss. Changes in insulin sensitivity using an OGTT, body composition, plasma lipids, reproductive hormones, health-related QOL, and menstrual cycle regularity were measured. The results showed an improvement in insulin sensitivity ($p = 0.03$) and menstrual cycle regularity (95% compared with 63% for the low GI and healthy diet, respectively). There were no significant changes among the biochemical measures, except for fibrinogen which showed significant differences between diets ($p < 0.05$) (Marsh, 2010). According to these results, benefits can be seen with low glycemic index diets (Ghosh, Murphy, & Elsheikh, 2008; Smith, Mann, Braue, Mäkeläinen & Varigos, 2007; Brand-Miller, Hayne, Petocz & Colagiuri, 2003), but these improvements often pale in comparison to diets that strictly reduce dietary carbohydrates.

Another study sought to compare the effects of a low GI diet and a low carbohydrate (LC) diet on anthropometric and biochemical parameters in overweight and obese women with PCOS (Ghosh et al., 2008). Twenty four women participated in the six-month study and were randomized to either diet. Results of the study included significant decreases in weight ($p = 0.007$), and marked improvements in body composition, insulin, lipid and testosterone levels, and menstrual cycle regularity in the LC versus the low GI diet. The researchers also noted that while reduction of body

36
weight led to improvements in body composition and metabolic markers independent of the type of diet being followed, the LC achieved a greater degree of improvement than the low GI diet (Ghosh et al., 2008).

The logic behind the low GI diet explains its potential effectiveness, and some researchers have found statistically significant metabolic improvements, whereas others have not (Brand-Miller et al., 2003). Studies finding no significant metabolic improvements using low GI diets may be due to the fact that while low GI diets have the potential to be low in dietary carbohydrate, overconsumption of low GI and medium GI foods can elicit a great insulin response (i.e., starchy foods and dairy products). Thus, diets focused on foods that do not promote this insulin response show greater improvements in improving the symptoms and chronic disease risk factors associated with PCOS.

Low Carbohydrate Diets

Dietary carbohydrate is the major determinant of postprandial glucose and insulin levels, and recent studies have shown that a low carbohydrate, ketogenic diet can lead to weight-loss and improvement in IR (Westman, Yancy, Mavropoulos, Marquart & McDuffie, 2008). While it may be plausible that consuming less than 20 g of carbohydrate per day, a common carbohydrate limit in ketogenic diets, would lead to better glycemic control than a “low glycemic diet”, the idea has not been widely tested. One study, however, sought to determine whether an ad libitum low carbohydrate, ketogenic diet (LCKD) improved glycemic control better than a calorie-restricted low GI
diet in obese subjects over a 24 week period (Westman et al., 2008). The results showed that the LCKD group had a greater reduction in hemoglobin A\textsubscript{1c} ($p = 0.009$), greater reduction or elimination of medication (95.2\% versus 62.1\% in the LCKD and low GI, respectively; $p < 0.01$), greater weight loss (-11.1 kg vs. -6.9 kg; $p = 0.008$), and greater mean improvement in HDL cholesterol (+5.6 mg/dL vs. 0 mg/dL; $p < 0.001$) than the low GI diet groups. Diabetes medication was also reduced or eliminated in 95.2\% vs. 62\% in the LCKD and low GI diets, respectively ($p < 0.01$).

The greater weight loss seen in the LCKD group is surprising because the average daily caloric intake on the LCKD was 1550 ± 440 kcal per day compared to 1335 ± 372 kcal (mean ± standard deviation) per day in the low GI diet group. Thus, despite an average intake of approximately 200 kcal more per day, the LCKD experienced a significantly greater weight loss compared to the low GI diet group. Another surprising effect was that even after adjustment was made for weight loss, statistical significance remained. This suggests that the greater effect of the LCKD in this study was due solely to its lower carbohydrate content (Westman et al., 2008).

In another study by Mavropoulos et al. (2005), researchers studied the specific effects of a LCKD on the risk factors and symptoms associated with PCOS. Eleven women with a mean body mass of >27 kg/m\textsuperscript{2}, mean age of 34.5 years, and a clinical diagnosis of PCOS were recruited. The patients were instructed to limit carbohydrate intake to 20g or less for 24 weeks as well as complete a PCOS-related quality of life questionnaire (PCOSQ). Results showed that all subjects lost weight, with an overall mean body weight change from baseline to 24 weeks of -12\% and mean decrease in BMI
of 4.0 kg/m² (p = 0.006). There were also significant reductions in percent free testosterone (p = 0.04) and fasting serum insulin (p = 0.002). Although the dietary treatment was high in fat (particularly saturated fat) and cholesterol, no adverse lipid changes were recorded. The domain scores from the PCOSQ also showed a trend for improvements in domains of “hair,” “infertility,” and “menstruation” (p=.06 for all three domains). Although this pilot study showed that adherence to a LCKD led to an improvement in body weight, percent free testosterone, fasting serum insulin, and symptoms in women with PCOS over a 24 week period, further research is needed to determine if the benefits were from the weight loss alone or from carbohydrate restriction (Mavropoulos et al., 2005; Chavarro et al., 2009). However, previous studies have shown that the benefits of carbohydrate restricted diets are due to the sheer reduction of dietary carbohydrates, independent of weight loss (Westman et al., 2008).

Despite the effectiveness seen with low carbohydrate diets, primary care providers are hesitant to prescribe these diets to patients because they are often high in saturated fat, total fat, and cholesterol, and low in fiber; dietary characteristics previously thought to promote heart disease. While carbohydrate-induced hypertriglyceridemia is beginning to gain widespread recognition as playing a colossal role in the prevalence of heart disease (Hellerstein, 2002), health care providers still cling to the age-old advice that recommends substituting dietary fat with carbohydrate to prevent cardiac risk factors. Studies show that plasma triglyceride concentrations increase in a dose-dependent manner, and increases in triglycerides can be seen even after increases as little as 10% in dietary carbohydrate. The cardiovascular outcomes related to carbohydrate-induced
hypertriglyceridemia is consistent and reproducible, and may be one of the highest priorities in public health nutrition (Hellerstein, 2002). Studies assessing the effects of replacing dietary carbohydrates with saturated fat on the development of heart disease are producing surprising results.

One study looked at the effects of a high saturated fat/starch avoidance (HSF-SA) diet on serum lipids in obese patients with and without PCOS (Hays et al., 2003). Twenty-three patients (15 with PCOS) were instructed to consume one half of all daily calories as saturated fat, primarily in the form of red meat, cheese, and eggs. Fresh fruit and non-starchy vegetables were allowed in restricted amounts at each meal, but dietary starch and starchy vegetables were forbidden. The results showed that subjects had a mean weight loss of $5.5 \pm 2.1$ kg ($p < 0.001$) representing a loss of $5.2\% \pm 2.5\%$ of total body weight (TBW) ($p < 0.001$), decrease in waist size ($-2.2 \pm 1.0$ inches; $p < 0.001$) and hip size ($-2.6 \pm 0.9$ inches; $p < 0.001$), and a decrease in total body fat ($-0.7\% \pm 1.8\%; p = 0.02$). Patients with PCOS had a TBW loss of $14.3\% \pm 20.3\%$ ($p < 0.008$). There was also a significant decrease in mean fasting glucose levels ($p = 0.04$), insulin levels ($p = 0.006$), total triglyceride levels ($p < 0.001$), VLDL concentration and size ($p < 0.001$ and $p < 0.001$, respectively), and an increase in HDL size ($p = 0.01$). Changes in LDL and HDL concentrations were no significant, but there was an increase in LDL size ($p = 0.02$). It is important to note that markers of metabolic syndrome include high levels of small and dense LDL, low levels of large HDL, and high levels of large VLDL. Thus, the increase in LDL and HDL particle size, and decrease in VLDL particle size in the previous study are improvements in the lipid profile (Hays et al., 2003).
Overwhelming evidence supports the atherogenic effects of the addition of saturated fat to an otherwise low-fat, high carbohydrate diet (Hellerstein, 2002; Hays et al., 2003). The previous study found that if dietary carbohydrate is replaced with saturated fat, instead of the two being combined, not only are adverse atherogenic effects not seen, but significant improvements to the lipid profile can be expected (Hays et al., 2003; Hays, Gorman & Shakir, 2002; Samaha, Iqbal, Seshadri, Chicano, Daily, McGrory, Williams, et al., 2003; Mavropoulos et al., 2005; Westman et al., 2008;).

According to these studies, apprehension of primary care providers in prescribing low carbohydrate diets should not be based on the saturated fat and cholesterol content of the diet and its possible atherogenic effects. However, diets low in carbohydrate, especially LCKD, are often very low in fruits and vegetables, and thus dietary fiber, nutrients, and antioxidants. This low fiber, nutrient, and antioxidant content should be the main reason behind the hesitation of primary care providers to prescribe this type of dietary regime, because of their beneficial effects on inflammation, cancer, and heart disease (Basu, Devaraj, & Jialal, 2006). There is also a common misunderstanding among health care providers and the general public that diets with reduced carbohydrate automatically result in extreme amounts of saturated fat. While there is an increase in total fat, the increase in saturated fat content is not always as extreme as some popular carbohydrate restricted dietary regimes (i.e., Atkins). Perhaps there is a middle ground that exists between low GI diets and LCKD diets; a dietary regime that focuses on nutrient rich, non-starchy vegetables and fruits, lean meats and poultry, fatty fish, and
healthy fats from nuts, seeds, and fatty fruits, at the expense of starch, dairy, and added sugars (Cordain, Eades, & Eades, 2003).

**Low Insulinemic Diet**

A low insulinemic diet would be one that focused on the most nutrient rich and least insulinemic foods. Glycemic Index has been used as a method to measure the glycemic response of foods, but its method is flawed because it does not take into account serving size. Therefore, researchers determined that multiplying the GI of a food by its carbohydrate content in 10g portions would give a more representative view of a particular food, known as its glycemic load (GL) (Atkinson, Foster-Powell & Brand-Miller, 2008). Table 1 lists the GL of commonly eaten carbohydrate containing foods. Dairy products are not listed because although they typically maintain lower GIs and GLs, they are highly insulinotropic with insulin indices similar to white bread (Cordain, 2002; Hoyt et al., 2004; Östman et al., 2001; Danby, 2008).
Table 1. Glycemic Loads (GL) of commonly eaten carbohydrate containing foods. Data adapted from Cordain (2002).

<table>
<thead>
<tr>
<th>Grains/Beans</th>
<th>GL</th>
<th>Vegetables</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagel</td>
<td>38.4</td>
<td>Sweet Potato</td>
<td>13.1</td>
</tr>
<tr>
<td>Rice Cakes</td>
<td>66.9</td>
<td>Carrots</td>
<td>7.2</td>
</tr>
<tr>
<td>Navy Beans</td>
<td>19</td>
<td>Beets</td>
<td>6.3</td>
</tr>
<tr>
<td>Brown Rice</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White Rice</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All Bran Cereal</td>
<td>32.5</td>
<td>Banana</td>
<td>12.1</td>
</tr>
<tr>
<td>Whole Wheat Bread</td>
<td>31.8</td>
<td>Pineapple</td>
<td>8.2</td>
</tr>
<tr>
<td>White Bread</td>
<td>34.7</td>
<td>Grapes</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Apple</td>
<td>6</td>
</tr>
<tr>
<td>Sweets</td>
<td>GL</td>
<td>Fruits</td>
<td>GL</td>
</tr>
<tr>
<td>Jelly Beans</td>
<td>74.5</td>
<td>Orange</td>
<td>5.1</td>
</tr>
<tr>
<td>Table Sugar</td>
<td>64.9</td>
<td>Pear</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Fruits and vegetables have universally low GLs, as well as higher nutrient contents and soluble fiber. Therefore, for the purpose of maintaining a diet with the highest amount of nutrients for the lowest insulin response, greater emphasis should be placed on the consumption of non-starchy fruits and vegetables in lieu of dairy and starch-containing foods.
It is thought that the reason starch containing foods have a higher glycemic response is because glucose derived from digestion of foods raises blood glucose more than fructose or galactose. Starch is a polymer of glucose, whereas fruits and most vegetables are made up of glucose and fructose. Thus, non-starch containing foods may raise blood glucose less than starch-containing foods because of their different monosaccharide composition. As briefly discussed, one study sought to determine the circulating 24hr glucose response to three different types of mixed meals (Gannon et al., 1998). The first type of test meal was representative of the American Diabetes Association (ADA) recommendation for people with diabetes and the general public, consisting of high carbohydrate content (55%) with an emphasis on starch (HS). The second type of test meal was designed to approximate the normal American diet (i.e., usual carbohydrate, usual starch) with 40% carbohydrate (US). The third type of test meal was designed to contain an amount of carbohydrate similar to the US test meals (43%), but with non-starch containing foods (i.e., fruits and non-starchy vegetables) (LS). The calorie intake for the three test meals was similar (2,052 kcal, 2,098 kcal, and 2,052 kcal for the HS, US, and LS test meals, respectively). Results showed that glucose area response was positively associated with starch ($p = 0.02$), and the LS meal had an insulin area response of 60% of the area measured after the HS meals and 54% of the US meals ($p < 0.05$). It is also important to note that after consumption of the LS meals, the glucagon area response was 350% greater than after the HS meals when overnight fasting values were used as a baseline (Gannon et al., 1998). Therefore, a diet based on non-
starchy sources of carbohydrates has potential to help prevent excess insulin secretion and reduce symptoms of PCOS.

