THE ASSOCIATION OF ADIPOKINES AND INSULIN RESISTANCE IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

By

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ABSTRACT

THE ASSOCIATION OF ADIPOKINES AND INSULIN RESISTANCE IN WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

Thesis under the direction of Gary D. Miller, PhD., Associate Professor of Health and Exercise Science.

A metabolic disorder with increasing concern to premenopausal women is Polycystic Ovarian Syndrome (PCOS). PCOS leads to numerous health-related concerns, including insulin resistance. The cause of insulin resistance in women with PCOS is unknown, but adipokine levels may play a role. **PURPOSE:** The primary purpose of this pilot study was to evaluate the relationships between measures of insulin resistance and adipokines (leptin, adiponectin, and visfatin) in women with PCOS. Secondarily this study assessed association between adipokines and markers of obesity in women with PCOS. **METHODOLOGY:** Plasma concentrations of insulin, leptin, adiponectin, and visfatin were made from a morning fasting venous blood sample in women with PCOS. A 2-hour OGTT was performed and plasma insulin and glucose were assessed at baseline and 60, and 120 minutes of the OGTT. Obesity measurements (body fat, mass, and waist circumference) were also determined during the visit. The two measures of insulin resistance were the area under the curve for insulin (I-AUC) during the OGTT, and HOMA, which was calculated based on fasting insulin and glucose levels. **STATISTICAL ANALYSIS:** Linear regression examined associations between adipokines and insulin resistance, and Pearson correlations determined the relationships between adipokines and obesity indices. **RESULTS:** I-AUC was negatively correlated with adiponectin ($r = -.684$) and positively correlated with visfatin ($r = .775$), but not with
leptin (r = .029). Only leptin was correlated with obesity markers. **CONCLUSION:**
Lower adiponectin and higher visfatin concentrations are related to insulin resistance in women with PCOS. A further understanding of these relationships may contribute to the identification of the pathogenesis of both insulin resistance and the syndrome as a whole, ultimately allowing for better identification and treatment. In addition to an alleviation of the symptoms associated with PCOS, proper treatment would decrease risk for cardiovascular disease and diabetes among these women.
INTRODUCTION

The prevalence of overweight and obesity has reached its highest level, with 67% of American adults falling in these categories with a Body Mass Index (BMI) of $\geq 25$ kg/m$^2$ or $\geq 30$ kg/m$^2$ for overweight and obesity, respectively, in 2006 (19). Additionally, the onset of obesity is occurring earlier in life. It is estimated that 52.1% of young adults aged 18-29 years are overweight or obese; this number has been steadily increasing over the past three decades (19).

Obesity has the potential to contribute to various chronic diseases such as cardiovascular disease and diabetes. These diseases can affect people of all ages, but are less common in adults aged 18-44 years compared to those 45 years and older (19). Although the percentage of people with these diseases is lower in younger adults, they are still at risk for metabolic conditions. Polycystic Ovarian Syndrome, or PCOS, is a metabolic disorder which affects this younger age group, specifically premenopausal women. It is highly probable that PCOS is the most common metabolic disorder in women of reproductive age (16).

PCOS leads to numerous health-related concerns, including those of reproduction, dyslipidemia, hypertension, and insulin resistance. Although PCOS can develop in women across a variety of body masses, approximately 44% of women with PCOS are obese (16). Obese women with PCOS tend to have more profound metabolic complications, such as higher levels of androgens and greater degree of insulin resistance than the already elevated levels seen in non-obese women with PCOS. In addition to the immediate concerns mentioned above, women with PCOS, whether normal weight or
obese, are at increased risk for both cardiovascular disease (102) and diabetes (66) compared to those without the syndrome.

Adipokines are hormones produced by adipose tissue. These hormones have various roles, including a potential contribution to many metabolic consequences that can eventually lead to cardiovascular disease and diabetes. Leptin, the first adipokine to be discovered, is positively correlated with obesity. Adiponectin is another adipokine thought to play a role, based on its relationship to inflammation, atherogenesis, and insulin resistance. Visfatin, a somewhat newly identified adipokine, is thought to have insulin-like properties. Abnormal metabolism of one or more of these adipokines may potentially play a role in the development of metabolic issues, and may ultimately help identify the pathogenesis of PCOS, particularly by affecting insulin sensitivity. If the main factor involved in insulin resistance in PCOS was determined, it may lead to the understanding of the pathogenesis of the syndrome itself, as well as directing research efforts towards potential methods of treatment.
REVIEW OF THE LITERATURE

POLYCYSTIC OVARIAN SYNDROME

Polycystic Ovarian Syndrome (PCOS) is a condition of ovarian dysfunction that affects 5-10% of premenopausal women in the United States. PCOS is diagnosed by the presence of two of the three Rotterdam Criteria. The Rotterdam Criteria are detailed and described in Table 1. When diagnosing the syndrome, it is important to rule out other metabolic abnormalities that cause these criteria. The actual pathogenesis of PCOS is not entirely understood, but there are various characteristics of the syndrome which potentially play a role in its development.

**Table 1. Diagnosis of PCOS**

<table>
<thead>
<tr>
<th>CRITERIA</th>
<th>EXPLANATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amenorrhea or Oligomenorrhea</td>
<td>Amenorrhea: Cessation of menses Oligomenorrhea: Fewer than 9 menses/year, dysfunctional uterine bleeding, and/or a cycle lasting &lt;24 days or &gt;35 days.</td>
</tr>
<tr>
<td>Hyperandrogenism</td>
<td>Clinical: Hirsutism, Acne, Alopecia Biochemical: Elevated testosterone</td>
</tr>
<tr>
<td>Polycystic Ovaries</td>
<td>One or both ovaries with the presence of 12 or more follicles in each ovary, measuring 2-9 mm in diameter, and/or increased ovarian volume (&gt;10 mL)</td>
</tr>
</tbody>
</table>

As seen in Table 1, amenorrhea/oligomenorrhea is one of the diagnostic criteria for PCOS. This criterion is caused by anovulation which potentially leads to a variety of health issues such as an increased risk for endometrial cancer (1). Anovulation may also increase risk for breast and ovarian cancers (16), and has additionally been shown to
contribute to dyslipidemia (107). Although these consequences are of concern in PCOS, the main complication associated with anovulation is infertility. Infertility is generally what instigates women with PCOS to seek medical attention. Although women with PCOS tend to focus on infertility as their major concern, there are numerous other metabolic consequences that arise from the syndrome, most of which lead to an increased risk for both diabetes and cardiovascular disease.

Hyperandrogenism, recognized by an excess production or secretion of male hormones, is also shown in Table 1 as a diagnostic criterion. Hyperandrogenism also has various potential consequences for women with PCOS, such as increasing triglycerides and LDL cholesterol, ultimately contributing to dyslipidemia (107). Additionally, hyperandrogenism indirectly contributes to anovulation via increased production of Luteinizing Hormone (LH). LH can then feed back and further contribute to hyperandrogenism, resulting in a seemingly unending cycle. Although women with PCOS may not realize that the metabolic effects of hyperandrogenism are taking place, they do notice the bothersome cosmetic effects such as acne and hirsutism. Another potential metabolic consequence associated with hyperandrogenism is increased resistance to the effect of insulin.

Although insulin resistance is not one of the diagnostic criteria for PCOS, it affects up to 50% of women with PCOS (34), and contributes to both the anovulation and hyperandrogenism that form the foundation of the syndrome. In addition to contributing to the pathogenesis of PCOS, insulin resistance itself leads to an increased risk for both cardiovascular disease and diabetes.
The risk of cardiovascular disease and diabetes is increased in women with PCOS due to the anovulation, hyperandrogenism, and insulin resistance that are characteristic of the syndrome. These metabolic consequences interact with each other, making the actual pathogenesis of PCOS difficult to determine. Insulin resistance is the consequence of interest for this thesis. The cause of insulin resistance in PCOS is not fully known, although various ideas have been proposed. Additionally, insulin resistance in PCOS exhibits a few unique characteristics, further complicating the condition.
INSULIN RESISTANCE

Overview

The term insulin resistance is widely used in clinical practice and refers to insensitivity of insulin receptors to endogenous insulin. Hyperglycemia, as well as normoglycemia, may be present with insulin resistance depending on the ability of the pancreas to continue producing insulin to lower blood glucose. For those with normal glucose levels and insulin resistance, higher than normal levels of insulin are required to maintain the normal glucose levels. Moller and Flier define insulin resistance as any subnormal biologic response to a given concentration of insulin (75).

Various screening tests and classifications have been implemented to evaluate glucose and insulin responses. The American Diabetes Association suggests using fasting plasma glucose (FPG) or a 75-gram oral glucose tolerance test (OGTT) to evaluate whether a person has, or is at risk for, glucose metabolism problems such as insulin resistance. Table 2 shows the classifications of glucose metabolism based on these tests.
### Table 2. American Diabetes Association Glucose Classifications

<table>
<thead>
<tr>
<th>Fasting Glucose Level (mg/dL)</th>
<th>Classification</th>
<th>2-Hour OGTT Glucose Level (mg/dL)</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 99</td>
<td>Normal Fasting Glucose</td>
<td>≤ 139</td>
<td>Normal Glucose Tolerance</td>
</tr>
<tr>
<td>100 - 125</td>
<td>Impaired Fasting Glucose</td>
<td>140 – 200</td>
<td>Impaired Glucose Tolerance</td>
</tr>
<tr>
<td>≥ 126</td>
<td>Diabetes</td>
<td>≥ 200</td>
<td>Diabetes</td>
</tr>
</tbody>
</table>

Of the two recommended measures, the American Diabetes Association prefers for clinicians to evaluate FPG because it is easy to use, more acceptable to patients, and less costly. It is important to note that the OGTT is more sensitive and reasonably more specific than FPG, but it is poorly reproducible and rarely performed in practice (3). Similarly, the World Health Organization recommends that both a FPG and OGTT be performed in order to evaluate impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). The World Health Organization has similar diagnostic criteria to the American Diabetes Association, although IFG is classified as a FPG of ≥110 mg/dl (compared to ≥100 mg/dl) (2).

Although the above measures are beneficial in identifying problems with glucose metabolism, these tests do not take insulin levels into consideration, making it difficult to identify whether the dysregulation of glucose metabolism is at the level of the pancreas in which decreased insulin production is occurring or if the issue is at the cell level in which
Insulin resistance is occurring. Measuring insulin levels during an OGTT can aid in the identification of the underlying problem.

Insulin Resistance is a complex matter because of the various causes that have been identified. There are potential defects that are intrinsic to the target cells, such as defects in the insulin-receptor gene or other genes involved in insulin action. There are also secondary factors such as certain physiologic states (including stress, starvation, puberty, advanced age, and obesity) and hormonal/metabolic factors (including growth hormones, catecholamines, and free fatty acids) (75). Obesity is one of the most common causes of insulin resistance, particularly because it affects both sensitivity to insulin and secretion of insulin.

