THE IMPACT OF LIFESTYLE ON THE REPRODUCTIVE, METABOLIC, AND PSYCHOLOGICAL WELL-BEING OF WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

by

Dylan Cutler

B.Sc., St. Lawrence University, 2012

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Reproductive and Developmental Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

May 2019

The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, the dissertation entitled:

THE IMPACT OF LIFESTYLE ON THE REPRODUCTIVE, METABOLIC, AND PSYCHOLOGICAL WELL-BEING OF WOMEN WITH POLYCYSTIC OVARY SYNDROME (PCOS)

submitted by Dylan Cutler in partial fulfillment of the requirements for

the degree of Doctor of Philosophy

in Reproductive and Developmental Sciences

Examining Committee:

Dr Anthony Cheung
Co-supervisor

Dr Mohamed Bedaiwy
Co-supervisor

Dr Lori Brotto
Supervisory Committee Member

Dr Timothy Rowe
University Examiner

Dr Yvonne Lamers
University Examiner

Additional Supervisory Committee Members:

Dr Sheila Pride
Supervisory Committee Member

Supervisory Committee Member

Supervisory Committee Member

Abstract

Introduction

Polycystic ovary syndrome (PCOS) affects the reproductive, metabolic, and psychological health of 6 to 18% of women worldwide. The impact of lifestyle is poorly understood in research and practice. This dissertation aims to elucidate how dietary intake, physical activity, and psychological well-being relate to the array of symptoms and characteristics of PCOS.

Methods

Women diagnosed with PCOS were compared to women without PCOS with subfertility in four observational studies. Data collected included: dietary intake, physical activity, psychological well-being symptoms (depression, anxiety, stress, and quality of life), anthropometrics, metabolic and reproductive hormonal assays. A protocol for a randomized controlled trial involving a lifestyle intervention is presented.

Results

Women with PCOS had similar caloric intake and physical activity as women without PCOS, despite being more overweight ($P < 0.001$). In women with PCOS, those with insulin resistance (IR) consumed less fiber ($P < 0.05$), a greater glycemic load ($P = 0.03$) and less magnesium ($P < 0.05$) than without IR. Fiber intake was negatively correlated with IR (rho = -0.35, $P < 0.005$), fasting insulin (rho = -0.37, $P < 0.005$), glucose tolerance (rho = -0.23, $P < 0.05$), testosterone (rho = -0.35, $P < 0.005$), and dehydroepiandrosterone sulfate (rho = -0.27, $P = 0.02$). Magnesium intake was negatively correlated with IR (rho = -0.32, $P < 0.01$), C-reactive protein (rho = -0.47, $P < 0.001$), and testosterone (rho = -0.30, $P < 0.01$), but positively correlated with HDL cholesterol (rho = 0.29, $P = 0.01$). Symptoms of anxiety were significantly

higher in women with PCOS, particularly those with hyperandrogenism ($P < 0.01$). In women with PCOS and increased depressive symptoms, vitamin D intake was significantly decreased ($P < 0.02$).

Conclusions

While caloric intake of women with PCOS could not explain obesity, increasing fiber and magnesium intakes may reduce IR, hyperandrogenemia, and dyslipidemia. Women with PCOS, particularly hyperandrogenic phenotypes, experience increased symptoms of anxiety potentially related to hirsutism, which challenges societal expectations of women's appearance. A lifestyle focused on a high fiber, low glycemic diet, supplementation, physical activity, and managing emotional distress may improve some symptoms of PCOS.

Lay Summary

Polycystic ovary syndrome (PCOS) is a multisystem disorder that impacts the reproductive, metabolic, psychological, and social facets of women's lives. While management has typically focused on targeting physical symptoms, a relative lack of attention on lifestyle factors may likely be thwarting the overall well-being of women with PCOS. This dissertation assesses dietary intake and identifies nutritional insufficiencies, including decreased fiber and magnesium intakes, which may be exacerbating levels of insulin, androgens, and lipids in women with PCOS. Secondly, this thesis identifies that symptoms of depression, anxiety, and stress are increased in women with PCOS. Some physical and social explanations for increased anxiety in women with hyperandrogenic PCOS are presented. Thirdly, relationships between nutrient intake and psychological well-being are assessed. Lastly, a lifestyle-based intervention is proposed for improving ovulation and quality of life by incorporating a low glycemic diet, physical activity, stress management, and myo-inositol.

Preface

Dylan Cutler (DA Cutler) participated in the design of all studies for this dissertation, the recruitment and research follow-up of over 300 participants, and was instrumental in data collection, initial data analysis and interpretation, and manuscript draft preparation.

The work presented in this dissertation has led to the following publications and/or manuscripts in preparation:

1. **Cutler DA**, Pride SM, Cheung AP. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12. Published.

This work is associated with Chapter 2. DA Cutler participated in the study design, was instrumental to participant recruitment and follow-up, the collection of nutritional data, analyses, initial interpretation of results and write-up of the manuscript. SM Pride contributed to the study design and critically reviewed the manuscript. AP Cheung conceived the study and was responsible for clinical management, methodology, and reviewing the final manuscript.

2. **Cutler DA**, Cheung AP. Anxiety is associated with hyperandrogenism in women with polycystic ovary syndrome: a cohort study. Manuscript in preparation.

This work is associated with Chapter 3. DA Cutler contributed to the study design, was instrumental in participant recruitment and follow-up, the collection of psychological data,

analysis of data, initial interpretation of results and the write-up of the manuscript. AP Cheung also designed the study, reviewed and critiqued results and analyses, and reviewed the final manuscript.

3. **Cutler DA**, Shaw AK, Pride SM, Bedaiwy MA, Cheung AP. A randomized controlled trial comparing lifestyle intervention to letrozole for ovulation in women with polycystic ovary syndrome: a study protocol. *Trials*. 2018 Dec;19(1):632. Published.

This work is associated with Chapter 4. DA Cutler collaborated with AP Cheung, AK Shaw and Dr. Bronwyn Williams to design this trial. DA Cutler played an important role in developing the 12-week intervention and was instrumental in developing the wellness book for the intervention group. She also conducted analyses for power and sample size calculations, literature review, and prepared the manuscript. SM Pride and MA Bedaiwy provided advice, support and assistance. AP Cheung was responsible for clinical management, methodology, and revisions.

Ethics approvals for these studies were obtained from the University of British Columbia Children's and Women's Research Ethics Board in Vancouver, Canada (certificate numbers: H13-02964 and H16-01388). The work in Chapter 4 was registered as a clinical trial at ClinicalTrials.gov (NCT02630485). Written informed consent was obtained from each participant (Appendix A). All identifiable information was obscured through attribution of identification numbers to ensure confidentiality.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

AE-PCOSS: Androgen Excess & PCOS Society

ASRM: American Society for Reproductive Medicine

BMI: body mass index

CC: clomiphene citrate

COCP: combined oral contraceptive pill

CRP: C-reactive protein

CVD: cardiovascular disease

DHEA-S: dehydroepiandrosterone sulfate

ED: eating disorder

ESHRE: European Society of Human Reproduction and Embryology

FSH: follicle-stimulating hormone

GL: glycemic load

hCG: human chorionic gonadotropin

HOMA-IR: homeostasis model of assessment for insulin resistance

HPA: hypothalamic-pituitary-adrenal

IR: insulin resistance

IR-PCOS: insulin resistant polycystic ovary syndrome

IVF: in-vitro fertilization

LH: luteinizing hormone

mFG: modified Ferriman-Gallwey

NAFLD: nonalcoholic fatty liver disease

OGTT: oral glucose tolerance test

OHSS: ovarian hyperstimulation syndrome

PCO: polycystic ovaries

PCOS: polycystic ovary syndrome

SHBG: sex hormone-binding globulin

T2D: type 2 diabetes

WHR: waist to hip ratio

17-OHP: 17-hydroxyprogesterone

Acknowledgements

First and foremost, I must thank each participant for graciously contributing to the advancement of our understanding of PCOS. I am grateful for their time they generously devoted and feel fortunate for our meaningful interactions.