Tables 2 and 3 compare a sample menu for a low insulinemic type dietary plan and a USDA dietary guidelines healthy eating plan, as well as their respective nutrition and percent recommended dietary allowance/average intake (%RDA/AI).

Table 2. Sample menu for a low insulinemic diet and its respective nutrition.

<table>
<thead>
<tr>
<th>Low Insulinemic Sample Menu</th>
<th>Nutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td></td>
</tr>
<tr>
<td>Coffee with 1 cup almond milk</td>
<td>Calories 1,679</td>
</tr>
<tr>
<td>Omelet</td>
<td>Fat 90g</td>
</tr>
<tr>
<td>2 eggs + 2 egg whites</td>
<td>Sat. Fat 16g</td>
</tr>
<tr>
<td>¼ tsp olive oil</td>
<td>PUFA 30g</td>
</tr>
<tr>
<td>½ cup onions, bell peppers,</td>
<td>MUFA 30g</td>
</tr>
<tr>
<td>tomato, spinach</td>
<td>Carbohydrate 108g</td>
</tr>
<tr>
<td>1 cup strawberries</td>
<td>Fiber 42g</td>
</tr>
<tr>
<td></td>
<td>Protein 142g</td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td></td>
</tr>
<tr>
<td>Chicken Caesar salad w/ 2 T Caesar dressing</td>
<td>Fiber 168%</td>
</tr>
<tr>
<td>4oz grilled chicken + 6 cups romaine lettuce</td>
<td>Vit A 219%</td>
</tr>
<tr>
<td>(substitute 1oz sunflower seeds for croutons)</td>
<td>Vit B-6 228%</td>
</tr>
<tr>
<td>(no parmesan cheese)</td>
<td>Vit B-12 426%</td>
</tr>
<tr>
<td></td>
<td>Vit C 471%</td>
</tr>
<tr>
<td></td>
<td>Vit D 119%</td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nutrition</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td><strong>Apple</strong></td>
<td></td>
</tr>
<tr>
<td>1 oz almonds (~22 nuts)</td>
<td></td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td></td>
</tr>
<tr>
<td>6 oz salmon</td>
<td></td>
</tr>
<tr>
<td>2 cups green beans + 1 pat butter</td>
<td></td>
</tr>
<tr>
<td>1 cup blackberries</td>
<td></td>
</tr>
<tr>
<td><strong>Snack</strong></td>
<td></td>
</tr>
<tr>
<td>Hot Cocoa</td>
<td></td>
</tr>
<tr>
<td>1 cup almond milk</td>
<td></td>
</tr>
<tr>
<td>2 T unsweetened cocoa powder</td>
<td></td>
</tr>
<tr>
<td>splenda to taste</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vit E 480%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calcium 99%</td>
</tr>
<tr>
<td></td>
<td>Copper 211%</td>
</tr>
<tr>
<td></td>
<td>Iron 106%</td>
</tr>
<tr>
<td></td>
<td>Magnesium 161%</td>
</tr>
<tr>
<td></td>
<td>Manganese 256%</td>
</tr>
<tr>
<td></td>
<td>Niacin 254%</td>
</tr>
<tr>
<td></td>
<td>Pant Acid 150%</td>
</tr>
<tr>
<td></td>
<td>Phosphorous 244%</td>
</tr>
<tr>
<td></td>
<td>Riboflavin 190%</td>
</tr>
<tr>
<td></td>
<td>Selenium 289%</td>
</tr>
<tr>
<td></td>
<td>Thiamin 145%</td>
</tr>
<tr>
<td></td>
<td>Zinc 105%</td>
</tr>
</tbody>
</table>

Table 3. Sample menu for a USDA dietary guidelines healthy diet. Diet created for a woman 19-30ys and its respective nutrition and %RDA/AI.
<table>
<thead>
<tr>
<th>Lunch</th>
<th>Protein</th>
<th>%RDA/AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 slices whole wheat bread</td>
<td>83g</td>
<td></td>
</tr>
<tr>
<td>2 oz deli turkey meat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 slice low fat cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 oz tomato, lettuce, onion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 T mustard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cup low fat yogurt</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 oz pretzels</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 apple</td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ oz almonds (~11 almonds)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 ½ oz grilled chicken</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 medium potato + 1 pat butter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salad</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 cup romaine lettuce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>½ cup diced tomatoes, mushrooms, onion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 T olive oil/vinegar dressing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 cup rice</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>%RDA/AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit A</td>
<td>90%</td>
</tr>
<tr>
<td>Vit B-6</td>
<td>138%</td>
</tr>
<tr>
<td>Vit B-12</td>
<td>112%</td>
</tr>
<tr>
<td>Vit C</td>
<td>166%</td>
</tr>
<tr>
<td>Vit D</td>
<td>65%</td>
</tr>
<tr>
<td>Vit E</td>
<td>49%</td>
</tr>
<tr>
<td>Calcium</td>
<td>108%</td>
</tr>
<tr>
<td>Copper</td>
<td>145%</td>
</tr>
<tr>
<td>Iron</td>
<td>60%</td>
</tr>
<tr>
<td>Magnesium</td>
<td>104%</td>
</tr>
<tr>
<td>Manganese</td>
<td>228%</td>
</tr>
<tr>
<td>Niacin</td>
<td>169%</td>
</tr>
<tr>
<td>Pant Acid</td>
<td>124%</td>
</tr>
<tr>
<td>Phosphorous</td>
<td>207%</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>156%</td>
</tr>
<tr>
<td>Selenium</td>
<td>161%</td>
</tr>
<tr>
<td>Thiamin</td>
<td>134%</td>
</tr>
<tr>
<td>Zinc</td>
<td>110%</td>
</tr>
</tbody>
</table>
After comparing the two diets, it is obvious that a diet which obtains dietary carbohydrates solely from fruit and vegetable sources can not only meet the necessary RDAs/AIs, but may surpass it in some cases. It is also interesting that a low insulinemic diet meets these RDAs/AIs in fewer calories than the USDA dietary plan with twice the fiber. It is logical to assume that diets with low calories, high fiber and nutrients, and very low GL carbohydrates would be beneficial to all populations, specifically women with PCOS.

One study investigated whether a diet similar to that of a low insulinemic diet confers health benefits (Frassetto, Schloetter, Mietus-Snyder, Morris & Sebastian, 2009). The pilot study included nine overweight, non-obese (mean BMI of 27.8 kg/m²), sedentary, healthy volunteers. Participants were instructed to eat a diet consisting of lean meats, poultry, fish, vegetables, low sugar fruits, nuts, seeds, and oil, with the exclusion of grains, cereals, legumes, honey, and dairy for 10 days. Meals and snacks were provided to them and calories were figured by a dietitian to ensure no weight loss, since weight loss can often show beneficial effects independent of dietary change. Results compared with baseline showed significant reductions in blood pressure ($p < 0.05$), plasma insulin versus time area under the curve (AUC) during an OGTT ($p = 0.006$), total cholesterol ($p = 0.007$), LDL cholesterol ($p = 0.003$), and triglycerides ($p = 0.01$). HDL increased an average of +4%, but was not significant. This pilot study shows that even short term consumption of a low insulinemic diet significantly improves the blood lipid profile, insulin sensitivity, glucose tolerance, and blood pressure independent of weight loss in healthy sedentary humans (Frassetto et al., 2009).
In one randomized, cross-over study on thirteen subjects with T2D, participants were instructed to eat a diet similar to that of a low insulinemic diet (i.e., lean meat, eggs, poultry, fish, vegetables, fish, nuts, and seeds) or a diabetes diet designed in accordance with dietary guidelines during two consecutive three-month periods (Jönsson, Granfeldt, Ahrén, Branell, Pålsson, Hansson, et al., 2009). Compared to the diabetes diet, the low insulinemic diet resulted in lower mean values of HbA1c ($p = 0.01$), triacylglycerol ($p = 0.003$), diastolic blood pressure ($p = 0.03$), weight ($p = 0.01$), BMI ($p = 0.04$), and waist circumference ($p = 0.02$), and higher mean values of HDL cholesterol ($p = 0.03$). It is important to note that no advice was given to restrict food intake in either diet, yet the low insulinemic diet had a lower reported energy intake (1581 kcal versus 1878 kcal for the low insulinemic diet and diabetes diet, respectively; $p = 0.005$). This agrees with the notion that this type of diet is satiating and results in a reduced caloric intake, thereby facilitating weight loss (Jönsson et al., 2009; Jönsson, Granfeldt, Erlanson-Albertsson, Ahrén, & Lindeberg, 2010).

**Conclusions**

Although the exact cause of PCOS is unknown, improving the hormonal environment that leads to the signs and symptoms of PCOS is of primary concern. Dietary recommendations should focus on carbohydrate and insulin control, with special attention placed on those patients exemplifying bulimic characteristics. Emerging research is beginning to show that fathers and brothers of women with PCOS have an increased risk for cardiovascular events (Taylor, Kar, Kunselman, Stetter, Dunaif, &
Further studies are needed to determine whether a low insulinemic diet would reduce cardiovascular risk factors in this population as well.

According to the literature, current diet and lifestyle changes for treatment of PCOS involve either a low GI diet or a LCKD. LCKDs result in greater improvements than low GI diets, but their highly restrictive nature may hinder long-term compliance. Low GI diets maintain an emphasis on dairy, grains and other starch-containing foods, which may have insulinotropic properties, thus contributing to the inconsistent results seen with administration of this diet on signs and symptoms in PCOS. A middle ground is needed that incorporates each diet by removing those disadvantageous characteristics of each: the removal of starch and dairy from the low GI diet and the inclusion of greater amounts of fruits, vegetables, and lean proteins to the LCKD. This low insulinemic diet would be composed of those foods with the highest nutrient qualities and that invoke the smallest insulin response, in *ad libitum* amounts.

Considering the detrimental effects of hyperinsulinemia on the hormonal environment and its subsequent signs and symptoms in PCOS, it is hypothesized that a low insulinemic diet would improve weight and WHR, insulin sensitivity, binge eating behaviors, signs and symptoms of hyperandrogenemia, chronic disease risk factors, and quality-of-life in women with PCOS. The purpose of this study is to determine the effect of the reduction of insulinemic foods on the anthropometric, biochemical, and metabolic outcomes in overweight and obese premenopausal women with PCOS.
CHAPTER 3

METHODS

A prospective study was conducted to determine the effects of a low insulinemic diet on the hyperinsulinemic and hyperandrogenic state, and its subsequent effects on signs and symptoms of PCOS.

Subjects

Following approval by the Texas Tech University Health Science Center Institutional Review Board, 24 overweight and obese women (BMI ≥25kg/m² and ≤40 kg/m²) with PCOS were recruited from a gynecological/obstetrical and fertility clinic at Texas Tech Health Science Center (TTUHSC) under the supervision of a Reproductive Endocrinologist (REI). Women diagnosed with PCOS were be asked by the REI if they would like to participate in an 8-week dietary intervention aimed at improving the symptoms and co-morbidities associated with PCOS. Subjects agreeing to participate in
the study were contacted by a Registered Dietitian to provide additional details regarding the intervention and to set up an appointment.

**Inclusion Criteria**

Eligible women were those between 18-45 years of age with a BMI ≥25kg/m^2 and ≤40 kg/m^2. Diagnosis suggestive of PCOS was based on oligo- and/or amenorrhea and the presence of hyperandrogenism (clinical and/or biochemical). Participants were screened for eligibility by the REI. Oligomenorrhea was determined by cycle length (<21 d or >35d, or variation between consecutive cycles of >3 d), and amenorrhea was determined as lack of a menstrual period ≥12 months. Clinical (presence of android obesity, hirsutism, acne, or androgenic alopecia) and biochemical hyperandrogenism (testosterone > 55 ng/dl) was assessed by the REI and TTUHSC laboratories. Presence of hirsutism was quantified by the REI via the Ferriman-Gallwey (FG) score.