**Insulin Resistance and Obesity**

Obesity is well documented to contribute to insulin resistance via decreased sensitivity and abnormal secretion. Cytokines, free fatty acids, and adipokines are all potential mechanisms behind the decreased insulin sensitivity seen in obesity. In particular, intra-abdominal obesity negatively affects a person’s insulin sensitivity (47). Increased levels of the cytokine TNF-α are found in obese people, and have been shown to negatively affect glucose metabolism (47). Additionally, increased free fatty acids can lead to reduced peripheral glucose uptake (9). Adipokines, hormones produced by adipose tissue, have also been shown to decrease insulin sensitivity, with adiponectin being the main one known at this time (55).
In addition to obesity’s negative effect on insulin sensitivity, insulin secretion from the beta cells in the pancreas is also impaired, possibly as a result of the cytokines and free fatty acids (47).

It is estimated that 30-75% of women with PCOS are obese, thus obesity was originally thought to play a large role in the mechanism for insulin resistance in these women, but this is not necessarily the case. The pathogenesis of insulin resistance in PCOS is not fully known, but there are most likely multiple aspects involved.

**Insulin Resistance and PCOS**

Insulin resistance is a common area of research in women with PCOS, as it is estimated that 20-40% (32), possibly even 50% (34), of these women are affected with some degree of insulin resistance – this prevalence rate is much higher than that reported for other women aged 20-44 (29, 52). Furthermore, it has been estimated that 10% of the cases of impaired glucose tolerance in premenopausal women can be attributed to insulin resistance as a result of PCOS (32).

Insulin resistance in women with PCOS has a few distinctive characteristics. For one, not all tissues in the body are resistant to the effect of insulin (64). This tissue-specific insulin resistance is not beneficial – in fact, it may further contribute to the problems associated with the syndrome. The two main tissues in the body that are insulin resistant are muscle and adipose tissue. The resistance in these tissues ultimately results in hyperinsulinemia. This increase in insulin concentration and insulin production results in more insulin available to be taken up by the ovary, adrenal glands, and the liver because these tissues remain sensitive to the effect of insulin. The increased insulin at the
level of the ovary and the adrenal glands stimulates an increase in androgen production. Similarly, the increase at the liver leads to an increase in free testosterone via a decrease in SHBG production. The mechanism responsible for the tissue-specific insulin sensitivity is unknown (24, 64).

Another unique quality of insulin resistance in PCOS is that glucose-intolerant women often have normal fasting glucose levels (37, 65). This makes diagnosis more difficult than that of diabetes or IGT in the general population, as measuring fasting glucose is not sensitive enough to identify the problem.

Additionally, insulin resistance has been shown independent of obesity in women with PCOS. Obesity does still play a role though, as obese women have a greater degree of insulin resistance, demonstrating an additive effect (21, 33-35, 77, 85). Given that insulin resistance is a unique feature of PCOS, independent of weight, the prevalence of glucose intolerance is significantly higher in obese PCOS women as compared to age-, ethnicity-, and weight-matched ovulatory control women (approximately 30% vs. 10%, respectively) (32). The precise underlying cause of insulin resistance in PCOS is unknown, although hyperandrogenism and anovulation have been proposed and well studied.

**Insulin Resistance in PCOS: Potential Interactions with Diagnostic Criteria**

Hyperandrogenism, a diagnostic criterion for PCOS, is one potential cause of insulin resistance in PCOS. A connection between hyperandrogenism and hyperinsulinism was first reported in 1980 when Burghen et al. found that obese women with PCOS had higher insulin levels than obese controls with normal androgen levels,
and that these high insulin levels were correlated with the high androgen levels (14).
Numerous investigators have provided additional supporting data for a relationship between hyperandrogenism and hyperinsulinism (58). Although hyperandrogenism correlates with hyperinsulinemia, treatment of hyperandrogenism has not been shown to improve hyperinsulinemia in women with PCOS (75), demonstrating that hyperandrogenism is not the only factor in PCOS-related insulin resistance.

Dunaif et al. also indicated that hyperinsulinemia is a unique feature of PCOS and not solely attributable to hyperandrogenism itself. This conclusion was formed based on a study comparing glucose tolerance (measured by glucose and insulin responses to a 40g/m² oral glucose load over 120 minutes) in lean and obese (classified as ≥ 20% above Ideal Body Weight) anovulatory, hyperandrogenic women with PCOS to glucose tolerance in lean and obese ovulatory hyperandrogenic women. Fasting insulin levels were significantly higher in the obese PCOS group compared to the obese hyperandrogenic women (27±17 μU/mL vs. 17±10 μU/mL), but no difference was found between the lean groups. When the mean summed insulin levels during the OGTT were compared, both lean and obese women with PCOS had significantly higher levels compared with the respective ovulatory hyperandrogenic women (lean: 187±110 μU/mL vs. 107±50 μU/mL; obese: 402±240 μU/mL vs. 179±101 μU/mL), proposing that insulin resistance is not related to hyperandrogenism alone, and may actually be related to ovulation (33).

Similarly, both Conway and Robinson showed that insulin resistance is more profound in women with PCOS who have an irregular menstrual cycle. Overall, Conway et al. found that both lean (BMI < 25 kg/m²) and obese (BMI > 25 kg/m²) women with
PCOS had higher fasting insulin values compared with lean controls, but found that when stratified by menstrual cycle regularity, lean women with oligomenorrhea or amenorrhea had increased values compared to women with PCOS that had a normal cycle. Using regression analysis, it was demonstrated that while testosterone accounted for the variability of the number of menstrual cycles per year, fasting insulin was an independent contributor and possible mediator to the relationship, with higher concentrations being associated with a decreased number of cycles (28). Robinson and colleagues suggest a similar relationship of reduced insulin sensitivity to oligomenorrhea in PCOS, and found this to be independent of hyperandrogenism (90). These data support abnormal menstruation as having a possible link with hyperinsulinism in women with PCOS; although a causal association could not be determined.

Although hyperandrogenism and anovulation have both been thought to cause insulin resistance, it has also been proposed that insulin resistance feeds back and potentially leads to hyperandrogenism and/or anovulation. The premise behind hyperinsulinism leading to hyperandrogenism is based on three main mechanisms. Hypersecretion of insulin by the pancreatic beta cell: 1) stimulates ovarian and adrenal androgen biosynthesis as a trophic hormone, 2) suppresses SHBG levels, leading to an increase in the number of bioavailable androgens, and 3) alters the pattern of circulating gonadotropins via direct effect on the hypothalamus and pituitary gland, ultimately causing a rise in luteinizing hormone (64). Additionally, hyperinsulinism may lead to anovulation via 1) an increase in intraovarian androgen production, 2) alteration of gonadotropin secretion, and/or 3) a direct effect on follicular development (78).
The interactions of hyperandrogenism, anovulation, and insulin resistance make it difficult to pinpoint their relationships. The complexity of PCOS and its associated consequences leads to the determination that insulin resistance is a multi-faceted issue that still has a lot to be determined. Although these hormonal interactions take place, there is also data to support an additional problem at the cellular level.

**Causes of Insulin Resistance in PCOS: Potential cellular mechanisms**

The actual mechanism for reduced insulin action at the cell level in women with PCOS is still being assessed, but it does seem to be a combination of increased secretion and reduced clearance of insulin. It has been proposed that the increase in secretion from the beta-cells occurs in an attempt to compensate for the reduced clearance (79).

Reduced binding of insulin to its receptor has been proposed as a mechanism for decreased clearance (58), but this is not confirmed in all studies. Dunaif et al. and Ciraldi et al. indicate that there may be a postreceptor defect, showing that normal binding occurs along with reduced insulin-mediated glucose transport (23, 35). Women with PCOS show a reduction in insulin-mediated glucose disposal of approximately 35-40%, which is similar to that seen in T2DM (35). The means by which the transport is reduced are not fully known at this time, but decreased expression of GLUT-4 (an insulin-dependent glucose-transporter protein) may play a role (91).

There is still a lot to be determined regarding the pathogenesis of insulin resistance in PCOS. This uncertainty regarding the cause of insulin resistance, and moreover, the effect of insulin resistance on these women, warrants further study. Adipokines have been proposed as having a role in insulin resistance in the general
population, and have recently been moderately incorporated into research in PCOS. Further study on the role of adipokines in PCOS-related insulin resistance is crucial, as it may be a large contributor to the pathogenesis of the disorder. Insulin resistance is a detriment to health in the general population because of the associated increased risk of cardiovascular disease and diabetes, but potentially has an even greater effect on individuals with PCOS because of its additional negative effects on hyperandrogenism, ovulation, and fertility.
**ADIPOKINES**

White adipose tissue (WAT) was originally thought to function exclusively for energy storage, but is now identified as a multi-functional organ. Of most interest in this paper is the function of WAT as an endocrine organ – particularly with respect to adipokines. Adipokines are proteins that are synthesized by and secreted from adipose tissue (which includes adipocytes and immune cells like macrophages) (104).

In 1952, G.C. Kennedy proposed a physiologic means by which energy stores, food intake, and energy expenditure were adjusted in rats – mainly via the hypothalamus (59). This idea was the first of its kind but led to further investigation by other researchers. This concept was confirmed by various scientists, as it was found that forced overfeeding reduced voluntary food intake, while fasting decreased energy expenditure. The concept behind a feedback mechanism for regulating fat stores was reinforced by G. R. Hervey in 1959 (54).

Further recognition of an actual obesity gene (*ob*) and a complementary diabetes gene (*db*), along with their effect on energy expenditure and obesity, provoked more research. These genes were then cloned in mice in 1994, confirming that the *ob* gene was needed for production of the “satiety factor” while the *db* gene was needed in order to produce a response to the satiety factor. The establishment of adipose tissue as an endocrine organ was ultimately initiated through the cloning of the *ob* and *db* genes, but it is the product of the *ob* gene, leptin, that was actually responsible for this development (50). It was further determined that this product’s effect was active on the brain (4, 15). The discovery of leptin led to the advance that WAT is a functional endocrine organ. This prompted WAT, and specifically adipokines, to become widely studied.
More than fifty adipokines have been identified, with many different roles. Adipokines are known to affect appetite and energy balance, immunity, insulin sensitivity, angiogenesis, inflammation and acute-phase response, blood pressure, lipid metabolism, and glucose homeostasis (104). Leptin, adiponectin, and visfatin are the three adipokines of interest for this thesis.
LEPTIN

Overview

Leptin, the first protein to establish adipocytes as endocrine cells, serves many different functions in the body. Leptin secretion is regulated by the sympathetic nervous system and plays a crucial role in the control of body metabolism, immune function, haematopoiesis, angiogenesis, renal function, reproduction, and growth and development (89), although its effect on satiety is what it is best known for. Leptin is primarily synthesized in WAT, but can also be found in various other tissues (4). Within WAT, leptin gene expression is dependent on short-term regulation of certain factors such as food, fever, cytokines, and sympathetic nervous system products (89). Leptin concentrations increase in response to insulin, glucocorticoids, cytokines, estrogens, glucosamine, overfeeding, fever, and obesity. Conversely, testosterone, thiazolidinediones, fasting, cold, and exercise have been shown to decrease leptin levels (4, 89). Sympathetic nervous system products such as noradrenaline, adrenaline, isoprenaline, and β3-agonists have also been shown to decrease leptin production (4, 89).