I would like to thank my supervisor, Dr Anthony Cheung, for his mentoring which allowed me to grow into the scientist I am today. I appreciate the staff at the Grace Fertility Centre who have generously welcomed and encouraged me. I would also like to thank my co-supervisor, Dr Mohamed Bedaiwy, and my committee, Drs Sheila Pride, Lori Brotto, and Rajavel Elango, for their feedback and wisdom throughout my degree.

I am grateful to have received the Four Year Doctoral Fellowship, from the University of British Columbia, which made this research possible.

With my utmost appreciation I must thank my family whose emotional support has been unwavering. To my mother, an inspiration who has defied the glass ceiling and continues to make waves all while supporting me with her positive reinforcement and belief. To my father, whose calm, rational mind continuously reminds me to focus on the big picture. Finally, to my sister who empathetically listens and holds me through storms then rejoices with me once they pass.

Thank you to my lovely friends for your fierce compassion and understanding through this journey. Lastly, thank you to my colleagues at WAVAW for providing a haven.

Dedication

This dissertation is dedicated to every woman and girl affected by PCOS. Your strength and resilience inspire me. I believe the array of struggles for women living with PCOS merit more awareness and deserve greater development of comprehensive treatment options.

Chapter 1: Literature Review

1.1 Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous, multisystem disorder that affects between 6 to 18% of women of reproductive age, resulting in the most common female endocrine disorder (1, 2). Currently, the clinically accepted inclusion criteria for the diagnosis of PCOS are the presence of at least two of the following three features (after the exclusion of other aetiologies): oligo-ovulation or anovulation, hyperandrogenism, and/or polycystic ovaries upon ultrasound assessment (3). Co-morbid conditions often associated with PCOS include obesity, dyslipidemia, insulin resistance (IR), decreased mental health, and long-term health risks, such as type 2 diabetes (T2D), cardiovascular disease (CVD) as well as an increased risk of endometrial cancer (4-6).

Despite that PCOS is the most common endocrine disorder, it remains poorly defined which has generated much confusion and uncertainty among scientists, clinicians, and patients (7). PCOS has been said to be "one of the biggest challenges in medicine" due to its intricacy, ability to progress, and lifelong implications for women, yet up to 70% of affected women remain undiagnosed (8-10). Several experts agree that "the needs of women with PCOS are not being adequately met and evidence-based practice gaps persist" (11). Research and clinical management have primarily focused on the treatment of the physical symptoms of PCOS using pharmaceuticals with varied results. A lack of attention to lifestyle factors might be hindering the long-term well-being of those affected by PCOS. Further, women with PCOS are commonly classified and treated as a single group while it is evident that PCOS is, in fact, a spectrum disorder with distinct phenotypes. A four-tier tailored approach to lifestyle in the form of

nutrition, physical activity, stress management, and dietary supplementation, may add significant benefit to current management, particularly for more severe PCOS phenotypes. This dissertation expands the existing knowledge of lifestyle's impact on the reproductive, metabolic, and psychological well-being of women with PCOS. In addition, it analyses how phenotypic subgroups are differentially impacted by lifestyle.

This first chapter is a summary of the literature pertaining to the experimental studies presented in Chapters 2 and 3, and the randomized controlled trial discussed in Chapter 4.

1.2 PCOS Diagnosis and Management

1.2.1 Historical Evolution of Knowledge

In 1935, PCOS was described by Stein and Leventhal as a "gynecological curiosity" which they observed in seven women (and was initially coined the Stein-Leventhal syndrome) (7, 12-14). These women presented with amenorrhea, hirsutism, and enlarged ovaries containing several small fluid-filled sacs and thickened tunica (15). Stein and Leventhal performed ovarian wedge resections on these seven women, and subsequently on an additional 75 women with similar symptoms, reporting a rate of 90% return of regular menstruation (16). There is evidence of PCOS being described even earlier when in 1721, Vallisneri, an Italian medical scientist wrote ''Young married peasant women, moderately obese and infertile, with two larger than normal ovaries, bumpy, shiny and whitish, just like pigeon eggs.'' (7, 17, 18). While the ovarian appearance was the main indicator of PCOS in these early centuries, this began to shift in the 1970s and 1980s when elevated serum levels of luteinizing hormone (LH) and testosterone were required for PCOS diagnosis (19, 20). In the 1980s, an elevated LH to follicle-stimulating hormone (FSH) ratio was added to diagnostic criteria but was then disregarded in the 1990s (7). New ultrasound technology provided a non-invasive diagnostic tool to observe the size and

morphology of the ovaries. Swanson et al first described the ultrasound appearance of polycystic ovaries and shortly thereafter Adams et al presented specific diagnostic ultrasound criteria to be used, which has remained consistent to this day (7, 21, 22). These criteria require more than 12 follicles of less than 10 millimeters around an enlarged stroma using either transabdominal or transvaginal ultrasound assessments (7).

1.2.2 The Diagnosis Debate

While women have seemingly been suffering from PCOS for centuries, only in 1990 was a set of diagnostic criteria proposed at a National Institutes of Health (NIH) conference which became widely accepted in research and clinical communities. These criteria became known as the "NIH criteria" (23, 24). These guidelines required that a patient presents with both hyperandrogenism (and/or hyperandrogenemia) and anovulation (oligo- or anovulation) while excluding other aetiologies producing similar symptoms such as androgen excess and anovulation. However, these criteria excluded women that may have normal serum androgen levels or those that ovulate regularly but have features of polycystic ovarian morphology on ultrasound examination. Therefore, in 2003, the European Society for Human Reproduction and Embryology (ESHRE) with the American Society for Reproductive Medicine (ASRM) met and an expert consensus formulated the "Rotterdam criteria" which required a patient to have at least two of the following three criteria: oligo-anovulation, hyperandrogenism and polycystic ovary morphology on ultrasound assessment (3). Hyperandrogenism can present biochemically (increased blood androgens) and/or physically (hirsutism or increased facial or body hair, acne, or alopecia). The Rotterdam criteria have been criticized as being too broad with the inclusion of more mild phenotypes of PCOS. As a result, in 2006, the Androgen Excess Society decided that hyperandrogenemia or hirsutism is an essential criterion for a PCOS diagnosis (in combination

with oligo-anovulation and/or polycystic ovary morphology). The “AE-PCOS criteria” eliminates milder phenotypes and places emphasis on hyperandrogenism as the main feature of PCOS (7). An important aspect of all three of these criteria is that non-classical congenital adrenal hyperplasia, androgen-secreting tumors, hyperprolactinemia, abnormal thyroid function, and Cushing’s syndrome must be ruled out. Currently, one set of diagnostic criteria has not been widely accepted for consistent use globally. Therefore, when one is evaluating current literature on PCOS, attention to the diagnostic criteria used is important as differing inclusion criteria can greatly impact the analysis and conclusions.