**Exclusion Criteria**

Women with adrenal enzyme defects such as Cushing’s Syndrome or adrenal virilizing tumors, patients with type II diabetes (T2D), evidence of late onset 21-hydroxylase deficiency, or any other medical condition requiring supervision were excluded from the study involving PCOS. Women looking to become pregnant or nursing during the length of the study, women with a confirmed eating disorder, and women with gastrointestinal absorption issues were excluded as well. These conditions were screened and diagnosed by the REI. Subjects were also expected to eliminate
insulin sensitizers, oral contraceptives, and cyclic progesterone for month prior to the study.

**Intervention**

At the baseline assessment visit, participants were provided a consent form, screening consent form, screener, and a questionnaire addressing their readiness to change by using an adapted version of a validated Stage of Change questionnaire (McConnaughy, Prochaska, & Velicer, 1983). The 32-item questionnaire uses a 5-point Likert-type format in which a score of 1 indicates strong disagreement and a score of 5 indicates strong agreement. Scores in each stage of change (precontemplation, contemplation, and action) were summed and the section with the highest score determined the participant’s stage of change. Only participants in the action stage of change were included and were instructed to begin the diet the same day. Participants were given intensive education on the diet and provided with an educational packet that includes details about the diet, as well as supplemental information, tips, recipes, and menus to aid in participant compliance.

Subjects were instructed to follow a low insulinemic diet throughout the 8-week study. The diet included *ad libitum* consumption of lean animal protein (meat, chicken, turkey, other fowl, fish, shellfish, and eggs), non-starchy vegetables, fruits (including fatty fruits), nuts, seeds, and oils. Subjects older than 21 years were allowed one 6oz glass of red wine per night, and all subjects were allowed up to 1oz of prepared or fresh, full-fat cheese per day. The diet excluded all grains (refined and whole), beans, pulses,
dairy products (except cheese and butter), and sugar (including cane sugar, beet sugar, raw turbinado sugar, evaporated cane juice, brown rice syrup, high-fructose corn syrup, corn sugar, honey, or agave nectar) because of their insulinotropic properties. Sugar substitutes were allowed for participants that wished to use them. Participants were not advised to count calories or carbohydrates, and they were encouraged to eat until they were satisfied, but not to overeat. Participants were instructed to continue their normal exercise routine for the duration of the study.

Dietary adherence was measured by food records and a self-report. Three day food records were collected at weeks 1, 4, and 7. Food logs were analyzed to determine each participant’s estimated food quotient (FQ) using Black’s Formula (Black, Prentice, & Coward, 1986). FQ has been used to predict fasting RER in previous studies (Black, et al., 1986). The researchers in this study aim to compare average post-study fasting RER and FQ.

**Anthropometric, Biochemical, and Metabolic Outcome Measures**

All subjects participated in anthropometric and biochemical measurements (n=20). For metabolic measurements, ten subjects were selected depending on their time, work, and family commitments, as well as driving distance from testing location due to the length of time (5.5 hours) needed to complete testing.

*Anthropometric Measurements*
At the baseline and week 8 anthropometric assessment visits, data collection included age, race, height, weight, body composition, waist circumference (WC; minimum circumference between the iliac crest and the rib cage), hip circumference (HC; maximum protuberance of the buttocks), waist-to-hip ratio (WHR), and BMI. Body weight was measured by a Tanita scale with subjects wearing light clothing, but with shoes removed. Body composition was measured using the BODPOD. To use the BODPOD subjects were in light clothing with all hair in a swim cap. Once inside the BODPOD, the subject sat on a bench and breathed normally. Body composition was then measured through air displacement.

**Biochemical Measurements**

After anthropometric assessments were completed at the Human Sciences Building, subjects met the dietitian at Dr. Phy’s clinic at TTUHSC for biochemical assessments. Dr. Phy conducted a subjective assessment for hirsutism using the Ferriman-Gallwey method at baseline and week 8 to reduce bias.

Laboratory tests for experimental subjects at week 0 and 8 included 25-hydroxy vitamin D, total and free testosterone, fasting glucose and insulin, glucose and insulin following a 75g 2-hr oral glucose tolerance test (OGTT) with blood samples collected at 120 minutes, hemoglobin A1c (HgbA1c), and a complete lipid panel. All laboratory and anthropometric tests were taken on the same day (with the exclusion of RMR and RER). The OGTT was performed after obtaining venous blood samples and anthropometric measurements. Serum free testosterone was measured by enzyme immunoassay; serum
insulin by immunoenzymatic assay; serum glucose by using a hexokinase reagent; and HgbA1c by immunoassay.

The dietitian administered questionnaires and provided the diet education while subjects were undergoing their 2-hr OGTT.

**Metabolic Measurements**

In 10 subjects, indirect calorimetry was used to measure resting metabolic rate (RMR) after a 12h fast and at least 24h free of structured exercise 1 week prior to the beginning of the study and within 1 week after final measurements were taken on week 8. Subjects were asked to schedule a day to conduct these measurements as soon as possible following measurements on week 8, and were asked to continue to follow the diet until these final measurements were taken. This data provided information on any metabolic differences that occur in response to meals after following the intervention diet for 8 weeks. This test was conducted before and after the diet intervention to ensure that it did not interfere with week 8 measurements.

To perform these measurements, participants were asked to lie on a cot with a pillow and to relax, and a plastic canopy hood was placed over the subject’s head to measure oxygen consumed (VO₂) and carbon dioxide expended (VCO₂). The hood forms a tight seal preventing any contamination from the room air aside from the one inlet valve. Respiratory gases were used to calculate EE using the Weir equation (Weir,
1949) and macronutrient oxidation using equations developed by (Frayn, 1983): Fat 
\[
\text{Fat (g/min)} = (1.67*\text{VO}_2 (\text{L/min})) - (1.67*\text{VCO}_2 (\text{L/min})); 
\text{Carbohydrate (g/min)} = (4.56*\text{VCO}_2 (\text{L/min})) - (3.21*\text{VO}_2 (\text{L/min})).
\]
For these calculations, the first 5 minutes of each 20-min segment was discarded to allow subjects to enter into a steady state.

Additionally, the metabolic cart was calibrated against methanol burns (Schoffelen, Westerterp, Saris, & Hoor, 1997) throughout the duration of the study. The percentage recoveries from each burn were used to develop correction factors for the corresponding metabolic cart data from each study visit. The average recovery percentages for \(\text{O}_2\) and \(\text{CO}_2\) were 99% and 96% respectively. DIT was calculated from EE (postprandial EE subtracted by baseline EE).

Following baseline RMR measurement, subjects drank a high saturated fat liquid meal, which had a base of 8 fl oz (237 ml) of chocolate Ensure® containing a total of 6 grams (g) of fat (1g of saturated fat [SFA], 3g of polyunsaturated fat [PUFA], and 2g of monounsaturated fat [MUFA]), 40g of carbohydrate, and 9g of protein. To increase the proportion of SFA to make the liquid meal a SFA-rich high fat meal, we added 32g of butter, 5g of coconut oil, and 19g of palm oil to the chocolate Ensure®. After the addition of dietary fat and lecithin, total grams of fat in the high-fat meal equaled 56g which constituted 68% of total calories (Table 4). The subjects were instructed to finish the liquid meal within 5 minutes.

Following ingestion of the high fat liquid meal, indirect calorimetry was used to measure respiratory gases for each participant in 30 minute increments for a total of 330 minutes (30 minutes for fasting measurements and 300 minutes (5 hours) for post-
This measured the level of macronutrient oxidation (fat and carbohydrate oxidation) that occurred with each meal for up to 5 hours.

Table 4. Nutrient breakdown for the high saturated fat liquid meal.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>691.2</td>
</tr>
<tr>
<td>Calories from fat</td>
<td>470.85</td>
</tr>
<tr>
<td>Calories from saturated fat</td>
<td>310.61</td>
</tr>
<tr>
<td>Protein</td>
<td>8.97</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>43</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>34.51</td>
</tr>
<tr>
<td>Monounsaturated Fat</td>
<td>10.56</td>
</tr>
<tr>
<td>Polyunsaturated Fat</td>
<td>3.78</td>
</tr>
<tr>
<td>% from fat</td>
<td>68.10%</td>
</tr>
<tr>
<td>% from saturated fat</td>
<td>44.90%</td>
</tr>
</tbody>
</table>
PCOS Questionnaire

A PCOS-specific questionnaire (PCOSQ) was administered to at weeks 0 and 8 to monitor for changes in subjective symptoms related to PCOS (Cronin, Guyatt, Griffith, Wong, Azziz, Futterweight, Cook, & Dunaif, 1998; Guyatt, Weaver, Cronin, Dooley, & Azziz, 2004). The PCOSQ includes 25 items from five health related quality of life domains: emotions (7 items), hair growth (5 items), body weight (5 items), infertility (5 items), and menstruation (4 items). Each item is rated on a seven-point scale in which a score of 7 indicates no problems or difficulties and a score of 1 indicates maximum impairment on that item. The mean score of all items in a domain provides a domain score for each subject. Subjects were asked to fill out their PCOSQ survey on week 0 and 7 to provide the researchers information on each individual’s PCOS-related symptoms.

Binge Eating Scale

At baseline and week 8, a Binge Eating Scale (BES) was administered to assess the degree and frequency of binge episodes in women that experience them (Gormally, Black, Daston, & Rardin, 1982; Timmerman, 2007). Its administration at week 8 provided information to the researchers regarding whether participants experienced improvement in binge eating degree and frequency over the 8-week study. The BES consists of 16-items with each item rated quantitatively (0 indicating no binge eating problem and 3 indicating severe binge eating problem). Subjects chose which score best describes their perceptions and feelings about their eating behavior. The BES was scored
by adding the individual values for the 16 items with a possible score range of 0 to 46. Scores $\leq 17$ signify mild or no binge eating problem, scores of 18-26 signify moderate binge eating problem, and scores $\geq 27$ signify serious binge eating problem. One reason the BES was an appropriate measurement for the purposes of this study is that it relates to objective and subjective binge eating and not total caloric intake, making BES a valid measure of determining severity of uncontrolled eating behavior.

**Statistical Analyses**

A pretest-posttest study design was chosen with the comparison of interest being the change in anthropometric and/or biochemical outcome measures from baseline to 8 weeks. A two-tailed paired $t$-test was used to test for statistical significance of anthropometric and biochemical outcomes variables. A repeated measures ANOVA was used to analyze diet and diet-time interaction in-resting (preprandial) and postprandial measurements, and results were compared with a paired $t$-test for area under curve (AUC). All data analyses were performed using SAS version 9.3 (SAS institute, Cary NC). A $p$ value of $\leq .05$ was used to determine statistical significance.
CHAPTER 4

RESULTS

Baseline Demographics

The mean age of the participants was 29.7±3.8 years. Of the 24 participants, 14 were white, 8 were Hispanic, 1 was Pacific Islander, and 1 was Native American (Table 5).

Table 5. Participant Demographics (n=24)

<table>
<thead>
<tr>
<th>Ethnicity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>29.7±3.9</td>
</tr>
<tr>
<td><strong>White</strong></td>
<td>58.3%</td>
</tr>
<tr>
<td><strong>Hispanic</strong></td>
<td>33.3%</td>
</tr>
<tr>
<td><strong>Pacific Islander</strong></td>
<td>4.1%</td>
</tr>
<tr>
<td><strong>Native American</strong></td>
<td>4.1%</td>
</tr>
</tbody>
</table>
Anthropometric Outcome Measures

All participants had a reduction of total body mass, fat mass, percent fat, BMI, and WC and HC (Table 6). There was an average reduction in total body weight of -8.6 ± 2.3 kg ($p < 0.0001$), percent fat -1.5 ± 2.8% ($p = 0.02$), and BMI -3.3 ± 0.8 kg/m$^2$ ($p < 0.0001$) despite that the intervention was not designed as a weight loss diet (i.e., participants were not allowed to exercise above baseline and were instructed to eat approved foods ad libitum without calorie or carbohydrate counting). Participants had an average WC and HC reduction of -3.3±1.2 inches ($p < 0.0001$) and -2.5±1.0 inches ($p < 0.0001$), respectively. The reduction in WC led to a small but significant improvement in WHR, reducing the ratio by -0.04±0.24 ($p = 0.003$). Hirsutism, determined by the subjective Ferriman-Gallwey scoring method, was significantly reduced from 11.9±6.7 points to 9.8±6.5 points ($p = 0.007$).

Table 6. Anthropometric Data ($n = 24$). BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio; FM: fat mass; FFM: fat free mass.