Leptin and Adiposity

Leptin concentrations are increased in obesity, therefore blood leptin levels correlate with BMI and adiposity (27, 67-69, 92, 94). In a study of 136 normal-weight (BMI 23.0 ± 2.5 kg/m²) and 139 obese (BMI 35.1 ± 7.2 kg/m²) men and women, Considine et al. found a correlation between serum leptin concentrations and percent body fat of r = 0.85. Significantly different mean leptin concentrations were also found, with concentrations of 7.5 ± 9.3 ng/mL and 31.3 ± 24.1 ng/mL in the lean and obese
subjects, respectively (27). The size of the adipocyte itself also plays a role in leptin concentration, with larger adipocytes containing more leptin than smaller adipocytes (51). Moreover, overweight/obese humans may even have increased concentrations of leptin per unit body fat. Havel et al. found almost a 2-fold difference; 1.01 ± 0.07 ng leptin/mL per percent body fat in overweight/obese postmenopausal women (BMI > 27.8 kg/m²) compared with 0.57 ± 0.06 ng leptin/mL per percent body fat in those of normal weight (BMI < 27.8 kg/ m²) (53). Leptin expression is more prominent in subcutaneous adipose tissue than in visceral adipose tissue when assessed with respect to fat distribution (41, 56, 72, 74, 76, 106). In a study of 77 obese males and 70 females (BMI range 30-55 kg/m², mean 42.3±5.9 kg/m²), leptin concentration was moderately correlated to subcutaneous fat (r = 0.49), while it showed almost no correlation to preperitoneal fat (r = -0.01) (74). Although there is an association between leptin and body fat, there are ample data that show changes in leptin levels independent of changes in adiposity. These changes may be contributed to changes in insulin concentration.

**Relationship between Leptin and Insulin**

It has been suggested that insulin may play a crucial role in the change in leptin concentrations over time. Havel et al. studied the effect of weight loss on leptin concentrations in normal weight and overweight/obese postmenopausal women and found that although changes in leptin concentration were well correlated with BMI, the change in plasma leptin was also correlated with the change in fasting insulin concentration, independent of change in BMI (r = 0.45) (53). A similar relationship has been shown in lean and obese men. Segal et al. compared leptin levels in insulin
resistant lean men (16 ± 1 % body fat) to fat-matched insulin sensitive lean men (15 ± 1 % body fat) and insulin resistant obese men (31 ± 3 % body fat) (with the same degree of insulin resistance as the insulin resistant lean men) and found insulin to have an independent relationship with leptin. Insulin sensitive lean men had lower levels of leptin than insulin resistant lean men (1.90 ± 0.4 ng/mL versus 4.35 ± 1.21 ng/mL), while insulin resistant obese men had the highest levels (9.27 ± 1.4 ng/mL), thus indicating an additional contribution of obesity to the effect of insulin resistance (96).

Boden and colleagues report that relatively large concentrations of insulin for relatively long periods of time (24 hours in this particular study) are needed in order to see a significant rise in leptin concentration (10). Chronically increased insulin concentrations are often seen in obese individuals, thus indicating that a prolonged increase in insulin may help to explain the increased concentrations of leptin observed with obesity.

Although there is a relationship between prolonged hyperinsulinemia and increased leptin concentrations, there is convincing data to support that there is no short-term effect of insulin on leptin levels (30, 60, 88, 95). Pratley et al. demonstrated this in a comparison of the effect of a two step acute increase in insulin on leptin levels in insulin sensitive versus insulin resistant Pima Indians. The first 100-minute insulin increase was at a level of potential physiologic hyperinsulinemia, while the second 100-minute increase was at a supraphysiologic level. A hyperinsulinemic-euglycemic glucose clamp was used, therefore glucose levels remained constant. The increase in insulin did not have a significant effect on leptin levels at either time point for either group. Leptin levels for the insulin sensitive group at 0, 100, and 200 minutes were 22 ± 5 ng/mL, 21 ±
6 ng/mL, and 21 ± 6 ng/mL, respectively. Leptin levels for the insulin resistant group were 39 ± 8 ng/mL, 39 ± 8 ng/mL, and 34 ± 7 ng/mL, respectively (88).

Although prolonged hyperinsulinemia and other factors may increase leptin levels, the high leptin levels do not translate into an increased response in the hypothalamus, demonstrating that obese people tend to be resistant to the effects of leptin.

**Leptin Resistance**

Leptin resistance ultimately results in increased levels of circulating leptin, which can then lead to increased levels of feeding and obesity, and then even higher levels of leptin. There are various proposed mechanisms for leptin resistance. Banks et al. and Caro et al. support the hypothesis that leptin resistance is a result of deficiency at the blood brain barrier. Their data show that the system by which leptin enters the brain is saturable, therefore even highly elevated levels of plasma leptin are not able to increase its effect on the hypothalamus (7, 18, 57). Additionally, obesity-related leptin resistance may be a result of a downregulation of the leptin receptor in the hypothalamus due to the continuous excess of circulating leptin (71). A mutation in the leptin receptor at the level of the hypothalamus has also been proposed (26).

Although obese people are frequently mentioned with respect to increased leptin levels and leptin resistance, other conditions, such as PCOS, may also result in an alteration of adipokine levels.
Leptin and PCOS

One of the marked characteristics associated with PCOS is hyperinsulinemia, often accompanied by obesity. Leptin is a proposed mechanism in the pathogenesis of PCOS based on its potential relationship with both of these features of the syndrome. Data regarding leptin levels in women with PCOS is inconclusive. Some researchers have found no difference in leptin levels in women with PCOS compared with age- and weight-matched controls (63, 70, 93), while others have found that women with PCOS have higher concentrations (13, 38, 86). Rouru et al. compared leptin levels in 24 women with PCOS (BMI 27 ± 2.76 kg/m², all with hyperandrogenism and hyperinsulinism) to 19 healthy control women (BMI 24.1 ± 5.5 kg/m²) and found there to be no difference (13.4 ± 9.8 μg/L versus 10.4 ± 5.1 μg/L) (93). Contrastingly, El Orabi et al. found that leptin levels in 45 women with PCOS (33.03 ± 14.04 ng/mL) were significantly higher than levels in 20 weight matched controls (22.7 ± 6.5 ng/mL). It is important to note that when stratified by weight, the obese group was the only one to show a difference, while there was no difference between the nonobese groups (38).

Increased leptin concentrations have been shown to decrease follicular growth, while increasing the production and secretion of progesterone, estradiol, and testosterone in mice (101), ultimately bringing up the question of leptin’s role in PCOS-related infertility. Leptin may also have a direct effect on reproduction. This was originally proposed because peripheral tissues such as the ovaries have been shown to have leptin receptors (20, 25). Additionally, there is a chance that the potentially increased amounts of leptin may play a role in the insulin resistance that is characteristic of the disease, although the data are inconclusive.
Leptin, Insulin Resistance, and PCOS

A relationship between leptin and insulin resistance has been proposed in women with PCOS. Pehlivanov and Mitkov found a strong correlation between leptin and immune-reactive insulin (IRI) \((r = 0.592)\) and HOMA \((r = 0.537)\) in women with PCOS, while there was no correlation in the control group. (This relationship did become less strong \((r = 0.488)\) when adjusted for WHR, BMI, and waist circumference). When the PCOS group was stratified by HOMA, the group with a higher degree of insulin resistance had significantly higher leptin levels \((21.0 \pm 4.28 \text{ ng/mL versus } 8.78 \pm 1.44 \text{ ng/mL})\). After this stratification, there was not a correlation between leptin and HOMA in the group with a lesser degree of insulin resistance, but they were still correlated \((r = .591; r = 0.494 \text{ after controlling for waist/hip ratio, BMI, and waist circumference})\) for the group with a higher degree of insulin resistance. These results led the authors to conclude that insulin resistance itself may be related to the increased leptin levels seen in women PCOS, although temporal sequence cannot be determined from this study (86). Laughlin et al. found similar results with a strong positive correlation \((r = 0.81)\) between leptin and 24-hour mean insulin levels. This relationship was found in the women with PCOS, as well as the controls. Interestingly, even though the women with PCOS had significantly higher 24-hour mean insulin levels \((394 \pm 55 \text{ pmol/L})\) compared to controls \((196 \pm 19 \text{ pmol/L})\), leptin levels did not differ between the two groups \((24.1 \pm 2.6 \text{ ng/mL for PCOS; } 21.5 \pm 3.5 \text{ ng/mL for controls})\) (63). The similar leptin levels may have been significantly different if the groups were stratified by level of insulin resistance, but these data were not reported.
The relationship between insulin resistance and leptin in PCOS has not been fully established due to the inconclusive nature of the literature, as Erturk et al. did not find a significant correlation between leptin and insulin resistance in women with PCOS after controlling for BMI (39). Further research is needed to determine whether abnormal leptin concentrations are present in women with PCOS, and also if leptin concentrations have a role in the pathogenesis of the many facets of PCOS, including insulin resistance. Although leptin is a commonly studied adipokine, adiponectin has gained recent recognition for its potential role in insulin resistance and PCOS.
Adiponectin is a protein hormone that is exclusively produced by adipocytes. Unlike other adipokines, adiponectin concentrations actually decrease with increasing amounts of adipose tissue (5, 44, 73, 105). This inverse relationship has also been shown in children (100). In the average person, the concentration of adiponectin found in plasma is approximately three times higher than that of the majority of other circulating hormones. It is important to note that there are gender differences in adiponectin concentration, independent of body weight, with females having higher levels than males (11, 62).