1.2.3 A Spectrum Disorder

Balen et al presented the idea of PCOS as a spectrum disorder in 1995 after observing 1741 patients with PCO morphology (PCOM) on ultrasound (7, 25). The spectrum of findings ranged from mild where women may ovulate, are normo-androgenic, but have PCOM on ultrasound, to severe where women have oligo-anovulation, hyperandrogenism, acne, crown pattern baldness, and insulin resistance. The authors concluded that genetic factors are likely to dictate where a woman falls on this spectrum. However, positioning on the continuum may be more related to BMI or lifestyle factors.

1.3 Theories of Origin

The primary cause of PCOS is unknown, but there are many theories, which have been briefly summarized in Figure 1.1. As previously discussed, the lack of global agreement regarding PCOS diagnostic criteria remains a significant obstacle for research of this syndrome. Without consistent diagnostic criteria, literature is too heterogeneous to draw any definitive conclusions. In addition, the aetiology and complexity of the disorder bring further difficulties. Nonetheless, it is likely that the development of PCOS, and its various phenotypes, is best

understood as a complex "interaction between genetic, environmental, behavioral and psychological factors" (8).

1.3.1 Genetic Origins

While literature indicates that PCOS is commonly found among female relatives of women with PCOS, a hereditary pattern has yet to be elucidated (26). Although it is possible that autosomal dominant transmission occurs due to a single gene abnormality, it is more likely that several genes are involved and interact with environmental factors, resulting in multiple phenotypes of PCOS (26, 27). Candidate genes at up to 22 loci have been identified as being either up or down-regulated, but these have yet to be confirmed (28-30). These genes encode for a variety of biological processes such as ovary and adrenal steroidogenesis, insulin metabolism, energy regulation, inflammation and folliculogenesis (27, 29). Large genome-wide association studies, with results that have been replicated in other populations, have identified the following gene candidates: follicle stimulating hormone receptor, luteinizing hormone/choriogonadotropin receptor, insulin receptor, and DENN domain containing 1A (31).

1.3.2 Epigenetic Origins

One theory, based on the Barker hypothesis, proposes that PCOS predisposition begins in utero when the fetal hypothalamic-pituitary-ovarian axis is exposed to high levels of androgens (32, 33). This concept is supported by studies in rhesus monkeys and sheep, as well as clinical evidence of PCO in pre-pubertal girls (27, 33, 34). Since the fetus is protected by placental aromatase activity and plasma binding proteins, this androgen exposure is unlikely from the mother. Rather, this androgen excess is likely the fetal ovary's response to maternal human chorionic gonadotropin (hCG) (33). This could also occur during infancy when hypothalamic-

pituitary secretion is activated (33). While Franks et al's hypothesis suggest that PCOS is based on genetic predetermination, other genetic and environmental factors tie in to result in different presentations, known as phenotypes, of PCOS. This developmental theory states that culprit genes are programmed by high androgen exposure in utero (28, 33, 34).

Additional factors in utero that can impact health later in life may also be at play. For example, both small and large for gestational age babies are at increased risk for metabolic conditions later in life, such as T2D and CVD. Fetal growth restriction increases the risk of IR while large for gestational age increases the risk for obesity and impaired glucose tolerance. Considering that these metabolic complications are associated with PCOS, as well as an increase in total body fat and visceral fat, it is possible that disturbances of fetal growth have an additive effect in predisposing infants to PCOS (27).

In adolescence when the hypothalamic-pituitary-ovarian axis is activated, symptoms of PCOS begin to present themselves. Metabolic changes occur, which leads to changes in body fat distribution. Insulin resistance is a feature of normal puberty and adolescence causing insulin levels to rise. Increased insulin levels can by itself or by synergizing with LH enhance ovarian theca cell androgen production. In turn, both increased circulation insulin and androgens have a decreases hepatic production of sex hormone-binding globulin (SHBG). Reduced circulating SHBG increases serum free testosterone which is more biologically available to tissues. Serum insulin levels may be increased as well due to genetic predisposition or related to body fat increase during this time frame, further contributing to PCOS (27, 33).

1.3.3 Gut Microbiome

The gut microbiome has been shown to be altered in women with obesity and metabolic disease (35). When this microbial imbalance (or dysbiosis) occurs, it may result in greater

intestinal permeability to gut lipopolysaccharides (LPS) which can enter the bloodstream and cause systemic low-grade inflammation (36). In PCOS, this could potentially result in an increase circulating insulin levels with an accompanying increase in testosterone production by the ovarian theca cells (37, 38). However, few studies have been conducted identifying the gut microbiota of women with PCOS. One study identified that women with IR-PCOS have greater dysbiosis than non-IR-PCOS (38). While only 25 women participated in this study (17 with PCOS and 8 without PCOS) this was the first study to identify gut microbiome differences in relation to IR in PCOS. Other literature has identified hyperandrogenism to be negatively correlated with alpha diversity in the gut microbiome (39). Since more research is needed on the link between PCOS and the gut, conclusions have yet to be made.

1.4 Prevalence of PCOS

The true prevalence of PCOS is frankly unknown. Reports vary depending on the diagnostic criteria used and the population studied. Worldwide estimates report between 6 to 18% of women have PCOS. In Canada, approximately 1.4 million women are affected and the economic burden of PCOS in the United States is an estimated $4.36 billion annually (13, 40). Most of these healthcare costs are due to treating T2D and menstrual irregularity. Adding to the complexity of establishing the prevalence of PCOS is the lack of public awareness (evidenced by lower prevalence rates reported in database studies than reported in community studies) resulting in many not seeking healthcare or going undiagnosed (41).

1.4.1 Ethnic Disparities

While PCOS affects women worldwide, some evidence suggests higher rates in specific ethnic groups (41). Unfortunately, a problem that frequently arises when conducting meta-

analyses on PCOS populations is the varied diagnostic criteria being used. The 2003 Rotterdam criteria are the broadest and therefore yields the highest rates of PCOS while the 1990 NIH criteria are the strictest. A systematic review and a meta-analysis were recently conducted which included 13 studies and categorized ethnic groups as follows: African American, European White, Chinese and Middle Eastern (41). In this meta-analysis, prevalence using the Rotterdam criteria was 5.6% (CI 4.4-7.3%) for Chinese women and 16.0% (CI 13.8-18.6%) for Middle Eastern women. With the NIH criteria, prevalence rates resulted with 5.5% (CI 4.8-6.3%) for European White women, 7.4% (CI 6.3-8.7%) for African American women and 6.1% (CI 5.3-7.1%) for Middle Eastern women. Lastly, using the 2006 AE-PCOSS criteria the estimated prevalence was 12.6% (CI 11.3-14.2%) for Middle Eastern women (41). These prevalence rates suggest that African American women have the highest prevalence of PCOS while Chinese women have the lowest.

Symptom severity can also vary according to ethnicity. A cohort study including 162 women with PCOS in New Zealand found that obesity and IR (along with other metabolic co-morbidities which increase the likelihood of CVD) and were more prevalent in Maori and Pacific Islander women (42). Hirsutism was more severe in Maori and European women while previous literature has shown Japanese women to be less hirsute, nonobese, and with lower fasting insulin levels in comparison to either American or Italian women with PCOS (43). Obesity is more prevalent in American women with PCOS than Italian women, however, this may partly be non-disease related given the higher obesity rates in the United States compared to Italy (44). In addition, American women had more severe IR and worse lipid profiles enhancing their CVD risk (44). Hispanic women with PCOS have three-fold higher odds of developing nonalcoholic

fatty liver disease (NAFLD) and have an increased prevalence of dyslipidemia and IR (45). Meanwhile, acne was not a major symptom for Pacific Islander women, but it was more prevalent in European White women when compared to African American women with PCOS (42, 46).