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($\bar{x} \pm SD$)</th>
<th>Week 8 ($\bar{x} \pm SD$)</th>
<th>Change ($\bar{x} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>102.1±17.4</td>
<td>93.5±16.9</td>
<td>-8.6±2.3§</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>38.3±5.5</td>
<td>35.1±5.5</td>
<td>-3.2±0.9§</td>
</tr>
<tr>
<td>WC (inches)</td>
<td>43.2±5.1</td>
<td>39.9±4.8</td>
<td>-3.3±1.2§</td>
</tr>
<tr>
<td>HC (inches)</td>
<td>50.2±4.8</td>
<td>47.7±5.4</td>
<td>-2.5±1.0§</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86±0.07</td>
<td>0.84±0.06</td>
<td>-0.02±0.03†</td>
</tr>
<tr>
<td>FM (%)</td>
<td>46.3±6.8</td>
<td>44.6±4.7</td>
<td>-1.5±2.8*</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>50.7±4.6</td>
<td>52.5±4.7</td>
<td>+1.5±2.8</td>
</tr>
<tr>
<td>FG Score</td>
<td>11.9±6.8</td>
<td>9.8±6.5</td>
<td>-2.1±2.7†</td>
</tr>
</tbody>
</table>
Biochemical Outcome Measures

There was a significant reduction in fasting glucose, fasting and 2-hour insulin, fasting glucose-to-insulin ratio (GIR), HgbA1c, VLDL cholesterol, HDL cholesterol, triglycerides, and total and free testosterone (Table 7). Both average fasting and 2-hr serum glucose were normal and within non-diabetic ranges. Fasting glucose was reduced from 95.0±19.6 mmol/L to 86.0±8.4 mmol/L (p = 0.01) from pre- to post-study, but 2-hr glucose change was not significant (p = 0.25). Fasting insulin and 2-hour insulin were both significantly reduced by -16.9±13.6 (p < 0.0001) and -82.8±177.7 (p = 0.03), respectively. Figure 4a-d shows the improvement in fasting and 2-hr glucose and insulin levels. Fasting GIR was significantly improved +2.9±2.9 (p < 0.0001), but not 2-hr GIR (p = 0.89). HgbA1c was significantly reduced -0.25±0.3% (p= 0.001), despite that HgbA1c is typically viewed as an indicator of an individual’s three month overall average glucose levels. Considering the study lasted for only 8 weeks, the significant reduction in HgbA1c is interesting.

Three participants had elevated fasting blood glucose with normal HgbA1c, therefore the REI determined that these participants were slightly below the diagnostic criteria for NIDDM and could be included in the study. These three participants had an average reduction in fasting and 2-hr blood glucose and HgbA1c from 135.7±31.2mmol/L to 94.7±4.9mmol/L and 5.6±0.4% to 5.3±0.4%, respectively. These
same three participants plus four additional participants had elevated 2-hr blood glucose, which was reduced from 209.8±73.1 mmol/L to 131.6±52.1 mmol/L. Interestingly, fasting and 2-hr blood glucose and HgbA1c were reduced to normal ranges through the dietary intervention and weight loss, without exercise or medications.

There was an average reduction in all cholesterol measurements, despite the fact that participants were allowed ad libitum consumption of animal protein. While participants were encouraged to choose lean protein sources as often as possible, no restrictions were placed on sources of animal protein. There was a significant reduction in triglycerides, VLDL cholesterol, and HDL cholesterol of -54.6±59.3 mg/dl \((p < 0.0001)\), -8.9±13.3 mg/dl \((p < 0.0001)\), and -5.7±9.1 mg/dl \((p = 0.006)\), respectively. Changes in total cholesterol and LDL cholesterol were not significant \((p= 0.09 \text{ and } p= 0.59, \text{ respectively})\).

Total and free testosterone were significantly reduced from 53.3±24.5 nmol/l to 43.3±17.6 nmol/l \((p = 0.008)\) and 7.7±4.8 nmol/l to 6.0±2.1 nmol/l \((p = 0.04)\), respectively. Interestingly, 25-OH vitamin D levels were significantly increased by +4.4±6.4 \((p = 0.003)\) from pre- to post-study without supplementation or increased sun exposure. Fifty percent of the participants began the study in the summer and early fall months and concluded in the late fall and winter months, which excludes the possibility of seasonal variations of sun exposure and natural fluctuations of vitamin D.

64
**Table 7.** Laboratory Data (n = 24). GIR: Glucose-to-Insulin Ratio; HgbA1c: Glycated Hemoglobin; VLDL: Very Low Density Lipoprotein; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; TG: Triglycerides.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 8</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(x̅ ± SD)</td>
<td>(x̅ ± SD)</td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (mmol/l)</td>
<td>95.0±19.6</td>
<td>86.0±8.4</td>
<td>-8.9±17.1*</td>
</tr>
<tr>
<td>2-Hr Glucose (mmol/l)</td>
<td>128.0±67.7</td>
<td>114.9±33.7</td>
<td>-13.1±54.3</td>
</tr>
<tr>
<td>Fasting Insulin (μg/mL)</td>
<td>32.7±17.7</td>
<td>15.7±6.8</td>
<td>-17.0±13.6§</td>
</tr>
<tr>
<td>2-Hr Insulin (μg/mL)</td>
<td>225.8±229.4</td>
<td>142.9±93.4</td>
<td>-82.8±177.7*</td>
</tr>
<tr>
<td>Fasting GIR</td>
<td>3.7±2.5</td>
<td>6.5±3.6</td>
<td>+2.7±3.2§</td>
</tr>
<tr>
<td>2-Hr GIR</td>
<td>1.2±1.3</td>
<td>1.2±0.7</td>
<td>-0.0±1.3</td>
</tr>
<tr>
<td>HgbA1c (%)</td>
<td>5.5±0.4</td>
<td>5.2±0.4</td>
<td>-0.3±0.3†</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dl)</td>
<td>195.9±27.6</td>
<td>186.7±27.2</td>
<td>-9.25±25.5</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td>32.4±15.8</td>
<td>21.7±6.7</td>
<td>-9.3±13.4§</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>127.7±25.8</td>
<td>124.7±21.6</td>
<td>-2.3±20.5</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>47.6±13.0</td>
<td>41.9±10.1</td>
<td>-5.7±9.1†</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>162.8±79.1</td>
<td>108.2±34.0</td>
<td>-57.0±9.1§</td>
</tr>
<tr>
<td>Total Testosterone (ng/dl)</td>
<td>53.3±24.5</td>
<td>43.3±17.6</td>
<td>-10.0 ±17.0†</td>
</tr>
<tr>
<td>Free Testosterone (pg/dl)</td>
<td>7.8±4.8</td>
<td>6.0±2.1</td>
<td>-1.8±3.9*</td>
</tr>
<tr>
<td>25-OH vitamin D (nmol/l)</td>
<td>20.3±10.9</td>
<td>24.7±12.2</td>
<td>+4.4±6.4†</td>
</tr>
</tbody>
</table>

* = p < 0.05; † = p ≤ 0.01; § = p ≤ 0.0001.
Figure 4a-h. Change in pre- and post-diet glucose, insulin, triglyceride and VLDL, and fasting and total testosterone. a) pre-diet fasting and 2-hr glucose, b) post-diet fasting and 2-hr glucose, c) pre-diet fasting and 2-hr insulin, d) post-diet fasting and 2-hr insulin, e) pre-diet VLDL and Triglycerides, f) post-diet VLDL and triglycerides, g) pre-diet free and total testosterone, and h) post-diet free and total testosterone.

**Questionnaires**

*Binge Eating Scale*

Participants showed a reduction in their Binge Eating Scale (BES), indicating an improvement in binge-eating behaviors (Table 8). The BES consists of 16-items with each item rated quantitatively (0 indicating no binge eating problem and 3 indicating severe binge eating problem). Subjects chose which score best described their perceptions and feelings about their eating behavior. The BES was scored by adding the individual values for the 16 items with a possible score range of 0 to 46. Scores ≤17 signify mild or no binge eating problem, scores of 18-26 signify moderate binge eating...
problem, and scores ≥27 signify serious binge eating problem. After the 8-week study, BES reduced from 18.0±7.4 to 7.0±4.6 (p < 0.0001). Nine of the participants had a BES between 18-26 and three had a BES ≥27 at the beginning of the study, signifying that ~50% of participants had moderate to severe binge eating problems before the 8-week intervention. All participants had reductions in their BES to <17 at the end of the study, indicating a reduction in binge eating behaviors after the 8-week diet.

**PCOS-Specific Questionnaire**

The PCOS-specific questionnaire showed significant improvements from pre to post study (Table 8 and Figure 5). The PCOSQ includes 25 items from five health related quality of life domains: emotions (7 items), hair growth (5 items), body weight (5 items), infertility (5 items), and menstruation (4 items). Each item is rated on a seven-point scale in which a score of 7 indicates no problems or difficulties and a score of 1 indicates maximum impairment on that item. The mean score of all items in a domain provides a domain score for each subject. Scores significantly improved in all five domains: hair (+0.9±1.3; p =0.002), emotions (+2.0±1.4; p< 0.0001), menstrual (+1.5±1.2; p < 0.0001), weight (+2.9±1.5; p< 0.0001), and infertility (+2.2±1.7; p < 0.0001).
Table 8. Questionnaire Scores ($n = 24$). PCOSQ: PCOS Specific Questionnaire; BES: Binge Eating Score.

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($\bar{x} \pm SD$)</th>
<th>Week 8 ($\bar{x} \pm SD$)</th>
<th>Total Change ($\bar{x} \pm SD$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PCOSQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair</td>
<td>$3.5 \pm 1.9$</td>
<td>$4.4 \pm 1.7$</td>
<td>$+0.9 \pm 1.3^*$</td>
</tr>
<tr>
<td>Emotion</td>
<td>$3.1 \pm 1.3$</td>
<td>$5.1 \pm 1.0$</td>
<td>$+2.0 \pm 1.4^*$</td>
</tr>
<tr>
<td>Menstruation</td>
<td>$3.5 \pm 1.3$</td>
<td>$5.0 \pm 1.3$</td>
<td>$+1.5 \pm 1.2^\S$</td>
</tr>
<tr>
<td>Weight</td>
<td>$2.2 \pm 1.2$</td>
<td>$5.1 \pm 1.2$</td>
<td>$+2.9 \pm 1.5^\S$</td>
</tr>
<tr>
<td>Infertility</td>
<td>$2.8 \pm 1.5$</td>
<td>$5.0 \pm 1.5$</td>
<td>$+2.2 \pm 1.7^\S$</td>
</tr>
<tr>
<td><strong>BES</strong></td>
<td>$18.0 \pm 7.4$</td>
<td>$7.0 \pm 4.6$</td>
<td>$-11.1 \pm 7.6^\S$</td>
</tr>
</tbody>
</table>

* $= p < 0.01$, $\S = p < 0.0001$
Figure 5. Change in PCOSQ scores. Each item is rated on a seven-point scale in which a score of 7 indicates no problems or difficulties and a score of 1 indicates maximum impairment on that item. * = p < 0.01; ** = p < 0.0001.

Food Logs

Study participants were instructed to record food intake for 9 days during the course of the study; food intake was recorded on the Thursday, Friday, and Saturday that corresponded with weeks 1, 4, and 7. The dietitian analyzed food records and averaged all 9 days to give an average intake over the 8 week study. Food logs were analyzed using Mastercook Deluxe version 11.0. Table 8 gives an average of the participants overall energy and macronutrient intake over the 8-week study.

Table 9. Energy and Macronutrient Intake (n = 24).

<table>
<thead>
<tr>
<th>Kilocalories</th>
<th>1,382.7 ± 154.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (g)</td>
<td>72.3 ± 16.4</td>
</tr>
<tr>
<td>Saturated Fat (g)</td>
<td>18.3 ± 5.8</td>
</tr>
<tr>
<td>Monounsaturated Fat (g)</td>
<td>32.0 ± 8.1</td>
</tr>
<tr>
<td>Polyunsaturated Fat (g)</td>
<td>14.0 ± 5.6</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>90.9 ± 22.4</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>23.7 ± 6.5</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>96.7 ± 22.4</td>
</tr>
<tr>
<td>% Fat</td>
<td>46.9 ± 7.4</td>
</tr>
<tr>
<td>% Protein</td>
<td>28.1 ± 5.58</td>
</tr>
<tr>
<td>% Carbohydrate</td>
<td>26.6 ± 7.71</td>
</tr>
</tbody>
</table>
Metabolic Outcomes Measures

Baseline Demographics

The subset of subjects participating in the metabolic testing portion of the study (n=10) had an average age of 29.6 ± 4.6 years. Of the ten participants, 6 were White, 3 were Hispanic, and 1 was Native American (Table 10).

Table 10. Demographics of Subjects Completing Metabolic Testing (n = 10).

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>29.6 ± 4.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>60%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>30%</td>
</tr>
<tr>
<td>Native American</td>
<td>10%</td>
</tr>
</tbody>
</table>

Anthropometric Data

All subjects completing metabolic testing had a significant reduction in total body weight, percent body fat, WC and HC, and BMI (Table 11).
Table 1. Anthropometric Data of Subjects Completing Metabolic Testing (n=10). BMI: body mass index; WC: waist circumference; HC: hip circumference; WHR: waist-to-hip ratio. * = p < 0.05.