A definitive role for adiponectin has not been confirmed, but it may play a role in reducing atherogenesis and inflammation (81, 82, 110), ultimately offering prevention against cardiovascular disease. On the contrary, low levels of adiponectin may be related to the development of type 2 diabetes mellitus and its associated complications (55). In fact, Xydakis et al. incorporated characteristics of the previously mentioned conditions (cardiovascular disease and diabetes) as found in the metabolic syndrome into a study evaluating adiponectin’s association with the metabolic syndrome. Adiponectin concentrations were lowest in those subjects who met four or five of the five diagnostic criteria for metabolic syndrome, with a stepwise decrease in plasma adiponectin levels in parallel to the number of metabolic syndrome components present. It was even suggested that for every 1 μg/mL increase in adiponectin levels, the odds of having the metabolic syndrome decreased by 17%, demonstrating a protective effect (108). Although the metabolic syndrome as a whole has been studied with respect to adiponectin,
adiponectin’s association with insulin resistance is a main area of research, as promising results have been found.

**Adiponectin and Insulin Resistance**

Increases in adiponectin may mediate the improvement of insulin action and carbohydrate metabolism that occurs with weight loss (12, 55, 109). Bruun et al. assessed the relationship between adiponectin and measures of insulin resistance in nineteen obese men (BMI 38.7 kg/m²) after undergoing a diet-induced weight loss intervention (average loss of 20 kilograms). This weight loss was accompanied by a 43% increase in adiponectin levels (4.3 ± 0.4 mg/L vs. 3.0 ± 0.3 mg/L), which was negatively correlated to the change in fasting insulin concentration (r = -0.55) and change in HOMA (r = -.048). The change in insulin that occurred was found to be a significant predictor of changes in adiponectin (12). Various studies also support a genetic mechanism, with low adiponectin levels having a causal role in the development of diabetes (105).

Additional improvements in adiponectin levels also occur after the use of insulin-sensitizing agents such as thiazolidinediones. Yu et al. found that adiponectin levels not only increased in obese and diabetic participants, but also in normal subjects (who showed no other changes due to the thiazolidinediones) (111).

It is important to note that improvements in insulin action and carbohydrate metabolism have also been shown independent of increases in adiponectin, so it is unlikely that adiponectin is the only contributor (95, 108). Additionally, Phillips et al. showed that although glucose control improved after both thiazolidinedione and
metformin treatment, the metformin treatment did not increase adiponectin levels, although the thiazolidinedione did (87).

Due to the increased prevalence of insulin resistance in women with PCOS, along with a high percentage of women that are overweight/obese, there is a possibility that adiponectin may play a role in the pathogenesis of insulin resistance in PCOS and/or the syndrome in general.

**Adiponectin and PCOS**

Adiponectin levels are not definitively established in women with PCOS. Most studies show lower levels of adiponectin in women with PCOS compared to healthy women (6, 17, 40, 97, 98), but there are others that do not demonstrate a significant difference (46, 48). Carmina et al. examined adiponectin concentrations in 52 normal weight and overweight women with PCOS, and compared them to 45 age and weight-matched controls. Overall, PCOS women had significantly lower levels of adiponectin when compared to controls (8.2 ± 0.6 μg/mL vs. 10.5 ± 0.7 μg/mL). When grouped by weight, obese PCOS women (BMI 25-30 kg/m²) had lower adiponectin concentrations compared to normal weight PCOS women (6.9 ± 0.7 μg/mL versus 9.2 ± 1.1 μg/mL), while normal weight PCOS women still had significantly lower levels than both normal weight (13 ± 1.5 μg/mL) and overweight controls (11.4 ± 1.3 μg/mL). In both women with PCOS and control women, adiponectin was negatively correlated with BMI (r = -0.28 for PCOS, r=0.036 in normal), although there was no correlation with WHR in either group (17). In addition to the examination of adiponectin levels in women with
PCOS, adiponectin’s relationship with insulin resistance in PCOS has also be evaluated due to the relationship seen in the general population.

**Adiponectin, Insulin Resistance, and PCOS**

In the same women mentioned above, Carmina et al. examined the relationship between adiponectin levels and insulin resistance. In women with PCOS, adiponectin was not significantly correlated with insulin or insulin resistance (QUICKI). Differing results were found in controls, as adiponectin was correlated to insulin ($r = -0.36$) and insulin resistance (QUICKI) ($r = 0.32$), although only when BMI was not controlled. The overall outcome of this study shows decreased adiponectin concentrations in all women with PCOS (regardless of weight), but this difference was not accounted for by insulin resistance, leading to the presumption that insulin resistance is not the main means by which altered adiponectin secretion occurs, and vice versa (17).

Contrastingly, several authors have reported significant relationships between adiponectin and measures of insulin resistance. Sepilian et al. found significant negative correlations for adiponectin’s association with both insulin ($r = -0.040$) and insulin area under the curve after a meal ($r = -0.47$), even after controlling for BMI, in their sample of obese insulin resistant women with PCOS (97). These negative correlations have been supported by other researchers (46, 48, 97, 98).

Aroda et al. further examined the relationship of adiponectin and PCOS with respect to glucose tolerance and insulin resistance in a group of 31 obese women (BMI $> 30$ kg/m$^2$) compared to 6 age- and BMI-matched ovulatory controls. It was established that the women with PCOS had significantly lower serum adiponectin levels compared
with the controls (9.5 ± 0.7 μg/mL versus 17.4 ± 1.7 μg/mL). This difference becomes more apparent when the PCOS women were divided into a normal glucose tolerance (NGT) group and an impaired glucose response (IGR) group based on a 2-hour OGTT. The IGR group had fasting adiponectin concentrations of 8.4 ± 0.9 μg/mL. The NGT group had fasting levels of 11.13 ± 1.10 μg/mL. Circulating adiponectin levels were not correlated with fasting glucose values, but were significantly correlated with both the absolute 2-hour glucose values (r = 0.40) and the increment in 2-hour glucose from the OGTT (r = 0.418). Additionally, whole body glucose disposal rate was determined using the 3-hour hyperinsulinemic euglycemic clamp, and was found to have a statistically significant relationship with adiponectin (r = 0.394). Due to the strengthened relationship between glucose tolerance and adiponectin after the women were grouped by glucose tolerance, Aroda et al. propose that the degree of glucose tolerance and insulin resistance is a strong predictor of circulating adiponectin levels. The authors also make a good point that because of the effect of the degree of insulin resistance, heterogeneity may account for some of the difference shown in other literature (6).

The possible direct or indirect roles of adiponectin with respect to insulin resistance make it a potential contributor to the pathogenesis of PCOS, as well as the occurrence of insulin resistance in PCOS. Further study is needed to confirm the discrepancies in the literature regarding the relationship between adiponectin levels and insulin resistance in women with PCOS.

In addition to the potential roles of the adipokines leptin and adiponectin in the pathogenesis of PCOS-related insulin resistance, visfatin, a newly discovered adipokine, may be a significant contributor due to its insulin-like properties.
**Visfatin**

**Overview**

Visfatin (also known as pre-B cell colony-enhancing factor) is an insulin-like protein partly produced by visceral fat that is emerging in the literature. Fukuhara et al. first speculated that increased visfatin concentrations occur in parallel with increasing amounts of visceral fat (42), but this is not definitively shown in the literature. Berndt et al. found no correlation between visceral fat (measured by computed tomography scan) and visfatin concentration in 73 individuals with varying BMIs, fat distributions, insulin sensitivity levels, and glucose tolerance levels (8). Along with the inconsistency regarding visfatin’s relationship to fat distribution, there is contradictory data regarding visfatin’s relationship to BMI. In a study of 189 participants with a wide range of BMIs, fat distribution, insulin sensitivity, and glucose tolerance, plasma visfatin levels were positively correlated with BMI (r = 0.25) and percent body fat (r = 0.22) (8). Various other studies have shown no relationship between visfatin and BMI. Interestingly, data from Chen et al. show no significant relationship between visfatin and BMI, but a positive correlation between visfatin and WHR (r = 0.25), (22). Contrastingly, Pagano et al. actually found a negative correlation between visfatin and BMI (r = -0.37) in a group of 69 people, although this only held true for those in the obese group (obese n = 39, lean n = 30) (83).

Fukuhara et al. were also the first to examine a potential relationship between visfatin and insulin. They found a dose-dependent glucose-lowering effect of visfatin (via injection) in insulin-resistant and insulin-deficient mice. The effect was independent of changes in plasma insulin levels. Further supporting data were found based on both
fasting and feeding plasma glucose levels when visfatin\(^{+/-}\) generated mice were compared to wild type mice (42).

Unlike insulin, visfatin levels do not change acutely due to feeding in mice. Additionally, although visfatin and insulin have shown similar responses regarding activation of glucose uptake and inhibition of glucose release, this seems to occur only at similar concentrations; visfatin is found in much smaller concentrations (only 10% that of insulin during fasting and 3% during feeding), leading to an overall lower effect of visfatin compared to insulin (42). The mechanism by which visfatin works to decrease glucose levels has yet to be determined, though visfatin has been shown to bind to and activate the insulin receptor without competing with insulin itself. This may indicate that visfatin has a separate binding site (42, 105).

Haider et al. found contrasting results regarding the report by Fukuhara et al. that no significant fluctuation in plasma visfatin levels occur after feeding, and state that Fukuhara may not have seen an increase in visfatin because of the coupled increase of insulin that occurs with feeding. This was demonstrated in a study of 9 healthy men (BMI 23±1 kg/m\(^2\)) in which somatostatin was administered to prevent an insulin increase during glucose infusion. As glucose levels were raised from 5.0 mmol/l to 8.3 mmol/l, and then to 11.1 mmol/l, visfatin levels increased significantly from 0.5 ± 0.0 ng/mL to 0.9 ± 1 ng/mL, and then to 2.1 ± 0.3 ng/mL, respectively, leading to the conclusion that hyperglycemia promotes visfatin release, however this increase is prevented by hyperinsulinemia or somatostatin. Assessment of the regulation of visfatin led to the recognition of a negative feedback effect of insulin (49). Hyperinsulinemia as a regulator
of visfatin has led to research of visfatin and its relationship to insulin resistance and type 2 diabetes mellitus.

**Visfatin and Insulin Resistance**

Visfatin’s insulin-mimetic properties have led to studies assessing its relationship to insulin, insulin sensitivity, and glucose tolerance. Conflicting results have been found. Berndt et al. found no significant correlation between visfatin and fasting insulin concentration, fasting glucose, or 2 hour OGTT concentrations in their sample of 189 men and women (8). Chen et al. analyzed patients with type 2 diabetes mellitus and similarly found no correlation between plasma visfatin levels and insulin resistance when a multivariate model was used. (Waist/hip ratio was the only factor that remained significant in the multivariate model). Overall, participants with type 2 diabetes mellitus had significantly elevated levels of visfatin as compared to sex- and age-matched controls (although participants were not matched for waist circumference, WHR, fasting glucose, fasting insulin, and insulin resistance) (22). Visfatin levels have also been evaluated in people with type 2 diabetes mellitus and impaired glucose tolerance that had not yet been treated (compared to a group of people with normal glucose tolerance). Although serum visfatin levels were significantly higher in the group with type 2 diabetes mellitus compared to controls (32.2 ± 2.3 ng/mL versus 28.5 ± 2.07 ng/mL), there was no correlation between visfatin and fasting insulin concentrations or insulin resistance in any of the three groups. Interestingly, visfatin was also not significantly correlated with BMI, as it has been in various other studies (31).
**Visfatin and PCOS**

Although there is still a lot to be determined regarding visfatin, it is fairly well shown that visfatin concentrations are increased in patients with type 2 diabetes mellitus. Due to the similar characteristics of diabetes and PCOS, it has been proposed that visfatin may play a role in the pathogenesis of insulin resistance and hyperinsulinemia seen in many of these women. However, the literature on PCOS is inconsistent (as it is in the general population).