1.4.2 Misdiagnosed and Underdiagnosed

Dokras et al conducted an online survey to identify knowledge gaps among physicians managing PCOS (47). Questionnaires were emailed to 7,708 specialists consisting of gynecologists (ObGyns) and reproductive endocrinologists (REI-ObGyns) in the United States and Canada, and 630 of these physicians responded. The results indicated substantial knowledge deficiencies in several areas of PCOS-related care. For example, an alarming 68.3% of REI-ObGyns and 41.2% of ObGyns were unaware that the Rotterdam criteria are the current widely accepted criteria for diagnosing PCOS (which has been the case since 2006). Further, 27.7% of REI-ObGyn consultants were unable to report the diagnostic criteria they relied on to screen for patients for PCOS. These statistics explain frustrations expressed by women with PCOS who were either misdiagnosed or who waited more than two years and several doctor visits before receiving a PCOS diagnosis (48).

There is an on-going debate among physicians as to when a diagnosis of PCOS can be accurately made in the adolescent population (49). While early diagnosis can fast-track management and care, a wrong diagnosis can cause unnecessary psychological distress given the long-term implications of PCOS (ex. CVD, T2D, and infertility). Several of the clinical indicators suggesting PCOS are typical occurrences for girls in puberty, such as acne, increased terminal hair, and irregular cycles. In addition, a transvaginal ultrasound may not be feasible if

adolescents are not yet sexually active. Therefore, some suggest that clinicians wait two years post-menarche to establish the diagnosis of PCOS in an adolescent population (49, 50).

1.5 Renaming of PCOS

In 2012, the first Evidence-Based Methodology Workshop on Polycystic Ovary Syndrome was held in Maryland, United States hosted by NICHD and NIH Office of Disease Prevention. The purpose of this workshop was for independent experts to evaluate currently available evidence and to answer three questions: the first question was to clarify the pros and cons of the continued use of the Rotterdam criteria for diagnosis, the second to identify causes, predictors, long-term consequences related to PCOS, and finally to determine the best prevention and treatment strategies for this syndrome. A major consensus recommendation that resulted from this workshop was that PCOS receive a change of name. Its current name suggests that PCO morphology on ultrasound is a key factor. The danger with this assumption is that women with both chronic anovulation and hyperandrogenism are at risk of going undiagnosed. Further, this hyperandrogenic group is at greater risk for long-term health complications such as prediabetes, T2D, and CVD. PCOS is rarely recognized outside of obstetrics and gynecology. Dunaif and Fauser proposed a “two-state solution” to the naming debate (51). This classification would result in a new endocrine syndrome for chronic anovulation and hyperandrogenism. The other phenotypes with PCO morphology identified by ultrasound would continue to be called PCOS. The name ‘PCOS’, using the Rotterdam criteria, will continue to be used throughout this dissertation.

1.6 Phenotypic Differences

As suggested by the heterogeneity of patients included in the PCOS diagnosis, there are four major clinical phenotypes of PCOS (Table 1.1). Two of these four phenotypes are known to have long-term health risks due to metabolic disturbances (frank and classic PCOS), while the implications of the remaining two phenotypes are largely unknown (ovulatory and mild) (13). Previous research has failed to identify whether the various phenotypes of PCOS respond differentially to lifestyle changes.

1.7 Diagnosing Hyperandrogenism

Hyperandrogenism can be assessed clinically and biochemically, however, both have limitations. Clinical assessment includes evaluating cosmetic signs such as hirsutism, acne, and androgenic alopecia. Hirsutism is defined as terminal hairs growing in a "male-like" pattern and is assessed by the modified Ferriman-Gallwey (mFG) tool (10, 52). The mFG assesses nine body areas and gives visual representations with scores ranging from zero (no terminal hairs) to four (terminal hair consistent with a well-developed biological male) for each of the nine sites (10). While acne and androgenic alopecia can also be indicative of hyperandrogenism in women with PCOS, these are not used as diagnostic criteria for hyperandrogenism.

Biochemically, there are currently three main markers of interest: testosterone, androstenedione, and DHEA-S. In addition, 17-hydroxyprogesterone (17-OHP) is used to rule out an adrenal tumor or congenital adrenal hyperplasia. According to the most recent international guidelines for PCOS assessment, free testosterone provides the most accurate assessment of biochemical hyperandrogenism, followed by total testosterone, DHEA-S, and androstenedione (10). However, reliable direct assays for total or free testosterone do not exist.

Both direct radioimmunoassays and chemiluminescence immunoassays are not precise, sensitive or specific enough for use (10). Clinical laboratories can provide calculated bioavailable testosterone, calculated free testosterone and free androgen index. These tests should be performed during the early follicular phase (in cycling women) and preferably in the morning (10). DHEA-S and androstenedione can be slightly elevated in 40 to 70% of women with PCOS. The majority of DHEA-S is produced in the adrenal glands while androstenedione is released by both the adrenal glands and the ovaries.

1.8 Long-Term Health

A diagnosis of PCOS can have long-term health implications. A large population-based cohort study took place in Western Australia and included 2566 women with PCOS and 25,660 age-matched women without PCOS. The objective of the study was to determine the prevalence and reason for hospital admissions in these two groups. This study found that women with PCOS had significantly higher rates of obesity, T2D, hypertension, ischemic heart disease, asthma, stress/anxiety, depression, licit/illicit drug use, self-harm, and mortality. They were also more likely to be admitted to a hospital for menorrhagia, infertility, and miscarriage (53).

1.8.1 Obesity and Metabolic Syndrome

Obesity is reportedly the most concerning long-term health risk according to physicians, although interestingly, not necessarily the main concern for women seeking treatment for PCOS (47). Prevalence of overweight and obesity in PCOS is highly variable depending on age, ethnicity and geographic location but is estimated at 61% (95% CI: 54 - 68%) (54). The World Health Organization defines a person as obese if their body mass index (BMI) is greater than 30, while a BMI over 25 is classified as overweight. Obesity per se does not necessarily mean one is

in poor health, but obesity can increase health risks such as IR, dyslipidemia, metabolic syndrome, T2D, and CVD. For women with PCOS, obesity can further exacerbate hyperandrogenism and menstrual irregularities and a small reduction in body weight can both decrease androgen levels and induce regular cycling in some of these women (54, 55). Being overweight can also result in difficulty conceiving with reports that it may be up to three times less likely that a woman with obesity will conceive (56, 57). While the biological explanation for this association is not completely understood, some explanations include impaired ovarian follicular development, oocyte development, fertilization, and implantation (56, 58).

1.8.2 Adipose Tissue

The location of white adipose tissue may be more reflective of poor health than the total body fat mass (59). Central adiposity is a strong indicator of metabolic health, and therefore, waist-to-hip ratio (WHR) is a helpful tool for clinicians and researchers (60, 61). A WHR greater than 0.80 can increase the risk of metabolic disease in women. Further, visceral fat, as opposed to subcutaneous abdominal fat, is likely the most harmful in women (62). This layer has been hypothesized to release free fatty acids (FFAs) and inflammatory proteins which end up in the liver (60, 63). An increase in FFAs can prevent insulin from stimulating muscles to take up glucose while causing the liver to produce more glucose (60, 64). However, other factors may also be involved. For example, an increase in triglycerides in the liver must also be present for increased visceral adipose tissue to be linked with insulin resistance (60, 65). So, while it is known that visceral fat is associated with metabolic health complications, the biological pathway is yet to be determined.