<table>
<thead>
<tr>
<th></th>
<th>Baseline (x ± SD)</th>
<th>Week 8 (x ± SD)</th>
<th>Change (x ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>105.5 ± 14.5</td>
<td>98.0 ± 13.9</td>
<td>-8.6 ± 1.8*</td>
</tr>
<tr>
<td>BMI</td>
<td>38.5 ± 4.2</td>
<td>35.5 ± 4.5</td>
<td>-3.0 ± 0.6*</td>
</tr>
<tr>
<td>WC (inches)</td>
<td>44.6 ± 3.7</td>
<td>41.5 ± 2.9</td>
<td>-3.0 ± 1.3*</td>
</tr>
<tr>
<td>HC (inches)</td>
<td>51.5 ± 4.1</td>
<td>48.9 ± 4.3</td>
<td>-2.5 ± 1.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87 ± 0.04</td>
<td>0.85 ± 0.04</td>
<td>-0.02 ± 0.04</td>
</tr>
<tr>
<td>Fat Mass (kg)</td>
<td>84.2 ± 44.5</td>
<td>73.4 ± 37.0</td>
<td>-10.8 ± 9.1*</td>
</tr>
<tr>
<td>Lean Mass (kg)</td>
<td>83.8 ± 40.8</td>
<td>80.8 ± 40.0</td>
<td>-3.0 ± 5.6</td>
</tr>
<tr>
<td>FM (%)</td>
<td>49.3 ± 4.7</td>
<td>47.0 ± 4.8</td>
<td>-2.3 ± 2.5*</td>
</tr>
<tr>
<td>FFM (%)</td>
<td>50.7 ± 4.7</td>
<td>52.5 ± 4.8</td>
<td>+1.8 ± 2.9</td>
</tr>
</tbody>
</table>

Respiratory Exchange Ratio

A paired t-test found a significant reduction in area under the curve (AUC) RER (p < 0.0001) after the low insulinemic diet. A repeated measures ANOVA found that the low insulinemic diet was significant in reducing RER from 0.85±0.05 to 0.77±0.02 (p < 0.0001) from pre- to post-diet, and post-hoc testing with the conservative Bonferroni adjustment (alpha = 0.05/11 = 0.0045) found significance for the change in RER at all 30-minute intervals (Table 12). Fasting RER on week 8 was reflective of the food quotient (FQ) that was analyzed using Black’s Formula (Black et al., 1986). The FQ was
calculated after averaging the nutrition analysis of food logs provided by the subjects undergoing metabolic testing on the Thursday, Friday, and Saturday days that corresponded with weeks 1, 4, and 7. Average FQ and RER data can be seen in Figure 7.

Table 12. Respiratory Exchange Ratio ($n = 10$). Rest indicates fasting measurements that were taken for 30 minutes after an overnight fast and before consumption of high fat liquid meal. All subsequent measurements were taken postprandially in 30 minute increments. The Bonferroni method ($0.05/11 = p < 0.0045$) was used to determine significance. $^* = p \leq 0.0045$.

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($\bar{x} \pm SD$)</th>
<th>Week 8 ($\bar{x} \pm SD$)</th>
<th>$p$ - value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.85 ± 0.05</td>
<td>0.77 ± 0.02</td>
<td>.001$^*$</td>
</tr>
<tr>
<td>30 min</td>
<td>0.83 ± 0.05</td>
<td>0.75 ± 0.02</td>
<td>.002$^*$</td>
</tr>
<tr>
<td>60 min</td>
<td>0.89 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>&lt;0.0001$^*$</td>
</tr>
<tr>
<td>90 min</td>
<td>0.91 ± 0.04</td>
<td>0.79 ± 0.02</td>
<td>&lt;0.0001$^*$</td>
</tr>
<tr>
<td>120 min</td>
<td>0.90 ± 0.03</td>
<td>0.79 ± 0.02</td>
<td>&lt;0.0001$^*$</td>
</tr>
<tr>
<td>150 min</td>
<td>0.90 ± 0.03</td>
<td>0.80 ± 0.02</td>
<td>&lt;0.0001$^*$</td>
</tr>
<tr>
<td>180 min</td>
<td>0.88 ± 0.03</td>
<td>0.78 ± 0.04</td>
<td>0.0002$^*$</td>
</tr>
<tr>
<td>210 min</td>
<td>0.86 ± 0.04</td>
<td>0.78 ± 0.03</td>
<td>0.0004$^*$</td>
</tr>
<tr>
<td>240 min</td>
<td>0.85 ± 0.04</td>
<td>0.79 ± 0.02</td>
<td>0.002$^*$</td>
</tr>
<tr>
<td>270 min</td>
<td>0.83 ± 0.03</td>
<td>0.77 ± 0.01</td>
<td>0.0001$^*$</td>
</tr>
<tr>
<td>300 min</td>
<td>0.83 ± 0.03</td>
<td>0.78 ± 0.02</td>
<td>0.001$^*$</td>
</tr>
</tbody>
</table>
Figure 6a. Respiratory exchange ratio (RER) \((n=10)\). A) change in RER and AUC RER from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. \(P < 0.05\) was used to determine significance for AUC RER. The Bonferroni method \((0.05/11 = p < 0.0045)\) was used to determine significance in intervals of time. \(\* = p \leq 0.0045\)

Figure 7. Fasting Respiratory Exchange Ratio and Food Quotient \((n=10)\). FQ reflects an average of the Thursday, Friday, and Saturday food logs corresponding to weeks 1, 4, and 7. RER: fasting respiratory exchange ratio; FQ: food quotient.
Energy Expenditure

A paired t-test found that AUC energy expenditure (EE) was significantly reduced from 572.5±65.7 to 528.8±49 kcal \((p = 0.005)\), but was no longer significant after adjusting AUC EE per kilogram. The repeated measures ANOVA found that the diet significantly reduced EE \((p < 0.0001)\), but significance did not remain after adjusting EE per kg. Cross-sectional tests of time found a significant reduction in EE at 150 minutes and 210 minutes using the Bonferroni method, but no significance was found at any intervals after adjusting EE per kilogram (Table 13 and Figure 8a,b). This implies that reduction in total body weight is responsible for the reduced EE, but the diet itself did not reduce EE per kilogram of bodyweight. There was no significant difference in AUC diet induced thermogenesis (DIT), before or after adjusting for weight in kilograms.
Table 13. Energy expenditure (n = 10). Change in kilocalories burned per 30 minute interval from pre- to post-diet. Rest indicates average fasting measurements that were taken for 30 minutes after an overnight fast and before consumption of high fat liquid meal. All subsequent measurements were taken postprandially in 30 minute increments. The Bonferroni method (0.05/11 = p < 0.0045) was used to determine significance. * = p ≤ 0.0045

<table>
<thead>
<tr>
<th></th>
<th>Baseline (x̄ ± SD)</th>
<th>Week 8 (x̄ ± SD)</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>97.0 ± 11.8¹</td>
<td>89.0 ± 10.5</td>
<td>0.06</td>
</tr>
<tr>
<td>30 min</td>
<td>114.2 ± 21.3</td>
<td>106.9 ± 11.5</td>
<td>0.06</td>
</tr>
<tr>
<td>60 min</td>
<td>116.0 ± 12.1</td>
<td>106.8 ± 10.2</td>
<td>0.06</td>
</tr>
<tr>
<td>90 min</td>
<td>116.9 ± 12.6</td>
<td>108.3 ± 10.9</td>
<td>0.06</td>
</tr>
<tr>
<td>120 min</td>
<td>116.0 ± 12.0</td>
<td>108.4 ± 10.8</td>
<td>0.01</td>
</tr>
<tr>
<td>150 min</td>
<td>116.5 ± 12.4</td>
<td>111.2 ± 11.5</td>
<td>0.001*</td>
</tr>
<tr>
<td>180 min</td>
<td>115.3 ± 12.7</td>
<td>106.8 ± 9.3</td>
<td>0.01</td>
</tr>
<tr>
<td>210 min</td>
<td>117.2 ± 14.6</td>
<td>106.3 ± 8.7</td>
<td>0.001*</td>
</tr>
<tr>
<td>240 min</td>
<td>113.6 ± 12.8</td>
<td>104.9 ± 9.6</td>
<td>0.01</td>
</tr>
<tr>
<td>270 min</td>
<td>113.9 ± 13.8</td>
<td>103.5 ± 9.4</td>
<td>0.01</td>
</tr>
<tr>
<td>300 min</td>
<td>113.5 ± 12.5</td>
<td>99.3 ± 16.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹ average kilocalories expended per 30 minute interval
Figure 8a-c. Energy expenditure (n = 10). a) Change in kilocalories burned per 30 minute interval from pre- to post-diet, b) change in kilocalories burned per kilogram of bodyweight per 30 minute interval from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. *P < 0.05 was used to determine significance for AUC EE. The Bonferroni method (0.05/11 = *p < 0.0045) was used to determine significance in intervals of time. * = p ≤ 0.0045
Fat Oxidation

A paired t-test showed a significant increase in AUC fat oxidation (FAT) from 25.3±5.8 to 39.3±5.9 g (p < 0.0001) from pre- to post-diet. After adjusting AUC FAT per kilogram, the t-test suggested a mean increase of 0.16 g/kg (p < 0.0001) after the 8-week low insulinenic diet. The repeated measures ANOVA concluded that the low insulinenic diet significantly reduced fat oxidation before and after adjustment per kg (p = 0.0005 and p < 0.0001, respectively). Cross-sectional tests for time found significance in fat oxidation at all intervals between 60 minute and 210 minute using the Bonferroni method (0.05/11 = p < 0.0045). After adjustment for fat oxidation per kg, significance remained at all intervals between 60 minutes and 180 minutes (Figure 10a-b). This suggests that an 8-week low insulinenic diet significantly increases post-prandial fat oxidation between 1 and 3 hours after a high fat liquid meal, independent of weight loss.
Table 14. Fat oxidation ($n = 10$). Rest indicates fasting measurements that were taken for 30 minutes after an overnight fast and before consumption of high fat liquid meal. All subsequent measurements were taken postprandially in 30 minute increments. The Bonferroni method ($0.05/11 = p < 0.0045$) was used to determine significance. $^* = p \leq 0.0045$

<table>
<thead>
<tr>
<th></th>
<th>Baseline ($\bar{x} \pm SD$)</th>
<th>Week 8 ($\bar{x} \pm SD$)</th>
<th>$p$ – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>$3.7 \pm 1.2^1$</td>
<td>$5.3 \pm 1.1$</td>
<td>0.02</td>
</tr>
<tr>
<td>30 min</td>
<td>$6.5 \pm 1.8$</td>
<td>$8.9 \pm 1.5$</td>
<td>0.008</td>
</tr>
<tr>
<td>60 min</td>
<td>$4.1 \pm 1.8$</td>
<td>$8.1 \pm 1.5$</td>
<td>0.0002*</td>
</tr>
<tr>
<td>90 min</td>
<td>$3.7 \pm 1.5$</td>
<td>$7.6 \pm 0.9$</td>
<td>0.0002*</td>
</tr>
<tr>
<td>120 min</td>
<td>$4.1 \pm 1.5$</td>
<td>$8.4 \pm 1.3$</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>150 min</td>
<td>$3.8 \pm 1.3$</td>
<td>$7.6 \pm 1.5$</td>
<td>0.0003*</td>
</tr>
<tr>
<td>180 min</td>
<td>$4.6 \pm 1.0$</td>
<td>$8.1 \pm 2.1$</td>
<td>0.001*</td>
</tr>
<tr>
<td>210 min</td>
<td>$5.7 \pm 1.7$</td>
<td>$7.7 \pm 1.7$</td>
<td>0.003*</td>
</tr>
<tr>
<td>240 min</td>
<td>$6.0 \pm 1.7$</td>
<td>$7.6 \pm 1.2$</td>
<td>0.009</td>
</tr>
<tr>
<td>270 min</td>
<td>$6.8 \pm 1.2$</td>
<td>$8.0 \pm 0.8$</td>
<td>0.01</td>
</tr>
<tr>
<td>300 min</td>
<td>$6.7 \pm 1.2$</td>
<td>$7.6 \pm 1.2$</td>
<td>0.06</td>
</tr>
</tbody>
</table>

$^1$ average grams of fat oxidized per 30 minute interval
Figure 10a-b. Fat oxidation (n = 10). A) Change in grams of fat oxidized per 30 minute interval from pre- to post-diet, b) change in grams of fat oxidized per kilogram of bodyweight per 30 minute interval from pre-to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. \( P < 0.05 \) was used to determine significance for AUC. The Bonferroni method (0.05/11 = \( p < 0.0045 \)) was used to determine significance in intervals of time. * = \( p \leq 0.0045 \).
Carbohydrate Oxidation