In a study of lean and obese women with PCOS compared with age- and BMI-matched ovulatory controls, Kowalska et al. found that the PCOS group had significantly higher serum visfatin concentrations (67.14 ± 36.99 ng/mL versus 53.56 ± 32.48 ng/mL) (61), which has been supported elsewhere in the literature (45, 103). The rationale for the increased levels of plasma visfatin in women with PCOS is still being explored, but a few concepts have been suggested. Tan et al. suggest that the increased visfatin levels seen in PCOS patients may be due to: 1) impaired visfatin signaling in target tissues, 2) an overall dysregulation in visfatin biosynthesis, 3) a compensatory response in insulin resistant tissues, and/or 4) visfatin as a marker of tissue-specific inflammatory cytokine action (103). Chan et al. support the first two suggestions as causes for increased levels in patients with T2DM (22), which has led to further study regarding the impact of increased visfatin concentrations as a possible contributor to insulin resistance in PCOS.
Visfatin, Insulin Resistance, and PCOS

In the same study as mentioned above, Kowalska et al. found that when grouped by weight, the higher visfatin concentrations found in the lean PCOS group were significantly associated with lower insulin sensitivity (measured with a euglycemic hyperinsulinemic clamp) in that group \((r = -0.30)\), although significance was lost after controlling for serum FFA. Insulin sensitivity was not associated with a difference in the visfatin concentrations in the obese PCOS group, although both groups had significantly lower levels of insulin sensitivity compared to the controls. Interestingly, obesity only had a significant effect on visfatin levels in controls, while there was no effect of obesity on visfatin levels in those with PCOS (61).

As previously mentioned, the literature is inconclusive. Gen et al. reported nonsignificant correlations between visfatin and measures of insulin resistance in women with PCOS (45), while Tan et al. did find significant results. A positive correlation was found between plasma visfatin and both fasting insulin \((r = 0.58)\) and insulin resistance \((r = 0.59)\) (as measured by HOMA) in overweight \((\text{BMI} 25-30 \text{ kg/m}^2)\) and obese \((\text{BMI} 30-35 \text{ kg/m}^2)\) women. This positive association was found in both women with PCOS and control women individually, as well as both groups combined. After further analysis, it was determined that HOMA was a significant predictor of visfatin concentrations, while insulin itself was not, based on multiple regression analysis including insulin, HOMA, testosterone, and sex hormone-binding globulin. It is important to note that this was a smaller sample than the previously mentioned studies (103).

The insulin-mimetic properties of visfatin, as well as the potential associations between visfatin and insulin resistance, present a potential role of visfatin in the
pathogenesis of the insulin resistance seen in women with PCOS, although further study is needed.

LIMITATIONS IN THE LITERATURE

The major limitation of the current literature regarding adipokines and insulin resistance in PCOS is its equivocal nature. The differences in data reported could be due to the predominant use of a static measure of insulin resistance, which uses only fasting insulin and glucose values, versus a dynamic measure which assesses a person’s insulin reaction in response to a glucose load. The present study addresses this limitation by incorporating I-AUC based on a 2-hour OGTT. Additionally, no previous studies have assessed the three adipokines leptin, adiponectin, and visfatin simultaneously in one sample.

IMPLICATIONS

As stated, the current PCOS literature is equivocal with regards to insulin resistance and adipokines metabolism; therefore it is necessary to continue the study of this complex syndrome in order to further understand its pathogenesis. Insulin resistance is one of the major metabolic consequences that occur in women with PCOS, but the cause is unknown. A further understanding of PCOS-related insulin resistance would lead to a better understanding of the treatment that is necessary, ultimately aiding in the relief of symptoms and the prevention of long-term consequences such as cardiovascular disease and diabetes.
STUDY OBJECTIVES

The current study’s main objective was to evaluate the relationships between insulin resistance (via a dynamic measure) and adipokines (leptin, adiponectin, visfatin) in women with PCOS. Secondarily, we sought to assess the correlations between adipokines and markers of obesity in women with PCOS.
METHODOLOGY

PARTICIPANT RECRUITMENT

The data used in the analyses were baseline statistics from an ongoing pilot study assessing the effect of exercise on adipokines and various other measures related to PCOS. Participants were recruited based on the diagnosis of PCOS via patient charts at Wake Forest University Baptist Medical Center. Each potential participant’s chart was assessed for the medical condition exclusions specified in Table 3, as well as for any other medical issues that would be contraindicated for the study. If the recruiter had a questionable eligible participant, the endocrinologist on the study was the one to make the decision whether that person would be eligible to participate. When a potential participant was identified, permission to contact her was obtained from her physician. Upon approval, the patient was phoned for further screening to identify her interest in the study as well as current activity levels (patient needed to be sedentary) and the ability to participate in the exercise intervention. Interested and eligible participants provided informed consent at their baseline visit.

Two-hundred and two potential participants were screened. Eleven participants agreed to participate and signed an informed consent. Table 4 displays an itemization of the numbers and reasons that women were not eligible or did not participate in the study.
Table 3. Medical Condition Exclusions

- Diabetes
- Active Cancer
- Pulmonary Disease
- Cardiovascular Disease
- Liver Dysfunction
- Renal Dysfunction
- Hematological Dysfunction
- Uncontrolled Hypertension

Table 4. Recruitment Itemization

<table>
<thead>
<tr>
<th>Recruited (11)</th>
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<tbody>
<tr>
<td>Under 18 years old</td>
<td>4</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>1</td>
</tr>
<tr>
<td>Pregnant</td>
<td>4</td>
</tr>
<tr>
<td>Previously diagnosed with diabetes</td>
<td>59</td>
</tr>
<tr>
<td>Other contraindicative medical issues</td>
<td>12</td>
</tr>
<tr>
<td>Previously underwent weight loss surgeries</td>
<td>3</td>
</tr>
<tr>
<td>Personal physician did not approve to participate</td>
<td>2</td>
</tr>
<tr>
<td>Currently physically active</td>
<td>5</td>
</tr>
<tr>
<td>Not able to get in touch with</td>
<td>32</td>
</tr>
<tr>
<td>Disconnected phone number</td>
<td>24</td>
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<tr>
<td>Moved out of town</td>
<td>9</td>
</tr>
<tr>
<td>Not interested/not enough time</td>
<td>12</td>
</tr>
<tr>
<td>Other</td>
<td>24</td>
</tr>
</tbody>
</table>

Testing Procedures

All testing was performed at the General Clinical Research Center at Wake Forest University Baptist Medical Center (by their staff) after informed consent was obtained by the study coordinator. Clinical measures including height, weight, waist circumference,
and body composition were assessed. Height and weight were measured using standard techniques in which the participant removed her shoes and outer garments before being measured. Waist circumference was measured on a horizontal plane around the abdomen at the level of the iliac crest, making sure that the measuring tape was taught and parallel to the floor, but not compressing the skin. Body composition was evaluated using bioelectrical impedance analysis, in which the participant laid down on a table with electrodes placed on the wrists and ankles.

A 12-hour fasting blood draw was performed to obtain samples for future adipokine assays, as well as to obtain fasting blood glucose and insulin concentrations. A 2-hour 75mg oral glucose tolerance test was then completed with plasma glucose and insulin samples collected at 60 and 120 minutes.

**Outcome Measures**

**Insulin Resistance**

The main method by which insulin resistance was quantified was insulin area under the curve (I-AUC), a dynamic method. Two static methods, homeostasis model assessment of insulin resistance (HOMA) and fasting insulin, were also calculated. (These methods are considered static because they only use fasting glucose and/or fasting insulin values.)

HOMA and fasting insulin were chosen because they are commonly used in PCOS literature. Although the static measures are commonly used, I-AUC provides a better quantification of insulin resistance because it incorporates the fasting, 60 minute, and 120 minute insulin values from the OGTT.
I-AUC was calculated using SAS® version 9.1.3 via the trapezoidal method based on the fasting, one hour, and two hour insulin values obtained. HOMA was calculated using the formula fasting insulin (μU/mL) x fasting glucose (mmol/L) / 22.5.

**Adipokines**

Leptin and adiponectin concentrations were determined using ELISA human assay kits. The leptin and adiponectin kits were purchased from Millipore Corporation, Billerica, Massachusetts. Visfatin concentrations were determined using a Visfatin C-terminal Human Enzyme Immunoassay Kit (range 0.1-1000ng/mL) from Phoenix Pharmaceuticals, Incorporated from Burlingame, California.

**Obesity Markers**

Obesity was quantified using BMI, waist circumference, and percent body fat as described above.

**STATISTICAL ANALYSES**

Data were checked for normality using a one-sample Kolmogorov–Smirnov test. All data were normally distributed. Descriptive statistics (including means and standard deviations) were determined for age, BMI, waist circumference, body composition (percent body fat), and concentrations of leptin, adiponectin, and visfatin.

Partial correlations were used to identify the relationships between adipokines and insulin resistance, as BMI was controlled due to its potential association with both insulin resistance and the adipokines. Pearson correlations were used to determine the
relationships between adipokines and obesity markers. Multiple linear regression determined the amount of variance in insulin resistance explained by the adipokines in our sample of women with PCOS. Pearson correlations were initially run to make certain that the adipokines were correlated with each other. Significance level was set at \( p<0.05 \) for all statistical tests. All analyses were performed using SPSS® 16.0.
RESULTS

DEMOGRAPHICS

Participant characteristics, including age, race, body mass, waist circumference, and percent body fat, are shown in Table 5. The sample was 64% white and 36% black with a mean age of approximately 32 years. As a whole, the participants were obese, with a mean BMI of 36.1 kg/m², a mean waist circumference of 113.49 cm, and mean body composition of 45.9% fat.