1.8.3 Insulin Resistance

While obesity can contribute to IR, adverse metabolic health also affects women with PCOS who are not overweight or obese. This group of women is commonly referred to as 'lean PCOS'. Overall, IR affects about 65 to 70% of all women with PCOS and 20 to 25% of women with lean PCOS (66). IR can increase androgen levels both directly, via increased ovarian androgen production, and indirectly, by decreased SHBG production. Impaired glucose tolerance is increased in women with PCOS (OR = 3.26, 95% CI: 2.17-4.90), and varies based on ethnicity (Asian = 5 times more likely, American = 4 times more likely, and European = 3 times more likely) (67). When left unmanaged, impaired glucose tolerance can progress to T2D. A systematic review and meta-analysis found that the prevalence of T2D was increased in women with PCOS by almost three times (OR = 2.87, 95% CI: 1.44-5.72) (67).

1.8.4 Nonalcoholic Fatty Liver Disease

Women with PCOS are 2.54 times more likely to have NAFLD than women without PCOS (95% CI: 2.19–2.95). Reports indicate that between 40 to 55% of all women with PCOS have NAFLD and 17% of women with lean PCOS (68, 69). Unfortunately, only 41% of ObGyn/REI specialists are aware of this increased risk (47). Common features between NAFLD and PCOS are obesity, diabetes, IR and metabolic syndrome (70). Women with the hyperandrogenic phenotype are more likely to have NAFLD than normoandrogenic phenotypes, suggesting that androgen excess is an additional factor in the development of NAFLD (70).

1.8.5 Cardiovascular Disease

Women with PCOS may be at an increased risk for CVD. Several markers of CVD are present in women with PCOS such as altered lipid/glucose metabolism, hypertension, systemic

inflammation and vascular dysfunction (71). For example, dyslipidemia, is present in up to 70% of women with PCOS and it is independent of obesity, exercise, smoking, alcohol use and ethnicity (72). Some literature reports a four times higher likelihood of women with PCOS developing CVD, however, reports have been critiqued for being from a young population of women and therefore with a relatively low incidence of CVD (73, 74). The most recent meta-analysis concluded that Chinese women with PCOS have a significantly increased risk of coronary heart disease (75).

1.8.6 Cancer

The risk of endometrial cancer for women with PCOS is up to three times greater than women without PCOS (76, 77). Chronic anovulation, obesity, and hyperinsulinemia are all predisposing factors for endometrial carcinoma. When anovulation or oligo-ovulation is present, the endometrium is exposed to high levels of estrogen combined with low levels of progesterone. In addition, obesity can increase endometrial cancer risk by increasing aromatase activity resulting in higher levels of estrogen, and from hyperinsulinemia, thus increasing insulin-like growth factor 1 (IGF-1) (78). Insulin dysregulation is a factor in both PCOS and endometrial cancer. When endometrial cancer cells are exposed to IGF-1, their growth is fast-tracked (76, 79). There are limited data on the risk of cervical, breast and ovarian cancers in women with PCOS (80). However, women with PCOS, similarly to the general population, who have not used oral contraceptives for more than three months may be at increased risk for ovarian cancer (76).

1.8.7 Infertility

Infertility is present in approximately 70 to 80% of women with PCOS primarily due to anovulation (81). Anovulation in women with PCOS has not been fully elucidated and likely involves dysregulation at the hypothalamic-pituitary level as well as intraovarian factors. One mechanism may involve anti-mullerian hormone (AMH) as PCOS is associated with an increase in circulating AMH levels produced by ovarian antral follicles. This directly affects hypothalamic GnRH neuronal activity causing increased LH output by the pituitary (82). AMH is secreted by small antral follicles in the ovary and has an important local effect on follicle growth with reduction of granulosa cell sensitivity to FSH. In PCOS, the increased number of small antral follicles results from increased local levels of androgens. Thus follicular growth is dysregulated with failure to advance to follicle selection and the emergence of a dominant follicle to proceed to ovulation. Other contributory factors would include IR with increased compensatory insulin levels and their impact on theca cell androgen production. The ASRM defines infertility as greater than twelve months of regular, unprotected sexual intercourse (83). It is often only after seeking fertility support that women are diagnosed with PCOS (84). Infertility can be devastating for individuals and couples. Levels of anxiety and depression in infertile subjects have been shown to be like those with life-threatening conditions, such as cancer or the human immunodeficiency virus (85). Psychologically, infertility diagnoses can increase feelings of losing control over one's life (86, 87). Relationships between couples with infertility can become strained, as well as between friends and family of the individual or couple. Infertility can challenge self-assessed femininity or masculinity, and self-esteem (86). Women with infertility are more likely to suffer from depression, while men are likely to suffer in silence (87). Unfortunately, psychosocial support interventions are rarely offered by medical providers

(87). In addition, when women with PCOS conceive, they are at greater risk of complications (which will be discussed further in Section 1.9.4) (88).

1.8.8 Psychological Well-being

While it is becoming evident, through cross-sectional analyses, that women with PCOS are more likely to suffer from poor psychological health than other women, Dokras et al reported that a significant percentage of medical specialists are uninformed of this connection (47). While 50 to 85% of ObGyn and REI-ObGyn physicians were aware of increases in body dissatisfaction, depression, and lower quality of life in women with PCOS, only 24% and 50% (respectively) reported knowledge of the association between PCOS and anxiety. Therefore, the connection between psychological health and PCOS is yet to be acknowledged by the medical community, which may be leaving many women with PCOS feeling unsatisfied with the level of emotional support and resources offered (48).

The literature has reported increased levels of depression, anxiety, and stress, as well as a decreased quality of life, for women with PCOS compared to non-PCOS (5). A large cross-sectional study found significant differences in the prevalence of depression, anxiety and perceived stress when 478 women with PCOS were compared to 8134 women without PCOS (self-reported) (89). The prevalence of depression in women with PCOS was 27% while in women without PCOS it was 19%. Other literature has reported the prevalence of depression as high as 40% in women with PCOS, in comparison to 12% of women in the general population, of reproductive age (90, 91). Likewise, symptoms of anxiety occurred in 50% of women with PCOS while only 39% of women without PCOS. Anxiety disorders were found in 12% of women with PCOS (90). Women with PCOS may also experience greater levels of daily stress as

indicated in a study by Damone et al where perceived stress scores were significantly greater for women with PCOS (1.06 +/- 0.61) than women without PCOS (0.88 +/- 0.53) (89). This study used the Perceived Stress Questionnaire which scores the level of perceived stress out of 4 (89). These differences in depression, anxiety, and stress, remained significant after adjusting for BMI, infertility and socio-demographic factors (89). In summary, 56% of women with PCOS have been found to have some form of mood disorder (90). It has been reported that women with PCOS are at a significantly increased risk for developing psychiatric morbidities, including depressive and anxiety disorders (92).