A paired t-test showed a significant reduction in AUC carbohydrate oxidation (CHO) from 91.3±19.3 to 40.2±7.2 g (p < 0.0001) from pre- to post-diet (Table 15). Significance remained after adjusting AUC CHO per kg of bodyweight and suggested a mean decrease of 0.41 g/kg (p < 0.0001) after the 8-week low insulinemic diet. The repeated measures ANOVA suggested that the low insulinemic diet was significant in reducing CHO oxidation, before and after adjustment for CHO oxidation per kg (p < 0.0001 and p < 0.0001, respectively). Cross-sectional tests of time concluded that there was a significant decrease in CHO oxidation at all intervals except resting using the Bonferroni method, both before and after adjustment for CHO oxidation per kg (Figure 9a-b).
Table 15. Carbohydrate Oxidation \((n = 10)\). Rest indicates fasting measurements that were taken for 30 minutes after an overnight fast and before consumption of high fat liquid meal. All subsequent measurements were taken postprandially in 30 minute increments. The Bonferroni method \((0.05/11 = p < 0.0045)\) was used to determine significance. \(\ast = p \leq 0.0045\)

<table>
<thead>
<tr>
<th></th>
<th>Baseline ((\bar{x} \pm SD))</th>
<th>Week 8 ((\bar{x} \pm SD))</th>
<th>(p – value^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>20.5 ± 3.3 (\dagger)</td>
<td>16.3 ± 2.5</td>
<td>0.005</td>
</tr>
<tr>
<td>30 min</td>
<td>14.9 ± 7.1</td>
<td>4.7 ± 1.46</td>
<td>0.002 (\ast)</td>
</tr>
<tr>
<td>60 min</td>
<td>20.5 ± 4.8</td>
<td>7.0 ± 2.3</td>
<td>&lt; 0.0001(^*)</td>
</tr>
<tr>
<td>90 min</td>
<td>21.7 ± 4.3</td>
<td>8.7 ± 2.6</td>
<td>&lt; 0.0001(^*)</td>
</tr>
<tr>
<td>120 min</td>
<td>23.3 ± 5.5</td>
<td>9.3 ± 2.5</td>
<td>&lt; 0.0001(^*)</td>
</tr>
<tr>
<td>150 min</td>
<td>21.4 ± 4.3</td>
<td>9.2 ± 2.3</td>
<td>&lt; 0.0001(^*)</td>
</tr>
<tr>
<td>180 min</td>
<td>19.3 ± 4.1</td>
<td>7.3 ± 3.8</td>
<td>&lt; 0.0001(^*)</td>
</tr>
<tr>
<td>210 min</td>
<td>16.8 ± 4.2</td>
<td>7.8 ± 2.7</td>
<td>0.0002(^*)</td>
</tr>
<tr>
<td>240 min</td>
<td>15.3 ± 5.6</td>
<td>8.0 ± 2.3</td>
<td>0.002(^*)</td>
</tr>
<tr>
<td>270 min</td>
<td>13.1 ± 3.9</td>
<td>6.7 ± 1.3</td>
<td>0.0002(^*)</td>
</tr>
<tr>
<td>300 min</td>
<td>13.3 ± 4.1</td>
<td>7.5 ± 2.4</td>
<td>0.0008(^*)</td>
</tr>
</tbody>
</table>

\(\dagger\) average grams carbohydrate oxidized per 30 minute interval
Figure 9a-b. Carbohydrate Oxidation (n=10). A) change in grams of carbohydrate oxidized per 30 minute interval from pre- to post-diet, b) change in grams of carbohydrate oxidized per kilogram of bodyweight per 30 minute interval from pre- to post-diet. Rest indicates fasting measurements and all subsequent intervals represent postprandial measurements. $P < 0.05$ was used to determine significance for AUC. The Bonferroni method ($0.05/11 = p < 0.0045$) was used to determine significance in intervals of time. $* = p \leq 0.0045$
CHAPTER 5
DISCUSSION

According to the results of this study, a low insulinemic diet has the potential to improve anthropometric, biochemical, and metabolic outcomes. Several additional outcome measures were unexpectedly improved as well including blood lipids, vitamin D status, and HgbA1c. Considering the dietary intervention spanned only 8 weeks with no additional exercise recommendations, medications, or supplements these improvements are interesting and worthy of further research. However, there was no control group in this study which makes it difficult to determine whether this dietary approach is optimal for women with PCOS.

Anthropometric Results

Participants were instructed to eat *ad libitum* of low insulinemic foods, yet averaged a daily caloric intake of ~1400 kcal. It is not surprising that this kilocalorie level led to significant weight loss, yet it is surprising that with no restrictions on food quantity participants were able to maintain this kilocalorie level throughout the study without hunger. According to the food logs, clients ate a large amount of protein, fiber, and fat, all of which lead to greater levels of satiety and can potentially explain the maintenance of a low kilocalorie intake. Further research is needed in comparing an *ad libitum* low insulinemic diet with an *ad libitum* low glycemic index and *ad libitum* low carbohydrate ketogenic diet to determine which diet leads to more significant anthropometric improvements. The significant change in the Ferriman-Gallwey score
was unexpected due to previous studies suggesting it takes 6 months to achieve improvements in hirsutism after weight loss (Homburg, 2008). More research is needed to compare a low insulinemic diet with a low glycemic index diet and a low carbohydrate ketogenic diet to determine the best treatment for women with PCOS.

**Biochemical Outcomes**

The results of this study support previously established beliefs that weight loss in women with PCOS improves biochemical outcomes (Escobar-Morreale et al., 2005). While it was hypothesized that a low insulinogenic diet would reduce fasting and 2-hr insulin, it was not expected to reverse elevated blood sugars in those six patients with fasting and/or 2-hr blood glucose levels outside of normal limits. The drop in HgbA1c was also surprising considering HgbA1c represents a three month average of blood glucose levels. This could have important implications due to the recent 2013 American Diabetes Association (ADA) statistics showing 25.8 million Americans of all ages suffer from type 2 diabetes, costing $245 billion dollars in combined direct and indirect costs per year. While additional research is needed, the results of this study implicate that using a low insulinemic diet could have potential improvements in populations clinically diagnosed with type 2 diabetes as well. Longer term studies are also needed to determine whether following a long term low insulinemic diet delays or prevents diabetes in the PCOS population.

Participants were allowed *ad libitum* intake of animal protein over the 8-week study. While the participants were encouraged to choose lean protein sources as often as
possible, no defined restrictions were placed on animal protein choices. Considering animal protein often is packaged with saturated fat and cholesterol, the reduction in lipid levels after 8-weeks is surprising. This data supports other studies suggesting that replacing carbohydrate with fat does not result in dyslipidemia (Hays et al., 2003). The significant reduction in triglycerides and VLDL can be explained by the potentially increased insulin sensitivity of the study participants. The plasma triglyceride level reflects the concentration of triglyceride-carrying lipoproteins (i.e., VLDL).

Hypertriglyceridemia may in part be due to 1) excess free fatty acids returning to the liver and 2) increased \textit{de novo} triglyceride production due to hyperinsulinemia, particularly in insulin resistant individuals (Berglund, Brunzell, Goldberg, Goldberg, Sacks, Murad, et al., 2012). Therefore, elevated triglycerides and VLDL may be due to defective lipolysis by hyperinsulinemia. The increase in fat oxidation seen in the metabolic measurements may explain the improvements in triglycerides and VLDL due to improved lipolysis.

Reductions in HDL are common after diet-induced weight loss, but are not particularly associated with increased cardiovascular risk (Aicher, Haser, Freeman, Carnie, Stonik, Wang, et al., 2012). The reduction may have been caused by the concomitant reduction in total cholesterol. Although statistically significant, the reduction in HDL did not reach pathological levels and may be viewed as lacking in clinical significance. More research is needed on any adverse effects of reduced HDL following diet-induced weight loss.

The results of this study were similar to previous findings that low serum 25-hydroxyvitamin D (25(OH)D) status is commonly found in women with PCOS, and that
weight loss often results in an increase in levels (Rock, Edmond, Flatt, Heath, Karanja, Pakiz, et al., 2012). This has been explained by Rock, et al., (2012) as being due to the fact that adipose tissue changes capacity of 25(OH)D activation and deactivation in obesity. Considering low circulating 25(OH)D metabolites have been implicated as a risk factor for several diseases, the increase in 25(OH)D seen in this study is an additional unforeseen benefit.

The significant reduction in free and total testosterone further strengthens the previously established direction of causation between insulin and androgen (Balen, 2004). This is also evidenced by the improvement in the Ferriman-Gallwey score and PCOS-Specific Questionnaire (PCOSQ) scores. According to these methods of assessing hyperandrogenemia, study participants experienced a significant improvement in all signs and symptoms of PCOS. The significant improvement in the Binge Eating Score (BES) is indicative of improvement in eating behaviors. Binge eating has been shown to be a barrier to dietary treatment adherence and sustained weight loss (Lillis, Hayes, & Levin, 2012). The improvement in binge eating behaviors following a low insulinemic diet is hopeful for continued weight loss or weight loss maintenance of the study participants. Considering the emotional and psychological stress that this disorder evokes on its victims, the quality of life improvements in the study participants after the dietary intervention brings hope.

While no direct causation can be inferred without additional research, it is important to note that out of the 24 participants, 15 of the participants desired pregnancy and had undergone various fertility treatments with no success prior to the study. Within
six months of completing the dietary intervention, 7 of the 15 participants achieved pregnancy and 1 achieved pregnancy during the study.

**Metabolic Outcomes**

There was a significant reduction in AUC RER, AUC EE, and AUC CHO as well as an increase in AUC FAT from pre- to post-diet before adjustment for body weight. AUC RER and AUC CHO remained significantly reduced and AUC FAT remained significantly increased after adjustment for body weight. However, considering all subjects lost weight and weight and diet are associated, separating the effects of diet and weight loss is difficult. Cross-sectional tests for time found that after adjusting for body weight, postprandial fat oxidation remained significantly increased between 1 and 3 hours after the high fat liquid meal, and postprandial CHO oxidation remained significantly decreased at all intervals between 30 minutes and 5 hours after the high fat liquid meal. This suggests that the 8-week low insulinenic diet significantly reduced carbohydrate oxidation and increased fat oxidation after consumption of a high-fat meal, independent of weight loss.

The close association between food quotient (FQ) and post-diet fasting RER indicate that subjects were able to adjust fat oxidation to meet fat intake over an 8-week period. Further studies are needed to determine whether a low insulinenemic diet improves metabolic flexibility by improving insulin resistance in women with PCOS, as well as studies designed to determine the length of time necessary for women with PCOS to adjust to substrate flux.
This is the first study to assess fasting and postprandial metabolic responses in women with PCOS before and after an 8-week diet intervention. This study provides further evidence of impaired fasting fat oxidation in women with PCOS (Di Sarra et al., 2013). In the study by Di Sarra et al., (2013), hyperandrogenemic women had a fasting RER of 0.73, which suggests participants in our study had a greater degree of impaired fasting fat oxidation with a fasting RER of 0.85. However, this could be explained by the greater BMI in our study participants (38.5 kg/m² versus 32.9 kg/m², respectively). The elevated RER at baseline and after consumption of a high fat load before diet intervention suggests that insulin resistance may be causing a shift in substrate metabolism towards a condition of predominant glucose oxidation, instead of adapting to substrate flux (i.e., fat).

Conclusions and Implications

An 8-weeks the low insulinemic diet improved anthropometric, biochemical, and metabolic outcomes in overweight and obese women with PCOS. Considering the participants were not advised to count calories, count carbohydrates, or increase exercise above baseline, the improvements in weight, insulin, A1c, and blood lipids is promising. Since women with PCOS are at increased risk for chronic diseases associated with insulin resistance such as type 2 diabetes and cardiovascular disease, the improvement in insulin sensitivity and hyperlipidemia is of significance. Further research is needed to determine whether the improvements seen can be contributed solely to the weight loss achieved by the participants due to a reduction in calories or from the reduction in...
insulinemic foods. However, considering the difficulties women with PCOS experience in trying to lose weight the diet seems like a promising and possibly sustainable solution.

Limitations and Strengths

This study has several limitations including small sample size, lack of a control group, and lack of pre-intervention dietary intake information. Sample size was limited due to availability of funding, but coincided with the post-hoc power analysis. The study was designed with no control group due to limited funding and instead used baseline values as control. In order to analyze differences in metabolic flexibility in the metabolic measurements, a FFQ could have been administered to estimate pre-intervention dietary intake to provide a pre-diet FFQ. Urinary nitrogen excretion was not collected, thus, all metabolic calculations assume that protein oxidation did not change from pre- to post-diet. Additionally, the sample selected was highly motivated due to the majority of participants desiring pregnancy, which may have biased outcomes. Further studies using a low insulinemic diet should be administered in women with PCOS or insulin resistance that do not desire pregnancy.