<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>MEAN ± SD</th>
<th>RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>31.7 ± 7.2</td>
<td>23 – 45</td>
</tr>
<tr>
<td>Race (White/Black)</td>
<td>7/4</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>36.1 ± 7.1</td>
<td>23.1 – 47.4</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>113.49 ± 19.6</td>
<td>78.6 – 148.25</td>
</tr>
<tr>
<td>Body Composition (% Fat)</td>
<td>45.9 ± 5.6</td>
<td>36.3 – 56.0</td>
</tr>
</tbody>
</table>

OUTCOME MEASURES

Mean ± S.D. values for fasting glucose and insulin, and calculations for HOMA, and I-AUC are listed in Table 6. Plasma concentrations of leptin, adiponectin, and visfatin concentrations are also listed in Table 6.
**Table 6. Clinical and Metabolic Parameters**

<table>
<thead>
<tr>
<th>Outcome Measure</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting Glucose (mg/dl)</td>
<td>90 ± 7.3</td>
</tr>
<tr>
<td>Fasting Insulin (μIU/mL)</td>
<td>13.7 ± 4.8</td>
</tr>
<tr>
<td>HOMA</td>
<td>3.06 ± 1.13</td>
</tr>
<tr>
<td>I-AUC (μIU/mL)</td>
<td>9,220.1 ± 4,484.6</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>88.6 ± 42.7</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>9.5 ± 5.2</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>17.6 ± 3.5</td>
</tr>
</tbody>
</table>

**Correlation Analysis**

Partial correlations were used to determine relationships between plasma adipokine concentrations and insulin resistance, while Pearson correlations determined the relationship between plasma adipokine concentrations and obesity markers in our sample.

**Adipokines and Insulin Resistance**

The correlations (and respective p values) between each adipokine concentration and each measure of insulin resistance are shown in Table 7. Partial correlations controlled for BMI. Leptin concentration was not significantly correlated with any measure of insulin resistance. Figure 1 displays a scatterplot for leptin and I-AUC. Adiponectin concentration was significantly negatively correlated with I-AUC, as seen in Figure 2. Adiponectin was not correlated with any of the other measures of insulin resistance.
Visfatin concentration was significantly correlated to all three measures of insulin resistance. Figure 3 displays visfatin’s relationship with I-AUC.

**Table 7. Correlations Between Adipokines and Insulin Resistance**

<table>
<thead>
<tr>
<th></th>
<th>Fasting Insulin (μIU/mL)</th>
<th>HOMA</th>
<th>I-AUC (μIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/mL)</td>
<td>r = .087</td>
<td>r = .225</td>
<td>r = .029</td>
</tr>
<tr>
<td></td>
<td>p = .811</td>
<td>p = .532</td>
<td>p = .937</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>r = -.333</td>
<td>r = -.217</td>
<td>r = -.684*</td>
</tr>
<tr>
<td></td>
<td>p = .347</td>
<td>p = .547</td>
<td>p = .029</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>r = .661*</td>
<td>r = .714*</td>
<td>r = .775*</td>
</tr>
<tr>
<td></td>
<td>p = .037</td>
<td>p = .020</td>
<td>p = .009</td>
</tr>
</tbody>
</table>
FIGURE 1. SCATTERPLOT FOR I-AUC AND LEPTIN

$r = 0.029$
$p = 0.937$
**Figure 2. Scatterplot for I-AUC and Adiponectin**

![Scatterplot](image)

$r = -0.684$

$p = 0.029$
It is important to note that our data contained two significant outliers based on the Grubbs test. One outlier existed for I-AUC and one outlier existed for adiponectin concentration.

**Adipokines and Obesity Markers**

Table 8 lists the correlations (and respective p values) for adipokine concentrations and obesity markers. Leptin concentration was significantly correlated to all three obesity markers (BMI, waist circumference, and percent body fat), while adiponectin concentration and visfatin were not correlated to any of the obesity markers, although a trend exists for a negative correlation between adiponectin concentration and
all three obesity markers, as well as a trend for a positive correlation between visfatin and percent body fat. Figures 4-6 demonstrate the relationship between percent body fat and leptin, adiponectin, and visfatin, respectively.

### Table 8. Correlations between Obesity Markers and Insulin Resistance

<table>
<thead>
<tr>
<th>Leptin (ng/mL)</th>
<th>BMI (kg/m²)</th>
<th>Waist Circumference (CM)</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r = .860*</td>
<td>r = .798*</td>
<td>r = .875*</td>
</tr>
<tr>
<td></td>
<td>p = .001</td>
<td>p = .003</td>
<td>p = .000</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>r = -.470</td>
<td>r = -.431</td>
<td>r = -.431</td>
</tr>
<tr>
<td></td>
<td>p = .144</td>
<td>p = .186</td>
<td>p = .186</td>
</tr>
<tr>
<td>Visfatin (ng/mL)</td>
<td>r = .155</td>
<td>r = .083</td>
<td>r = .510</td>
</tr>
<tr>
<td></td>
<td>p = .648</td>
<td>p = .809</td>
<td>p = .109</td>
</tr>
</tbody>
</table>
Figure 4. Scatterplot for % Body Fat and Leptin

$r = 0.875^*$
$p = 0.00$
FIGURE 5. SCATTERPLOT FOR % BODY FAT AND ADIPOnectin

$r = -0.431$
$p = 0.186$
**Multiple Regression Analysis**

Multiple linear regression was used to determine the contributions of the adipokines to I-AUC. The Adjusted R-Square for our model was .766, demonstrating that 76.6% of the variance in I-AUC can be attributed to adiponectin and visfatin. Visfatin and adiponectin were found to be significant predictors of I-AUC, with visfatin being the most significant, as shown by the standardized beta coefficient in Table 9. Our data show that a one ng/mL increase in visfatin leads to an 850.211 μIU/mL increase in I-AUC, while a one μg/mL increase in adiponectin leads to a 413.422 μIU/mL decrease in I-AUC.
**Table 9. Multiple Regression Analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized Coefficients</th>
<th>Standardized Coefficients</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beta</td>
<td>Standard Error</td>
<td>Beta</td>
</tr>
<tr>
<td>Constant</td>
<td>-1796.460</td>
<td>4127.619</td>
<td>.675</td>
</tr>
<tr>
<td>Visfatin</td>
<td>850.211</td>
<td>202.949</td>
<td>.660</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>-413.422</td>
<td>136.847</td>
<td>-.476</td>
</tr>
</tbody>
</table>
DISCUSSION

PCOS is a complex disorder that involves various metabolic abnormalities. Due to the inter-connected nature of these issues, it is not clear whether these abnormalities actually lead to PCOS, are a result of the disorder, or a combination of the two. Insulin resistance is a major metabolic consequence observed in women with PCOS, although there are a few characteristics of insulin resistance in this population that differentiate it from that of diabetes and/or pre-diabetes. Insulin resistance in PCOS is tissue-specific (64), occurs independent of obesity (21, 34, 35), and generally does not result in abnormal fasting glucose values (37, 65).

Although substantial research has been performed regarding insulin resistance in women with PCOS, the actual cause is still not known. Adipokines have been suggested to play a role, but the data are inconclusive as to whether a relationship truly exists. Additionally, the current literature is equivocal regarding whether there are altered adipokine concentrations in women with PCOS. Much of the previous work regarding the relationship between adipokines and insulin resistance in PCOS involves the use of HOMA as a means of quantifying insulin resistance, although it has been suggested that this measure may not be sensitive enough to identify insulin resistance in women with PCOS. Fulghesu et al. found that when HOMA was used to identify hyperinsulinemia in a group of women with PCOS, only 18% of women were found to be hyperinsulinemic, while 44% of subjects were determined to have hyperinsulinemia based on I-AUC of a 3-hour OGTT (43). The current study has attended to this issue of evaluating insulin resistance based on a static measure by using I-AUC, a dynamic measure incorporating
fasting, one hour, and two hour insulin values based on a 2-hour OGTT. Additionally, leptin, adiponectin, and visfatin were assessed simultaneously in the same sample, which has not previously been done.

The main purpose of this study was to evaluate the relationship between insulin resistance and adipokines (specifically leptin, adiponectin, and visfatin) in women with PCOS, using a dynamic measure of insulin resistance. The secondary purpose of this study was to assess the correlations between the adipokines and markers of obesity in our population, as there is still some discrepancy regarding adipokine levels in women with PCOS.

**LEPTIN**

The mean leptin concentration in the present study was 88.6 ± 42.7 ng/mL. This is higher than concentrations previously reported in this population, although a wide range of values have been described. Pehlivanov et al. found a mean leptin concentration of 15.03 ± 2.9 ng/mL in their group of slightly overweight women with PCOS (BMI 25.74 ± 0.89 kg/m²), while Erturk et al. found a mean leptin concentration of 55.1 ± 24.3 ng/mL in their sample of obese women with PCOS (BMI 33.8 ± 4.7 kg/m²). The high concentrations found in our participants are most likely the result of the high mean percent body fat of the group, as leptin has been well correlated to body fat percentage (39, 63).

Compared to the general population, many studies show that women with PCOS have higher leptin levels than controls (13, 38, 86), although some have not found a significant difference (63, 70, 93). A broad range of concentrations have been reported in
controls, with values ranging from 6.16 ng/mL in a normal weight sample (BMI 23.45 ± 0.69 kg/m²) (86) to 33.12 ng/mL in an obese sample (BMI 40.88 ± 1.87 kg/m²) (70).

Leptin was not significantly correlated with any of the measures of insulin resistance. Our findings for a lack of relationship between leptin and fasting insulin are similar to those of El Orabi et al., as they did not observe a relationship between insulin and leptin in their non-obese (r = -0.13) or obese (r = -0.4) group of women with PCOS (38). It is important to note that there is literature that conflicts with our findings (17, 39, 93), with the strongest correlation of leptin and fasting insulin of r = 0.71 found by Laughlin et al. in their group of obese women with PCOS (63). Our findings for a lack of a relationship between leptin and HOMA are not consistent with a few previous reports including those by Pehlivanov et al. (86) and Erturk et al. (39).

Although various researchers have found correlations between leptin and static measures of insulin resistance, it is important to look at data with respect to a more dynamic measure, such as I-AUC. Erturk et al. assessed a form of this measure involving a post-meal insulin area and its relationship to leptin, and found that there was not a significant relationship between these two variables (r = .309), which supports our findings. Based on the lack of relationship between leptin and a dynamic measure of insulin resistance, it seems likely that leptin does not play a role in the insulin resistance found in many women with PCOS.

Although our primary outcomes did not appear to be significant with respect to leptin, our secondary outcomes (obesity markers) are highly related to leptin in our sample of women with PCOS. Leptin was very strongly correlated with BMI (r = .769, p = .006), waist circumference (r = .712, p = .014), and % body fat (r = .871, p = .000).
The strong relationship between leptin and BMI has been well-reported in the literature, although a wide range of correlations have been reported (17, 38, 39, 63, 70, 86, 93). The present study’s correlations and strength of significance are among the highest of those previously reported. Additionally, the relationship between leptin and waist circumference has been previously reported in women with PCOS (39, 86), although the association in our sample is stronger than most.