1.8.8.1 Body Image and Femininity

While the medical literature supports an increased risk of developing psychological health conditions for women with PCOS, the basis for this is unclear. The current research is limited and presents conflicting reports to explain this increased distress. Some studies have found that obesity in women with PCOS is a strong determinant of decreased psychological health while other evidence suggests body image (obesity, hirsutism, acne) does not differ between women with and without depression and that BMI is independent of depression in PCOS (90, 93-95). Additionally, higher levels of serum androgens and lipids have been reported as strong predictors of depressive symptoms (96). However, in terms of quality of life (QoL), a recent meta-analysis determined that hirsutism and menstruation are the two strongest predictors of QoL in PCOS (97). Given that PCOS is a heterogeneous syndrome, it's plausible that some phenotypes have physical and/or biochemical characteristics that are more likely to negatively affect psychological health. Some potential factors that have been suggested include hyperandrogenism (which describes both an increase in androgen levels and a change in physical appearance), obesity or increases in weight, infertility, or fear of long-term health implications.

Unfortunately, much of the literature to date has significant gaps in methodology. For example, several other associations that could be affecting psychological health, such as BMI, infertility, or ethnicity, were not accounted for (89).

1.8.8.2 Female Identity

A few small qualitative studies, with sample sizes of no more than 30, have considered the lived experiences of women with PCOS (98-101). In 2002, Kitzinger and Willmott conducted one of the first studies to include women with PCOS using a feminist framework as opposed to a medical or psychiatric framework (102). Feminist standpoint theory in science seeks to incorporate the lived experience of women into their medical treatment (103). It is inspired by the contention that scientific study in medicine, in its aspirations to use objective scientific methods, has ignored or marginalized a woman's personal, lived experience of their medical condition, causing the underreporting or misreporting of symptoms and conditions. Standpoint theorists seek to put women back into the diagnosis and treatment of their medical conditions. In the context of PCOS, this approach involves interviewing women and recording the impact this syndrome has had on their lives as wives/partners, mothers, daughters, sisters, and friends. Kitzinger and Wilmott interviewed thirty women with PCOS and several themes emerged, including deeply-rooted stigmatization of PCOS symptoms. Women reported feeling "freakish", "abnormal" and not "proper" women which resulted in the authors coining PCOS as the "Thief of Womanhood" (102).

Nasiri Amiri et al conducted a small study, in 2014, interviewing twenty-three Iranian women with PCOS with regard to their identity and gender roles (101). This study demonstrated that symptoms of PCOS disturb one's perceived femininity. Women believed their beauty was

diminished by their obesity, hirsutism, hair loss and acne. Additionally, struggles with infertility and irregular cycles appeared to bring shame and insecurity in their identity as a woman (101). Other studies have echoed these findings showing that women felt less feminine because of a deepened voice, smaller breasts, greater muscle mass and reduced sexual drive/function (88, 98-100). While ethnicity and cultural norms have been identified as a possible limitation to the generalizability of the study by Nasiri Amiri et al, the variety of ethnic groups included in research to date suggests that female identity is an important factor for women with PCOS across all cultures.

1.8.8.3 Eating Disorders

The prevalence of eating disorders (ED) in women with PCOS is estimated to be 14% (90). To put this in perspective, only 1.5% of Canadian women, aged 15 to 24, were found to have an ED in 2002 (104). Binge ED is likely the most common ED in women with PCOS affecting 23.3% of this group (90). In the general population, binge eating affects approximately 2% of Canadians (105). Binge eating is associated with dieting to control weight while dieting for weight loss is a common treatment recommended for women with PCOS (106).

Anorexia nervosa is present in 0.5 to 4.0% of women in Canada and subclinical anorexia is present in 1.1% of women with PCOS (105, 107). The seriousness of this psychiatric illness cannot be overlooked as 10% of patients with anorexia will die within ten years of the disorder's onset (108).

Bulimia nervosa is defined as episodes of binge eating with behaviors including starvation, self-induced vomiting, laxative/diuretic abuse and over exercising in order to maintain weight (109, 110). In the general population of women in Canada, bulimia prevalence

is approximately 1.0 to 4.0% (105), while in women with PCOS clinical bulimia prevalence has been reported as 6% and subclinical bulimia at 11% (107, 109). The average lifetime duration of bulimia is an average of 8.3 years (111).

While the literature points towards a higher prevalence of ED in women with PCOS, there are limitations of the research to date. Specifically, diagnostic criteria for PCOS is inconsistent, sample sizes are small, and there may be bias in participant selection (women with ED may be less inclined to participate in research where they require discussing their experience).

The association between PCOS and ED has several possible explanations. ED develop from the following three triggers: genetic predisposition, poor body image and a change in dietary habits (112). As previously discussed, socially accepted views of femininity are challenged by the various symptoms of PCOS (irregular menstrual cycles, infertility, hirsutism, acne, and obesity). Therefore, a coping mechanism for this emotional distress and struggle with body image could be the disordered food intake (109). Lastly, with weight loss being the most frequent advice health care providers give their patients to improve their symptoms, this often results in changes in dietary habits and perhaps, extreme weight loss methods. Therefore, the requirements for the onset of an ED are met.

Twin studies have determined that the more diets individuals use, the more likely they are to suffer from disordered eating. Dieting to control weight, even under medical supervision, has been shown to promote weight gain over a three-year interval (106). Huijgen et al found that self-initiated diets were associated with PCOS, particularly the hyperandrogenic phenotype (113). In this explorative nested case-control study, 218 patients with PCOS and 799 subfertile

controls completed self-administered questionnaires. Participants declared whether they participated in a diet such as energy-restricted, vegetarian, vegan, macrobiotic or other. They were also evaluated according to the Preconception Dietary Risk score which evaluates a patient's nutritional adequacy in each of six food groups. Women with PCOS were more likely to use a self-initiated diet than the control group. Furthermore, women with the hyperandrogenic phenotype were the most likely to use a self-initiated diet, while also having significantly higher BMI and WHR. Hormonal and metabolic parameters also differed among dieters and non-dieters. Both AMH and the free androgen index (FAI) were positively associated with nutritional inadequacy scores (113).

1.9 Treatment and Management

Comprehensive management of women with PCOS has often been neglected in research, and therefore, has resulted in low to moderate quality evidence to date (11). The first international evidence-based guidelines for PCOS management were published in 2018 with funding by the Australian National Health and Medical Research Council of Australia, ESHRE and the ASRM (10, 11). These guidelines highlighted the importance of interdisciplinary care for women with PCOS. The following recommendations are based on these guidelines:

1.9.1 Lifestyle

A healthy lifestyle, including a focus on diet and exercise, is the first recommendation for women with PCOS to achieve and/or maintain a healthy weight and to improve hormonal health and quality of life (10). The focus for lifestyle intervention is typically weight reduction and blood sugar regulation. In women with PCOS who are overweight, a decrease in weight of 5 to 10% can improve clinical outcomes (10). Lifestyle changes are further discussed in Section 1.10.

1.9.2 Oral Contraceptive Pills

When treating women with PCOS, pharmaceuticals are often prescribed based on symptoms women present with, such as hirsutism, acne, hair loss or irregular menstrual cycles. Combined oral contraceptive pills (COCPs), along with lifestyle changes, are recommended as first-line management in adult women (and considered in adolescents) to suppress hyperandrogenism and regulate menstrual bleeding (10, 114). COCPs contain estrogen which increases the liver production of SHBG and progestogen which suppresses LH secretion. Increased circulating LH and the LH:FSH ratio is commonly present in women with PCOS. Not unlike other pharmaceutical interventions, side-effects have been reported from COCPs, some of which are so disruptive to women's lives that the benefits do not outweigh the risks (115). Unfortunately, some comorbidities associated with PCOS can also be associated with COCPs. For example, obesity, T2D, hypertension, dyslipidemia, and age are all factors that need to be considered when prescribing COCPs (114). "Long-term use of the OCP remains controversial due to potential adverse metabolic and cardiovascular effects (116-118)" (115).