The strengths of this study are that all laboratory analyses were done in the same laboratories and all anthropometric measurements were conducted by the dietitian, thus reducing bias. Also, after diet instruction on week 0 no contact was made between participants and the researchers aside from answering questions about the diet and acceptable foods until week 8. Food logs provided information regarding food intake and compliance to ensure all participants followed the diet as instructed.
LITERATURE CITED


Ching, H., Burke, V., & Stuckey, B. (2007). Quality of life and psychological morbidity in women with polycystic ovary syndrome: body mass index, age and the provision of patient information are significant modifiers. *Clinical Endocrinology, 66*(3), 373-379.


Coviello, A., Legro, R., & Dunaif, A. (2006). Adolescent girls with polycystic ovary syndrome have an increased risk of the metabolic syndrome associated with increasing androgen levels independent of obesity and insulin resistance. *JCEM, 91*(2), 492-497.


Di Sarra, D., Tosi, F., Bonin, C., Fiers, T., Kaufman, J., Signori, C., Zambotti, F., Dall’Alda, M., Caruso, B., Zanolin, M., Bonora, E., & Moghetti, P. (2013). Metabolic inflexibility is a feature of women with polycystic ovary syndrome and is associated with both insulin resistance and hyperandrogenism. *Journal of Clinical Endocrinology & Metabolism*, 98(6), 2581-2588.


may resolve after weight loss induced by bariatric surgery. *Journal of Clinical Endocrinology & Metabolism, 90*(12), 6364-6369.


National Institutes of Health. National Cancer Institute. Diet History Questionnaire II.


APPENDIX A

SCREENING CONSENT FORM

Patient’s or Authorized Person’s Signature

I, __________________ agree to complete the Stage of Change questionnaire to determine whether I am eligible for the study: “Study of The Association between the Reduction of Carbohydrates from Starch (STARCH) and hyperandrogenism in women with PCOS”.

I understand that my answers are confidential and will not affect my regular medical care. I understand that my participation is voluntary and I may choose not to participate at any time.

If I am eligible to participate in the study, I would like to be contacted at:

Cell phone: __________________

Home phone: __________________

Email address: ________________________________

Patient Signature: ________________________________

Date: ______________

*You can request additional information about the Study or discuss concerns related to the Study by calling the Study’s co-investigator, Ali Pohlmeier, at 806-441-7275.
APPENDIX B

PARTICIPANT SCREENER

Pt ID: _____________________________________

1. Are you between the ages of 18-45?  Yes  No

   What is your current age? _________

2. What is your current Height? ________________

3. What is your current Weight? ________________

4. Have you lost or gained more than 5% of your body weight in the past 3 months?  Yes  No

5. Are you pregnant, lactating, or planning to become pregnant before this study would finish?  Yes  No

6. Are you planning on starting an exercise program or altering your current exercise program before this study would finish?  Yes  No

7. Are you planning on beginning any supplement use between now and the time this study would finish?  Yes  No

8. Are you willing to discontinue use of metformin, birth control pills, and progesterone for one month prior to and during the 8-week study?  Yes  No
9. Are you on a medically prescribed diet?  
   Yes  
   No  
   If so, are you willing to discontinue it one month prior to beginning the study and during the 8-week study  
   Yes  
   No  
10. Do you have irregular or absent periods  
    Irregular (cycles lasting less than 21d or more than 35d)  
    Absent (lack of period for more than 12 months)  
   Yes  
   No  
11. Do you get anxiety in small or enclosed spaces?  
    (also known as claustrophobia).  
   Yes  
   No
APPENDIX C

STAGE OF CHANGE QUESTIONNAIRE

Please answer each question by circling a number between 1 (strongly disagree) and 5 (strongly agree) that best describes you right now.

_Precontemplation_

Item:
1. As far as I'm concerned, I don't have any problems that need changing.
   1  2  3  4  5
4. PCOS is not a problem for me. It doesn't make sense for me to be here.
   1  2  3  4  5
7. Being here is pretty much of a waste of time for me because I don’t see a connection between PCOS and my diet.
   1  2  3  4  5
10. I guess I have some issues with PCOS, but there's nothing that I really need to change about my diet.
    1  2  3  4  5
13. My diet may be connected to my problems with PCOS, but I don't really think it is.
    1  2  3  4  5
16. All this talk about diet change is useless. I will never change my diet.
    1  2  3  4  5
19. I have PCOS, but I don't spend time thinking about it or trying to fix it, especially by changing my diet.
    1  2  3  4  5
22. I would rather cope with my PCOS than try to change it.
    1  2  3  4  5

_Contemplation_

Item:
2. I think I might be ready to treat my PCOS by changing my diet.
   1  2  3  4  5
5. It might be worthwhile to work on changing my diet to help treat my PCOS.
   1  2  3  4  5
8. I've been thinking that I might want to change my diet to help with my PCOS.
   1  2  3  4  5
11. I'm hoping this study will help me to better understand how to change my diet to improve my PCOS.
   1  2  3  4  5
14. My PCOS is a problem and I really think I should work on it, even if it means changing my diet.

17. I wish I had more ideas on how to treat my PCOS through diet.

20. Maybe this study will be able to help me learn how to treat my PCOS through diet.

23. I hope that the researchers in this study will have good advice for treating my PCOS with diet.

Action Item:
3. I am ready to do something about my diet to help my PCOS as soon as possible.

6. I am finally ready to make some changes to my diet to help my PCOS.

9. I know at times making healthy food choices to treat PCOS will be difficult, but I'm willing to do my best.

12. I am really ready to work hard on making healthy food choices.

15. Even though I'm not always successful in making healthy food choices, I'm confident I can make the changes asked of me for 8 weeks.

18. I would like some help on how to make healthy food choices to treat PCOS so I can get started.

21. Anyone can talk about changing their diet; I'm actually ready to do something about it.

24. I am ready to actively work on making healthy food choices to treat my PCOS.
CONSENT TO TAKE PART IN A RESEARCH STUDY
Signed copy to be provided to subject or authorized representative
This is a research study for people who voluntarily choose to take part. Please take your time to make a decision, and discuss the study with your personal doctor, family and friends if you wish.

STUDY TITLE: Study of The Association between the Reduction of Carbohydrates-from-starch and Hyperandrogenemia (STARCH) in women with PCOS

INVESTIGATORS: Jennifer Phy, DO; Ali Pohlmeier, MS; Mallory Boylan, PhD, RD; and Jamie Cooper, PhD

CONTACT TELEPHONE NUMBERS: 806-441-7275 (at any time)
(You may contact the investigator(s) at the number(s) listed above if you develop any of the conditions listed in Question # 6 of this form or if you have any unexpected complications.)

INSTITUTIONS: Texas Tech University Health Sciences Center (TTUHSC)
Texas Tech University (TTU) Human Sciences Building

1. Why is this study being done?
This study is being done to determine the association between the reduction of carbohydrates from starch and sugar and hyperandrogenism and its related signs and symptoms in pre-menopausal women with polycystic ovary syndrome (PCOS).

2. How many people will take part in this study?
40 women diagnosed with PCOS.

3. Why am I being asked to take part in this research study?
You have been clinically diagnosed with PCOS by your OBGYN.

4. What will happen during this study? What will be done that is different from my usual care?
Sequence of procedures within the study (to be given at initial visit):
You have already completed the screening process (i.e., Stage of Change questionnaire). Today you will drink a high-fat liquid shake and we will study your body’s response by measuring the ratio of the air you breathe in to the air you breathe out (see figure 1 on page 3).
You will also receive a brief education on the diet and a diet instruction manual to take home and read through before you return to have your blood drawn.

In order to measure your body composition, you will be asked to wear a swimcap and sit in the BODPOD (see figure 2 on page 3).

Next Wednesday morning you will have your blood drawn after an overnight fast. During this time, you will be given continued education on the diet. 8 weeks after Wednesday’s appointment, you will return to have your blood drawn one last time (again, on a Wednesday morning). 3-4 days after your final blood draw appointment (scheduled at your convenience), you will repeat the high-fat liquid shake procedure and we will re-measure and determine any change in your body’s response.

You are also encouraged to attend bi-monthly support meetings; however, your attendance is voluntary. We will also ask you to complete questionnaires that ask you about your normal diet over the past month, your physical activity patterns, and your relationship to food. You will be asked to provide a food record every other week which should include a Thursday, Friday, and Saturday. You will be asked to stop medications that affect insulin levels as well as birth control pills. If you desire birth control, other methods will be discussed with you.

The diet will consist of unlimited lean meats, vegetables, fruits, nuts, seeds, and oils, as well as one serving (1oz) of cheese will be allowed each day. Subjects over 21 will be allowed ONE 5oz glass of RED wine each day if desired. You will not be asked to count calories or carbohydrates. ALL grains and grain products (i.e., bread, rice, pasta, corn, cereal, oatmeal), beans, potatoes, sugar, honey, and milk (and milk products other than cheese) must be avoided. You will be given an educational packet with specific instructions for the diet, as well as general tips and recipes.

5. How much of my time will this study take? How long will I be in the study?

First visit: approximately 4 hours (3-4 days before first blood draw)
Second visit: approximately 4 hours (First blood draw; day 1 of diet)
Third visit: approximately 4 hours (Last blood draw 8 weeks later)
Fourth visit: approximately 4 hours (3-4 days after last blood draw; last day of diet)
These four visits will be spread out over approximately 8 weeks. If you wish to attend the support meetings, they will last approximately 1 hour.

6. What are the risks and/or discomforts to me if I join this study?

The risks associated with the study are risks common to routine blood draw procedures, including bleeding, fainting or light headedness, hematoma (blood accumulating under the skin), or mild infection.
To measure the amount of calories your body burns at rest, you will be asked to lie flat on a bed while wearing a clear plastic hood to measure the air you breathe in and out for approximately 30 minutes. The plastic hood is spacious and will allow you to breathe comfortably.

7. **Will there be any added risks to me from this study if I am a female?**

You will be asked to eliminate birth control pills for one month prior to the start of the study and during the complete 8 week study. While you may experience an irregular period during the month prior to the start of the study, one purpose of the study is to determine whether the diet improves the regularity of your menstrual cycle.

8. **Are there any benefits to me if I take part in this study?**

There is a possibility of improved signs and symptoms of PCOS.

9. **What other choices do I have if I don’t take part in the research study?**

Your participation in this research study is voluntary. If you do not participate you will receive your usual medical care.

10. **What about confidentiality and the privacy of my records?**

We will keep your involvement in this research study confidential to the extent permitted by law. In addition to the staff carrying out this study, others may learn that you are in the study. This might include federal regulatory agencies such as the Food and Drug Administration (FDA) and the Office for Human Research Protection (OHRP), Texas Tech University Health Sciences Center (TTUHSC) representatives, and the TTUHSC Institutional Review Board (a committee that reviews and approves research). These people may review and copy records involving this research. A copy of this document may be placed in your medical record.

Study results that are used in publications or presentations will not use your name.

11. **Who is funding this study?**

The proposal has been submitted for application to the Laura W. Bush Institute for Women’s Health/University Medical Center - Seed Grant

12. **Will it cost me anything to take part in this research study?**

No.
13. Will I receive anything for taking part in this research study?

You will receive free laboratory testing.

14. Does anyone on the research staff have a personal financial interest in this study?

No.

15. What if I am hurt by participating in this study?

Texas Tech University Health Sciences Center and its affiliates do not offer to pay for or cover the cost of medical treatment for research related illness or injury. No funds have been set aside to pay or reimburse you in the event of such injury or illness unless specifically stated. If you have a research related illness or injury, the sponsor will provide payment for extra unanticipated tests, treatments, and hospitalizations unless such expenses were due to: (i) TTUHSC’s and Principal Investigator’s failure to strictly adhere to the terms of the Protocol; (ii) the negligence or misconduct of TTUHSC or its employees or agents; or (iii) a pre-existing medical condition or your underlying disease.

16. What are my rights as a voluntary participant?

Taking part in this study is your choice. You may choose not to be in it. If you decide not to be in the study, it will not affect any medical care, benefits or rights to which you are entitled. If you sign this form, it means that you choose to be in the study. If new information becomes available during the study that may affect your willingness to take part in the study, you will be told.

17. Can I stop being in the study?

You may leave the study at any time. If you do, discuss it with the investigator, who will help you leave the study in the safest way. If you leave the study, your right to standard medical care will continue. If you leave the study, we cannot remove any information we have collected to that point.

18. Can someone else end my participation in the study?

Under certain circumstances, the investigators, TTUHSC, or the study sponsor may decide to end your participation in this research study earlier than planned. This might happen if you are unable to follow the diet for the entire 8 weeks.
19. What if I have questions?