The obesity marker that was most highly correlated with leptin was percentage of body fat. Laughlin et al. found a similarly strong correlation ($r = 0.91, p < 0.0001$) in their combined group of overweight PCOS women and overweight control women (there was no difference between groups) (63). This relationship between leptin and body composition has also been displayed in normal weight women with PCOS (39).

It is fairly well shown that increased amounts of insulin, as well as obesity, can increase leptin concentration in the general population. Although a relationship between leptin and obesity exists in women with PCOS, it is unlikely that the insulin resistance often seen in women with PCOS is related to leptin concentration.

**ADIPONECTIN**

It has generally been shown that women with PCOS have decreased adiponectin concentrations compared to age- and weight-matched controls (6, 17, 40, 97, 98), with control values ranging from $8.5 \pm 3.9 \mu g/mL$ (97) to $17.4 \pm 1.7 \mu g/mL$ (6). The mean adiponectin concentration for the present study was $9.5 \pm 5.2 \mu g/mL$. This mean concentration is comparable to the means reported in other PCOS literature, with values ranging from $7.6 \pm 0.5 \mu g/mL$ (98) to $9.5 \pm 0.7 \mu g/mL$ (6).
Adiponectin’s relationship to PCOS-related insulin resistance has been an emerging topic in the literature, as low levels of adiponectin have been strongly associated with type 2 diabetes. Our finding of a significant correlation between adiponectin and I-AUC is similar to that of Sepilian et al. who found a strong correlation between adiponectin and I-AUC in their sample of women with PCOS (r = -0.47), although it is important to note that a three hour OGTT was performed in their study (versus the two hour OGTT used in the present study) (97).

Although HOMA and fasting insulin are often used as surrogate measures of insulin resistance, our data did not support significant relationships between adiponectin and these two variables. The current literature is inconclusive regarding these relationships, as most have reported significant relationships (though quite variable) between adiponectin and fasting insulin and/or HOMA (46, 48, 80, 97, 98), while others have not (17, 80). It is possible that fasting insulin and HOMA are not sensitive enough measures to quantify insulin resistance in women with PCOS, and thus, determine the correct relationship, therefore creating discrepancies in the literature. The differences found in the literature may also result from samples of differing degrees of insulin resistance, regardless of which test is being used.

The negative correlation that was found for our sample of women with PCOS indicates that women with higher degrees of insulin resistance have lower levels of adiponectin, and those women with low levels of adiponectin tend to have high levels of insulin resistance. The fact that this relationship was only found with I-AUC, the dynamic measure, and not with our two static measures demonstrates the importance of assessing insulin resistance dynamically, particularly when assessing its relationship with
adiponectin. Overall, our data support a strong negative relationship between adiponectin levels and insulin resistance in women with PCOS.

In addition to the inconsistencies in the relationship between adiponectin and insulin resistance in women with PCOS, adiponectin’s relationship with obesity markers is also uncertain. In the general population, it is well established that there is a strong negative correlation between adiponectin and BMI. Although some data support this relationship in women with PCOS (17, 46, 48, 98), others do not (97). Our data show that there are no significant relationships between adiponectin and BMI, waist circumference, or percent body fat, although there was a trend towards significance for all three.

**Visfatin**

The mean visfatin concentration for our sample was 17.6 ± 3.5 ng/mL. The concentrations reported in the PCOS literature are wide ranging, with values as low as 30.2 ± 10.4 ng/mL (103) and as high as 73.35 ± 11.54 ng/mL (84) being described. Our mean was noticeably lower than those previously reported.

Visfatin has been shown to have insulin-like action, thus a relationship with insulin resistance has been proposed. Our data support a strong relationship between visfatin and insulin resistance as shown by the significant correlations with fasting insulin (r = .661), HOMA (r = .714) and I-AUC (r = .775). Kowalska et al. found similar results for a significant relationship when measuring insulin resistance using a euglycemic hyperinsulinemic clamp (61), with a significant correlation of r = -0.27, but this has not been shown in all studies (45, 103). These studies that did not support a relationship did
not use a dynamic measure of insulin resistance, possibly playing a role in the lack of relationship found.

Our data support higher levels of visfatin in insulin resistant women with PCOS and lower levels of visfatin in women with lesser degrees of insulin resistance. Interestingly, Haider et al. reported that insulin had an inhibitory effect on visfatin, while glucose had a stimulatory effect, in their small sample of healthy, young adult males (49). This is noteworthy, as women with PCOS often have increased insulin levels, but normal glucose levels. It is possible that the dysregulation of glucose metabolism seen in women with PCOS has the potential to hinder this preventative effect of insulin (61). There are various rationales for why the positive relationship between visfatin and insulin resistance exists in women with PCOS. It has been suggested that visfatin concentrations may be increased in an attempt to compensate for the insulin resistance that is occurring, as well as to avoid additional increases in insulin resistance (61, 103). Additionally, it is possible that visfatin is involved in autocrine and paracrine processes of insulin sensitivity in addition to endocrine processes (103).

Visfatin was originally thought to have been produced by visceral fat, and therefore, proportional to visceral fat, though there is a lot of discrepancy in the literature regarding visfatin’s relationship to both visceral fat and subcutaneous fat, as well as other markers of obesity. Our data have added to the equivocal nature, as there was not a significant relationship between visfatin and BMI, waist circumference, or percent body fat, although there was a trend towards significance for percent body fat. Gen et al. (45) and Tan et al. (103) support our findings of a lack of relationship between BMI and/or waist circumference, but Kowalska et al. (61) found both of these obesity markers to be
associated with visfatin concentration ($r = .23$ and $r = .24$, respectively) in their sample of lean and obese women with PCOS. Interestingly, although a relationship was found for BMI and waist circumference, there was not for percent body fat (based on bioelectric impedance analysis), making their results entirely different than those found in our sample.

Waist circumference has been used as a rough estimation of visceral fat, as the abdomen is where visceral fat was originally thought to increase with obesity. Our data do not show any trend for a correlation between visfatin and waist circumference, therefore we conclude that the visceral fat in the abdomen must not be the greatest contributor of visfatin concentration. Percent body fat showed a trend for significance in our sample, therefore we presume that it is an increase in total fat that can lead to an increase in visfatin concentration, although the bioelectric impedance analysis is not specific enough to determine visceral versus subcutaneous fat.

**Multiple Regression**

The Adjusted R Square for our model signifies that 76.6% of the variance in I-AUC is associated with visfatin and adiponectin concentration. Additionally, it was determined that visfatin was the most significant contributor, with a one ng/mL increase in visfatin leading to an $850.211 \mu$IU/mL increase in I-AUC. Adiponectin also plays a significant role, as a one $\mu$g/mL decrease in adiponectin contributes to a $413.422 \mu$IU/mL increase in I-AUC in our sample.
**STUDY LIMITATIONS**

A few limitations existed based on our sample. The data used in these analyses were from a pilot study, therefore our sample was quite small (n=11), and there was not a control group for comparison purposes. It is necessary to assess the relationships found in this sample in a larger sample to determine if they still retain significance. Additionally, our small sample may not have included enough women with a high degree of insulin resistance, ultimately affecting our results. Another limitation based on our sample is that participants were recruited based on chart reviews, therefore our study staff did not verify diagnosis based on the Rotterdam Criteria. It is possible that some of these women did not truly meet the criteria for PCOS, or had been diagnosed long before the study, possibly affecting our results. Lastly, our sample primarily consisted of obese women. It would be advantageous to have more normal-weight women in order to assess the relationship between insulin resistance and adipokines across a wider span of obesity.

From a testing standpoint, there are two potential limitations. First, although an OGTT is a dynamic measure of insulin resistance, two hours of monitoring may not be enough to fully identify those women with impaired glucose metabolism. A three-hour OGTT has been shown to be more sensitive in identifying insulin resistance in women with PCOS (43). Second, bioelectric impedance analysis in some populations and conditions may not be comparable to underwater weighing. Assessing body composition using Dual X-Ray Absorptiometry would be more valid and reliable.
IMPLICATIONS

PCOS is possibly the most common endocrine disorder in premenopausal women (16), ultimately leading to numerous metabolic and other health-related consequences for those affected. Albeit common, the pathogenesis is unknown, making it difficult to identify effective treatment. Ineffective and/or incomplete treatment ultimately continues to leave these women at risk for larger problems such as diabetes and cardiovascular disease.

Results from our study demonstrate a relationship between insulin resistance and two adipokines: adiponectin and visfatin. It is possible that dysregulated adipokine concentration and metabolism are playing a role in PCOS-related insulin resistance, although temporal sequence can not be determine from our cross-sectional analysis. Further research studying the effect of alterations of these adipokines should be assessed with respect to insulin resistance in women with PCOS. If adiponectin and visfatin were found to be true predictors of insulin resistance in women with PCOS, physicians would have an effective screening method in order to identify insulin resistance at its early stages in these women, thus being able to treat it and prevent its further contribution to PCOS. On a broader level, the potential identification of the pathogenesis of insulin resistance and PCOS as a whole would eventually lead to improved treatment for the syndrome in general, ultimately decreasing the occurrence of numerous metabolic consequences associated with this syndrome, thus preventing the increased risk for cardiovascular disease and diabetes found in women with PCOS.
REFERENCES


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APPENDIX A

WAKE FOREST UNIVERSITY and
WAKE FOREST UNIVERSITY SCHOOL OF HEALTH SCIENCES
Influence of Exercise Training on Adipokines and Steroid Hormones in Women
with Polycystic Ovarian Syndrome

INFORMED CONSENT

INVESTIGATORS:

Gary Miller, PhD
Associate Professor
Health and Exercise Science
Wake Forest University

Kristen Gill Hairston, MD
Assistant Professor
Internal Medicine
Section of Endocrinology and Metabolism
Wake Forest University School of Medicine

You are invited to be in a research study. Research studies are designed to gain scientific knowledge that may help other people in the future. You are being asked to take part in this study because you have been diagnosed with Polycystic Ovarian Syndrome (PCOS). Your participation is voluntary. Please take your time to make your decision, and ask your study doctor or the study staff to explain any words or information that you do not understand. You may also discuss the study with your friends and family.

WHY IS THIS STUDY BEING DONE?
The purpose of this research study is to observe if an exercise training program affects levels of hormones and lipids in the blood of women with PCOS. Certain hormones like estradiol, along with those related to body fat and inflammation in your body are considered important in several conditions, including PCOS. We know that exercise training can be good for many aspects of your health, including heart disease, weight loss, and diabetes, but we are not sure if exercise affects hormones in women with PCOS.