One concern with oral contraceptives, which has been highly debated, is their potentially adverse effects on insulin sensitivity, and therefore, the potential to develop glucose metabolism disorders over extended periods of time (119, 120). In 2012, Piltonen et al conducted a study including 42 healthy women of normal weight who used either oral contraceptives, transdermal patches or vaginal rings. The results demonstrated significant increases, across all groups, in oral glucose tolerance tests and fasting serum insulin levels over an interval of nine weeks (while fasting glucose was unchanged) (121). The authors also found an increase in the inflammatory marker C-reactive protein (CRP) (121). While this study was limited by its sample size, a larger cross-sectional study including 1290 healthy women concluded that oral contraceptives increased

markers of insulin resistance such as 2-hour glucose, fasting insulin and triglyceride levels (122). In PCOS, specifically, similar trends have been noted. Mastorakos et al followed 36 adolescents with PCOS for a year: half were prescribed ethinyl estradiol combined with desogestrel and the other half were prescribed ethinyl estradiol combined with cyproterone acetate. In both groups, the homeostasis model of assessment for insulin resistance (HOMA-IR) was significantly increased after one year, and in the cyproterone acetate group, hyperinsulinemia was also increased (123). On the other hand, the OCP can be protective against endometrial and ovarian cancers (124).

1.9.3 Metformin

In addition to lifestyle modification, metformin is often recommended to women with PCOS, with or without COCPs, for the treatment of obesity, hyperinsulinemia, hyperandrogenism, and high triglycerides. The use of metformin can have adverse effects including gastrointestinal issues and low vitamin B12 levels (10).

1.9.4 Infertility Treatment

Infertility is a concern for 70% of women with PCOS due to infrequent or absent ovulation. Similar to non-fertility related treatment, lifestyle intervention is recommended for women with PCOS who are obese (81). Even modest weight loss may improve ovulation rates, however, there are no studies assessing the effect of lifestyle intervention on the live birth rate in women with PCOS (81, 125). Weight loss in obese women with PCOS may also reduce the risk of pregnancy complications, which are statistically higher in women with PCOS (81) These complications include preterm birth, congenital anomalies, perinatal mortality (possibly dependent on obesity), gestational diabetes mellitus (independent of obesity), preeclampsia

(independent of obesity, multiple pregnancy rate and parity), miscarriages, and the need for Caesarean delivery (dependent on obesity) (81, 126, 127).

1.9.4.1 Oral Ovulation Induction

The recommended first-line approach to PCOS-related infertility is ovulation induction medication, such as clomiphene citrate or letrozole (10). Conventionally, clomiphene citrate has been used for anovulatory infertility (128). It is an estrogen receptor modulator which competes with estrogen receptors in the hypothalamus and pituitary (81). This blocks estrogen negative feedback and consequently pituitary FSH secretion is increased. Finally, a dominant follicle is recruited (81). Ovulation rates are between 75 to 80% and the conception rate is 22% per cycle in non-PCOS populations (81). In women with PCOS, around 15% of women will not respond to the maximum dosage (81). The side-effects of clomiphene citrate include flushing, headaches, visual disturbances, abdominal discomfort, and the risk of twin pregnancy or higher-order multiples is increased (81, 128). Ovarian hyperstimulation syndrome (OHSS) is estimated between 1 to 6% of cases (81). Recent evidence has suggested that letrozole, an aromatase inhibitor, is more effective at achieving ovulation and live birth than clomiphene citrate (128, 129). While still off-label in Canada, the effectiveness and safety of letrozole, compared to CC, was demonstrated in this multicenter trial by Legro et al (129). However, up to 40% of women may still not ovulate with oral fertility treatments alone (81).

1.9.4.2 Gonadotrophins

When oral ovulation induction agents prove unsuccessful, the second line (and sometimes first line) recommendation for anovulatory infertility is the use of recombinant exogenous gonadotrophins (128). Recombinant follicle-stimulating hormone or human menopausal

gonadotropin stimulation is used with timed intercourse or intrauterine insemination (greater success rates) (81). Gonadotrophins provide an ovulation rate of about 70% with a clinical pregnancy rate of 20% per cycle, in PCOS populations (81, 130). Ovarian stimulation using exogenous gonadotropins increases the possibility of multiple pregnancy (5.7%) and the risk of OHSS, which is greater in women with PCOS (81, 131).

1.9.4.3 In-vitro Fertilization

The third line approach for infertility is in-vitro fertilization (IVF). Pregnancy rates for women with and without PCOS are similar for IVF cycles and range from 40 to 50% per cycle (128). In IVF cycles, there are increased risks of multiple pregnancy if multiple embryos are transferred and OHSS. In particular, patients with obesity will often require a higher dosage of rFSH stimulation (133, 134). The miscarriage rate is also higher in women with PCOS (35.8% vs 23.6%) (133, 134).

1.10 Indicators Supporting a Lifestyle-Based Approach

A review of the literature pertaining to the management of PCOS has identified that there is a lack of consideration for non-pharmaceutical approaches. Pharmacological treatments for PCOS can be disruptive or even harmful to patients' quality of life. Emerging evidence suggests that nutrition, physical activity, and stress have an impact on ovulation and affect the ovarian response to fertility treatments (135). Dietary intake and physical activity may influence metabolic health and reproductive hormones (136). While lifestyle intervention is recommended as a first-line treatment, 45% of women with PCOS have reported that they have never been provided information about lifestyle management (48). In addition, 62% of women reported that emotional support or counseling was never discussed with healthcare providers, which is significant when emotional distress is common for women with this stigmatizing disorder (5).

Therefore, attention to both the physical and psychological health of women with PCOS is lacking in research and clinical practice. Women with PCOS would benefit with an improved quality of life, and more scientific evidence is necessary to provide physicians with the expertise to guide patients to optimize their physical and psychological health. Given the aspects of PCOS, a comprehensive lifestyle-based approach may be a beneficial option for management, with the least harm.

1.10.1 Nutrition

While medical professionals can generally agree that a nutritious diet is of importance for women with PCOS, the evidence is limited as to what specific dietary approaches should be used. A review published in Nutrition Research Reviews in 2018 identified an unhealthy, typical Western diet as a substantial constituent of the 'deadly quartet' of metabolic risk factors in PCOS (along with hyperinsulinemia, hyperandrogenism, and low-grade inflammation) (137). In addition, nutritional interventions are far from being routinely implemented in clinical practice. Unfortunately, the available data examining habitual dietary intake and risk factors in PCOS is meager and conflicting.

1.10.1.1 Low Glycemic Diet

While most research has not identified a relation between daily caloric intake or macronutrient composition with PCOS status, a few studies have found minor nutrient and/or food group differences compared to women without PCOS. For example, a case-control study conducted on 200 overweight and obese women found that the PCOS group consumed, on average, more high-glycemic starchy foods and sweets (138). Similarly, a cross-sectional study including 61 women with PCOS and 44 control subjects found that glycemic intake was

increased in the classic PCOS phenotype and associated with obesity and adiposity (139). These are not the only indicators of glycemic load playing a factor in the presentation of PCOS. As insulin resistance has a significant role in PCOS, and women with PCOS often experience compensatory hyperinsulinemia following consumption of carbohydrates, a diet based on foods with a low glycemic load may be beneficial (140). In a randomized controlled trial, 29 women with PCOS followed a low-glycemic diet for 12 months while 21 women followed a healthy diet with a normal glycemic load (141). The group following the low glycemic diet showed a significant increase in insulin sensitivity, improved menstrual regularity and a more positive emotion score in a QoL survey. In a second trial, 21 women with PCOS followed an isocaloric low glycemic diet (140). The dietary intervention lasted 12 weeks and the outcomes were improved insulin sensitivity and improved circulating nonesterified fatty acid levels. Although the results of these trials were promising, both studies had significant dropout rates (140, 141). Marsh et al noted that these dropouts appeared to be heavier and more insulin resistant than their counterparts who remained in the study. Therefore, it may be difficult for those with more severe symptoms of PCOS to comply with a low glycemic diet over longer periods of time (141).