For questions about this study, contact the Investigator, Ali Pohlmeier at 806-441-7275. If you would like to speak to someone who is not involved in the study about your rights as a participant, research-related injuries, or any other matter related to the study, you can call the TTUHSC Research Protection Hotline: 1-800-396-0918.

Your signature indicates that this research study has been explained to you; you’ve been given the opportunity to ask questions; you accept your responsibility to follow the instructions given to you by the research team regarding study participation and, if applicable, research medication; you agree to take part in this study.

You will be given a signed copy of this form.

______________________________________
Printed Name of Subject

______________________________________    __________________________
Signature of Subject Date Time

______________________________________    __________________________
Signature of Parent/Guardian Date Time  or Authorized Representative

[ ] Subject was unable to read and understand the written consent. The elements of informed consent required by 45 CFR 46.116 and 21 CFR 50 have been presented orally to the subject or the subject’s authorized representative in a language understandable to the subject or representative.

______________________________________    __________________________
Signature of Witness to Oral Presentation Date Time
I have discussed this research study with the subject and his or her authorized representative, using language that is understandable and appropriate. I believe I have fully informed the subject of the possible risks and benefits, and I believe the subject understands this explanation. I have given a copy of this form to the subject.
Signature of authorized research personnel who Date Time
conducted the informed consent discussion

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER (“TTUHSC”)

STUDY TITLE: Study of The Association between the Reduction of Carbohydrates-from-starch and Hyperandrogenemia (STARCH) in women with PCOS

AUTHORIZED TO USE AND/OR DISCLOSE YOUR PROTECTED HEALTH INFORMATION for a RESEARCH STUDY

This form is intended to tell you about the use and/or disclosure (sharing) of your personal Protected Health Information (PHI) if you decide to participate in the research study described on the previous pages. The health information about you that may be used or disclosed is described below. This information is usually found in your medical records. Only the health information about you that is needed for this research study will be used or disclosed. When you consider taking part in this research study, you are also being asked to give your permission for your Protected Health Information to be released from your doctors, clinics, and hospitals to the research personnel approved for this research study. This Authorization specifically relates to the research study described in the attached Informed Consent document.

1. This Authorization is valid indefinitely or until such time as legal requirements will allow this Authorization to be destroyed.

2. If you choose to cancel this Authorization, please give notice in writing to:

Shauna Baughcum  
Institutional Privacy Officer  
Office of Institutional Compliance  
3601 4th St MS 8165  
Lubbock TX 79410

If you sign this Authorization, the following persons, groups or organizations may rely on this Authorization to disclose your Protected Health Information to the Principal Investigator and other research personnel who are conducting this Study:

• your treating physicians and healthcare providers and their staff,  
• associated healthcare institutions and hospitals where you have or may receive care.

While this research study is in progress, the Principal Investigator or research personnel working on this study will inform you whether or not you will be allowed to see the research related health information that is created about you or collected by the research personnel prior to the end of the study. After the study is finished you may request this information as allowed by the TTUHSC Notice of Privacy Practices.
The Protected Health Information that you authorize to be used or disclosed for research purposes may include your current or future health information from some or all of your health records, including:

- hospital records and reports
- admission history, and physical examination
- X-ray films and reports; operative reports
- laboratory reports, treatment and test results
  (including sexually transmitted diseases, HIV or AIDS)
- any other Protected Health Information needed by the research personnel listed above.

(* use separate form for disclosure of psychotherapy notes)

- immunizations
- allergy reports
- prescriptions
- consultations
- clinic notes
- mental health records
- alcohol / substance abuse records
APPENDIX E

EXERCISE QUESTIONNAIRE

During the past week, even if it was not a typical week for you, how much total time (for the entire week) did you spend on each of the following? (Please circle one number for each question.)

0 = none
1 = less than 30 minutes/week
2 = 30-60 minutes/week
3 = 1-3 hrs/week
4 = more than 3 hours/week

1. Stretching or strengthening exercises (range of motion, using weights, etc.) 0 1 2 3 4
2. Walk for exercise 0 1 2 3 4
3. Swimming or aquatic exercise 0 1 2 3 4
4. Bicycling (including stationary exercise bikes) 0 1 2 3 4
5. Other aerobic exercise equipment
   (Stairmaster, rowing, skiing machine, etc.) 0 1 2 3 4
6. Other aerobic exercise
   Specify_________________________ 0 1 2 3 4
APPENDIX F

BINGE EATING SCALE

Instructions. Below are groups of numbered statements. Read all of the statements in each group and mark on this sheet the one that best describes the way you feel about the problems you have controlling your eating behavior.

#1
a. I don’t feel self-conscious about my weight or body size when I’m with others.
b. I feel concerned about how I look to others, but it normally does not make me feel disappointed with myself.
c. I do get self-conscious about my appearance and weight which makes me feel disappointed in myself.
d. I feel very self-conscious about my weight and frequently, I feel intense shame and disgust for myself. I try to avoid social contacts because of my self-consciousness.

#2
a. I don’t have any difficulty eating slowly in the proper manner.
b. Although I seem to “gobble down” foods, I don’t end up feeling stuffed because of eating too much.
c. At times, I tend to eat quickly and then, I feel uncomfortably full afterwards.
d. I have the habit of bolting down my food, without really chewing it. When this happens I usually feel uncomfortably stuffed because I’ve eaten too much.

#3
a. I feel capable to control my eating urges when I want to.
b. I feel like I have failed to control my eating more than the average person.
c. I feel utterly helpless when it comes to feeling in control of my eating urges.
d. Because I feel so helpless about controlling my eating I have become very desperate about trying to get in control.

#4
a. I don’t have the habit of eating when I’m bored.
b. I sometimes eat when I’m bored, but often I’m able to “get busy” and get my mind off food.
c. I have a regular habit of eating when I’m bored, but occasionally, I can use some other activity to get my mind off eating.
d. I have a strong habit of eating when I’m bored. Nothing seems to help me break the habit.

#5
a. I’m usually physically hungry when I eat something.
b. Occasionally, I eat something on impulse even though I really am not hungry.
c. I have the regular habit of eating foods, that I might not really enjoy, to satisfy a hungry feeling even though physically, I don’t need the food.
d. Even though I’m not physically hungry, I get a hungry feeling in my mouth that only seems to be satisfied when I eat a food, like a sandwich, that fills my mouth. Sometimes, when I eat the food to satisfy my mouth hunger, I then spit the food out so I won’t gain weight.

#6
a. I don’t feel any guilt or self-hate after I overeat.
b. After I overeat, occasionally I feel guilt or self-hate.
c. Almost all the time I experience strong guilt or self-hate after I overeat.

#7
a. I don’t lose total control of my eating when dieting even after periods when I overeat.
b. Sometimes when I eat a “forbidden food” on a diet, I feel like I “blew it” and eat even more.
c. Frequently, I have the habit of saying to myself, “I’ve blown it now, why not go all the way” when I overeat on a diet. When that happens I eat even more.
d. I have a regular habit of starting strict diets for myself, but I break the diets by going on an eating binge. My life seems to be either a “feast” or “famine.”

#8
a. I rarely eat so much food that I feel uncomfortably stuffed afterwards.
b. Usually about once a month, I eat such a quantity of food, I end up feeling very stuffed.
c. I have regular periods during the month when I eat large amounts of food, either at mealtime or at snacks.
d. I eat so much food that I regularly feel quite uncomfortable after eating and sometimes a bit nauseous.
#9
a. My level of calorie intake does not go up very high or go down very low on a regular basis.
b. Sometimes after I overeat, I will try to reduce my caloric intake to almost nothing to compensate for the excess calories I’ve eaten.
c. I have a regular habit of overeating during the night. It seems that my routine is not to be hungry in the morning but overeat in the evening.
d. In my adult years, I have had week-long periods where I practically starve myself. This follows periods when I overeat. It seems I live a life of either “feast or famine.”

#10
a. I usually am able to stop eating when I want to. I know when “enough is enough.”
b. Every so often, I experience a compulsion to eat which I can’t seem to control.
c. Frequently, I experience strong urges to eat which I seem unable to control, but at other times I can control my eating urges.
d. I feel incapable of controlling urges to eat. I have a fear of not being able to stop eating voluntarily.

#11
a. I don’t have any problem stopping eating when I feel full.
b. I usually can stop eating when I feel full but occasionally overeat leaving me feeling uncomfortably stuffed.
c. I have a problem stopping eating once I start and usually I feel uncomfortable stuffed after I eat a meal.
d. Because I have a problem not being able to stop eating when I want, I sometimes have to induce vomiting to relieve my stuffed feeling.

#12
a. I seem to eat just as much when I’m with others (family, social gatherings) as when I’m by myself.
b. Sometimes, when I’m with other persons, I don’t eat as much as I want to eat because I’m self-conscious about my eating.
c. Frequently, I eat only a small amount of food when others are present, because I’m very embarrassed about my eating.
d. I feel so ashamed about overeating that I pick times to overeat when I know no one will see me. I feel like a “closet eater.”
#13
a. I eat three meals a day with only an occasional between meal snack.
b. I eat 3 meals a day, but I also normally snack between meals.
c. When I am snacking heavily, I get in the habit of skipping regular meals.
d. There are regular periods when I seem to be continually eating, with no planned meals.

#14
a. I don’t think much about trying to control unwanted eating urges.
b. At least some of the time, I feel my thoughts are pre-occupied with trying to control my eating urges.
c. I feel that frequently I spend much time thinking about how much I ate or about trying not to eat anymore.
d. It seems to me that most of my waking hours are pre-occupied by thoughts about eating or not eating. I feel like I’m constantly struggling not to eat.

#15
a. I don’t think about food a great deal.
b. I have strong cravings for food but they last only for brief periods of time.
c. I have days when I can’t seem to think about anything else but food.
d. Most of my days seem to be pre-occupied with thoughts about food. I feel like I live to eat.

#16
a. I usually know whether or not I’m physically hungry. I take the right portion of food to satisfy me.
b. Occasionally, I feel uncertain about knowing whether or not I’m physically hungry. At these times it’s hard to know how much food I should take to satisfy me.
c. Even though I might know how many calories I should eat, I don’t have any idea what a “normal” amount of food is for me.
APPENDIX G

PCOS-SPECIFIC QUESTIONNAIRE

To what extent have you felt growth of visible hair on your chin has been a problem for you during the last two weeks:

1. Growth of visible chin hair
   1  2  3  4  5  6  7

During the past two weeks, how much of the time have you felt:

2. Depressed as a result of PCOS
   1  2  3  4  5  6  7

3. Concerned about being overweight
   1  2  3  4  5  6  7

4. Easily tired?
   1  2  3  4  5  6  7

5. Concerned with infertility problems
   1  2  3  4  5  6  7

6. Moody as a result of PCOS
   1  2  3  4  5  6  7

In relation to your last menstruation, how much were the following issues a problem for you?

7. Headaches?
   1  2  3  4  5  6  7

8. Irregular menstrual periods?
   1  2  3  4  5  6  7
To what extent has growth of visible hair on your upper lip been a problem for you the last two weeks:

9. Growth of visible hair on upper lip?
   1  2  3  4  5  6  7

During the past two weeks, how much time have you:

10. Had trouble dealing with your weight?
    1  2  3  4  5  6  7

11. Had low self-esteem as a result of PCOS?
    1  2  3  4  5  6  7

12. Felt frustration in trying to lose weight?
    1  2  3  4  5  6  7

13. Felt afraid of not having children?
    1  2  3  4  5  6  7

14. Felt frightened of getting cancer?
    1  2  3  4  5  6  7

Over the last two weeks, to what extent have the following issues been a problem for you?

15. Growth of visible facial hair?
    1  2  3  4  5  6  7

16. Embarrassment about excessive body hair?
    1  2  3  4  5  6  7

During the past two weeks how much time have you:

17. Worried about having PCOS?
    1  2  3  4  5  6  7

18. Self-conscious as a result of PCOS?
    1  2  3  4  5  6  7
In relation to your last menstruation, how much have the following issues been a problem from you?

19. Abdominal bloating?  
   1  2  3  4  5  6  7

20. Late menstrual period?  
   1  2  3  4  5  6  7

21. Menstrual cramps?  
   1  2  3  4  5  6  7

How much of the time in the last two weeks did you:

22. Feel like you are not sexy because of being overweight?  
   1  2  3  4  5  6  7

23. Feel a lack of control over PCOS?  
   1  2  3  4  5  6  7

24. Have difficulties staying at your ideal weight?  
   1  2  3  4  5  6  7

25. Feel sad because of infertility problems?  
   1  2  3  4  5  6  7

To what extent has growth of visible body hair been a problem for you during the last two weeks:

26. Growth of visible body hair?  
   1  2  3  4  5  6  7