HOW MANY PEOPLE WILL TAKE PART IN THE STUDY?
30 women will be participating in this study.

WHAT IS INVOLVED IN THE STUDY?
For your first visit you will meet with the research staff and participate in initial screening and consenting. This will take about 2½ hours to complete. If you are pregnant, you will not be able to participate in the study. If you meet all of the eligibility requirements, you will be scheduled to go to the General Clinical Research Center (GCRC) of Wake Forest University Health Sciences (WFUHS) located at the North Carolina Baptist Medical Center during a morning time. You will be asked to fast for 12 hours (not have anything to eat or drink, besides water) before this visit. About 2 tablespoons of blood will be taken from a blood vessel in your arm at this time. This blood will be used to measure levels of hormones, like estrogen and testosterone, and to determine the amount of cholesterol and triglycerides in your blood. You will have your height and weight determined, along with your waist and hips with a measuring tape. A bioelectrical
impedance assessment (BIA) will also be performed. BIA is used to determine the amount of body fat in an individual. For this test, you will lie down on an exam table. Small electrodes will be placed on your hands and feet. A very small electrical current will pass through your body. The current is so small that you will not be able to feel it. The speed at which the current goes through your body is related to the amount of body fat you have. Your blood pressure will also be tested at this visit. You will also have a glucose tolerance test during your visit. For this, you will consume a syrupy drink containing sugar and have about 1½ teaspoons of blood drawn from a blood vessel in your arm a total of 2 times over a 2 hour period while you sit and relax. Information about your diet will also be obtained. For this, a Registered Dietitian will teach you how to keep a four-day food record. You will write down what you eat and drink for four days and return the record to the GCRC. You will be asked to complete a questionnaire about your current physical activity level. Your medical history will be obtained from patient records at the Endocrine Clinic.

At the end of this testing period, you will be randomized, like the flipping of a coin, into either an exercise training group or a healthy lifestyle control group. If you are randomized into the exercise training group you will take part in an ongoing community exercise program that is run through Wake Forest University called HELPS. This program takes place in the Clinical Research Center, which is located next to Groves Stadium. You will have an exercise program tailored specifically for you that is comprised of both aerobic (walking or stationary cycling) and strength training. You will attend this program 3 days a week and also be encouraged to exercise at least 2 additional days outside the facility. During the exercise training program, daily monitoring will include attendance, as well as heart rate, blood pressure, distance walked (minutes on stationary cycle), muscle strength, and perceived effort for the day.

If you are randomized into the healthy lifestyle group you will be asked to maintain your current level of physical activity over the next 3 months. At the end of the three months you will be given the option to perform an identical training program as the exercise group received for the previous 3 months.

At the end of the first 3 months, you will undergo a similar set of tests to that measured during the first visit.

**HOW LONG WILL I BE IN THE STUDY?**
If you are randomized to the exercise training group, you will be in the study for 3 months. If you are randomized to the healthy lifestyle group, you may be in the study up to 6 months if you elect to receive the 3 month training program following the 3 month testing point.

You can stop participating at any time. If you decide to stop participating in the study we would like you to talk to the investigators or study staff first to learn about any possible health or safety issues.

**WHAT ARE THE RISKS OF THE STUDY?**
Being in this study involves some risk to you. You should discuss the risk of being in this study with the study staff. Risks and side effects related to this study include:
There may be muscle or joint soreness following the exercise. There is a chance that your arm may become bruised or have an infection after blood draws. These symptoms usually get better quickly and are usually not serious.

There also may be other side effects that we cannot predict. You should tell the research staff about all the medications, vitamins and supplements you take and any medical conditions you have. This may help avoid side effects, interactions and other risks.

The risk of harm or discomfort that may happen as a result of taking part in this research study is expected to be only slightly more than in daily life or from routine physical exercise. You should discuss the risk of being in this study with the study staff.

Taking part in this research study may involve providing information that you consider confidential or private. Efforts, such as coding research records, keeping research records secure and allowing only authorized people to have access to research records, will be made to keep your information safe.

ARE THERE BENEFITS TO TAKING PART IN THE STUDY?
If you agree to take part in this study, there may or may not be direct benefit to you. It is well known that exercise is beneficial in many ways and could be a key component in preventing a wide range of chronic diseases. It is possible that you may lose weight as a result of this study. We hope the information learned from this study will benefit other people in the future.

WHAT OTHER CHOICES ARE THERE?
This is not a treatment study. Your alternative is not to participate.

WHAT ABOUT THE USE, DISCLOSURE AND CONFIDENTIALITY OF HEALTH INFORMATION?
By taking part in this research study, your personal health information, as well as information that directly identifies you, may be used and disclosed. Information that identifies you includes, but is not limited to, such things as your name, address, telephone number, and date of birth. Your personal health information includes all information about you which is collected or created during the study for research purposes. It also includes your personal health information that is related to this study and that is maintained in your medical records at this institution and at other places such as other hospitals and clinics where you may have received medical care. Examples of your personal health information include your health history, your family health history, how you respond to study activities or procedures, laboratory and other test results, medical images, and information from study visits, phone calls, surveys, and physical examinations.

Your personal health information and information that identifies you ("your health information") may be given to others during and after the study. This is for reasons such as to carry out the study, to determine the results of the study, to make sure the study is being done correctly, to provide required reports and to get approval for new products.

Some of the people, agencies and businesses that may receive and use your health information are the research sponsor; representatives of the sponsor assisting with the research; investigators at other sites who are assisting with the research; central
laboratories, reading centers or analysis centers; the institutional review board; representatives of Wake Forest University Health Sciences and North Carolina Baptist Hospital; representatives from government agencies such as the Food and Drug Administration (FDA), the Department of Health and Human Services (DHHS) and similar agencies in other countries.

Some of these people, agencies and businesses may further disclose your health information. If disclosed by them, your health information may no longer be covered by federal or state privacy regulations. Your health information may be disclosed if required by law. Your health information may be used to create information that does not directly identify you. This information may be used by other researchers. You will not be directly identified in any publication or presentation that may result from this study.

If this research study involves the treatment or diagnosis of a medical condition, then information collected or created as part of the study may be placed in your medical record and discussed with individuals caring for you who are not part of the study. This will help in providing you with appropriate medical care. In addition, all or part of your research related health information may be used or disclosed for treatment, payment, or healthcare operations purposes related to providing you with medical care.

Laboratory test results and other medical reports created as a result of your participation in the research study may be entered into the computer systems of Wake Forest University Health Sciences and North Carolina Baptist Hospital. These will be kept secure, with access to this information limited to individuals with proper authority, but who may not be directly involved with this research study.

When you sign this consent and authorization form you authorize or give permission for the use of your health information as described in the consent form. This authorization does not have an expiration date. You can revoke or take away your authorization to use and disclose your health information at any time. You do this by sending a written notice to the investigator in charge of the study at the following address:

Gary D. Miller, PhD, RD
Associate Professor of Health and Exercise Science
P.O. Box 7868
Winston-Salem, NC 27109
(Office: 758-1901, Fax: 758-4680; E-mail: millergd@wfu.edu)

If you withdraw your authorization you will be removed from this study. If you withdraw your authorization, no new health information that identifies you will be gathered after that date. Your health information that has already been gathered may still be used and disclosed to others. This would be done if it were necessary for the research to be reliable. You will not have access to your health information that is included in the research study records until the end of the study.

**WHAT ARE THE COSTS?**
There are no costs to you for taking part in this study. All the study costs, including any study procedures related directly to the study, will be paid for by the study. Costs for your regular medical care, which are not related to this study, will be your own responsibility.
**WILL YOU BE PAID FOR PARTICIPATING?**
You will receive no payment or other compensation for taking part in this study.

**WHO IS SPONSORING THIS STUDY?**
This study is being sponsored by the Departments of Health and Exercise Science and Endocrinology of Wake Forest University. The researchers do not hold a direct financial interest in the sponsor or the product being studied.

**WHAT ARE MY RIGHTS AS A RESEARCH STUDY PARTICIPANT?**
Taking part in this study is voluntary. You may choose not to take part or you may leave the study at any time. Refusing to participate or leaving the study will not result in any penalty or loss of benefits to which you are entitled. If you decide to stop participating in the study we encourage you to talk to the investigators or study staff first to learn about any potential health or safety consequences. The investigators also have the right to stop your participation in the study at any time. This could be because you become pregnant during the study period or you are unwilling or unable to undergo testing procedures at follow-up visits. You will be given any new information we become aware of that would affect your willingness to continue to participate in the study.

**WHOM DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?**
For questions about the study or in the event of a research-related injury, contact the study investigator, Gary Miller at (336) 758-1901, Kristen Hairston at (336) 713-7251 or after hours at (336) 764-8904.

The Institutional Review Board (IRB) is a group of people who review the research to protect your rights. If you have a question about your rights as a research participant, you should contact the Chairman of the IRB at (336) 716-4542.

You will be given a signed copy of this consent form.

**Signatures**
I agree to take part in this study. I authorize the use and disclosure of my health information as described in this consent and authorization form. If I have not already received a copy of the Privacy Notice, I may request one or one will be made available to me. I have had a chance to ask questions about being in this study and have those questions answered. By signing this consent and authorization form, I am not releasing or agreeing to release the investigator, the sponsor, the institution or its agents from liability for negligence.

________________________    ______________________
Subject Name (Printed)    Time

________________________    ______________________
Subject Signature    Date

________________________    ______________________
Person Obtaining Consent    Date
# APPENDIX B – Individual Participant Data

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SCHOLASTIC VITA

JUDITH ANN CUMMINGS

Birthplace: Danbury, Connecticut
Birthdate: November 30, 1986

UNDERGRADUATE STUDY

2004 - 2008
Auburn University
Auburn, Alabama
B.S., Kinesiology

GRADUATE STUDY

2008 - 2010
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M.S., Health and Exercise Science
Thesis: The Association of Adipokines and Insulin Resistance in Women with Polycystic Ovarian Syndrome

PROFESSIONAL EXPERIENCE

2009-2010
Study Coordinator, Exercise Interventionist
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Winston-Salem, North Carolina

2009-2010
New Patient Coordinator
Healthy Exercise & Lifestyle Programs
Wake Forest University
Winston-Salem, North Carolina

2008-2010
Exercise Leader, Exercise Testing Assistant
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Wake Forest University
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2008
Intern
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2006
Wellness Coach
Alpharetta Family YMCA
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CERTIFICATIONS

Certified Clinical Exercise Specialist, American College of Sports Medicine
Certified Health Fitness Specialist, American College of Sports Medicine
Certified Personal Trainer, National Strength and Conditioning Association
Healthcare Provider BLS, American Heart Association

PROFESSIONAL ORGANIZATIONS

American College of Sports Medicine