Additionally, a low glycemic diet has been shown to be safe during pregnancy and may help prevent large-for-gestational-age babies, thus, influencing long-term health outcomes (142).

1.10.1.2 Low Carbohydrate Diet

A recent systematic review identified five studies of good quality that examined the effect of low carbohydrate diets on reproductive outcomes in overweight and obese women with PCOS (143). Studies were included if they had a carbohydrate intake of less than 45% of daily caloric intake. Results showed improvements in insulin levels, reproductive hormones

(testosterone, SHBG and LH/FSH ratio), and improved ovulation rates over short periods of intervention (4-24 weeks). However, all the included studies of the review also featured energy restriction which may influence the findings (144). Moreover, it is unclear what the minimal carbohydrate intake should be, how long a low carbohydrate diet can be sustained, and the long-term health implications (143). For example, a small study completed by 11 women found that a low carbohydrate, ketogenic diet (less than 20 grams of carbohydrates a day) improved several parameters of PCOS over 24 weeks, including weight, free testosterone, LH:FSH ratio and fasting insulin (145). However, over half of the women did not complete the study suggesting that restricting carbohydrate intake to this level was unsustainable (143). There are also concerns regarding low carbohydrate diets as they can decrease food groups that can be protective of general health, ie. vegetables, fruits and whole grains (146).

1.10.1.3 High Fiber Diet

In 2017, a large Iranian case-control study included 281 women diagnosed with PCOS (according to the Rotterdam criteria) and 472 age-matched controls (147). A food frequency questionnaire was utilized to assess dietary intake. The study found that low fiber intake was inversely associated with PCOS. Given the importance of fiber in diabetes therapy, this finding is not surprising. Fiber intake has also been negatively correlated with serum levels of androstenedione in premenopausal women (148). Likewise, high fiber foods, such as fruits, vegetables, and whole grains, have been inversely associated with circulating androstenedione levels (148).

1.10.1.4 High Protein Diet

While diets high in protein are commonly suggested for achieving weight loss and controlling diabetes, there is limited evidence to support their use (146). A small randomized

controlled trial including 26 women with PCOS and obesity compared a hypocaloric high protein/low carbohydrate (30% protein, 40% carbohydrate) to a hypocaloric low protein/high carbohydrate (15% protein, 55% carbohydrate) diet over a one-month interval. While both groups experienced significant weight loss (likely due to a reduction of 1,000 kcal/day compared to their typical dietary intake), and improvements of reproductive and metabolic perturbations, there were no differences in body weight or markers of glucose homeostasis between the two dietary intervention groups (149). Further, there is some evidence suggesting that high protein diets can exacerbate IR as protein stimulates insulin secretion (150, 151). In addition, high protein diets should be cautioned as they can promote renal damage (152).

Protein intake has been assessed in populations with ovulatory infertility and significant differences were found based on the consumption of animal versus plant protein (153). In a large study by Chavarro et al including 18,555 women, it was found that obtaining 5% of overall calories from plant protein rather than animal protein resulted in a 50% less risk of ovulatory infertility (153). The effects of protein sources have yet to be studied in PCOS-specific populations.

1.10.1.5 Omega-3 Supplementation

Polyunsaturated fatty acid intake, such as omega-3, may have beneficial effects for women with PCOS. Randomized controlled trials in women with PCOS have shown that eight weeks of omega-3 supplementation (three grams a day) can regulate cycles, decrease testosterone, LH, LH:FSH ratio and increase adiponectin (154, 155). Omega-3 supplementation may also be effective at treating NAFLD in women with PCOS as a small randomized controlled trial found that four grams a day of omega-3 decreased liver fat content, triglycerides, systolic blood pressure, and diastolic blood pressure (156). There are currently no medications used to

treat NAFLD but weight loss is recommended by eating a healthy diet and exercising (157). Further trials are needed to determine if omega-3 supplementation could improve symptoms of PCOS and reduce the rate of CVD in this population.

1.10.1.6 Vitamin D Supplementation

Levels of serum 25-hydroxyvitamin D have been found to be significantly decreased in women with PCOS and obesity (158, 159). Vitamin D can be sequestered by fat cells, and therefore, supplementation is important to be considered for patients with obesity (160). Further discussion on the biological role of vitamin D will follow in Chapter 3, Section 2.

1.10.1.7 Mineral Supplementation

Deficiencies of several minerals, including chromium, magnesium, and selenium, have been identified in PCOS populations and therefore, preliminary trials have assessed supplementation (146). Chromium levels have also been shown to be lower in women with T2D. When women with PCOS received chromium picolinate supplementation for two months, they experienced improvement in glucose levels and insulin sensitivity. Chromium supplementation can also reduce hirsutism and body mass (161, 162). Serum magnesium levels have been found to be reduced in some women with PCOS, and similar conditions such as T2D, but trials on magnesium supplementation are limited (163, 164). Another mineral that has been found to be deficient in some women with PCOS is selenium (165). Selenium also negatively correlated with testosterone levels. More research is required to determine how these minerals affect women with PCOS and whether supplementation may or may not be supportive.

1.10.2 Physical Activity

Minimal research has been conducted addressing the benefit of physical activity for women with PCOS (115). Literature has established that weight loss in women with PCOS who are overweight or obese often improves several clinical features including irregular menstruation, anovulation, infertility, hirsutism and acanthosis nigricans (166). Physical activity contributes to weight loss and may be as effective as caloric restriction (167). However, exercise type, duration, and intensity need to be considered when determining an effective, sustainable weight loss method in this specific population of women (168, 169). Exercise interventions for weight loss in women with PCOS often have issues with high drop-out rates (up to 40 to 45%) and noncompliance (115, 170, 171). Furthermore, a lack of exercise has not been able to explain the higher rates of obesity in women with PCOS (172). Studies assessing habitual exercise indicate that the duration of exercise does not differ between women with and without PCOS, even though obesity rates are higher in women with PCOS (173).

Regardless of weight loss, increasing physical activity has benefits for cardiovascular, metabolic and reproductive health. In the general population, regular activity improves HDL-cholesterol and triglyceride levels, decreases visceral adiposity, improves blood pressure, improves insulin sensitivity, reduces total and central adiposity, and maintains bone mass (168). Several types of regular exercise in women with PCOS have been shown to lower risk factors of CVD (WHR, HDL cholesterol, triglyceride levels, blood pressure, and homocysteine levels) and T2D (IR) (170, 173). Menstrual and ovulation frequency can improve after exercise intervention and may be more beneficial than dietary restriction (115). While an objective duration of activity has not been established to improve reproductive health outcomes, exercise interventions that

have been successful have been of low to moderate intensity (168). In addition, evidence suggests that exercise can help relieve symptoms of primary dysmenorrhea (174).

Physical activity has been shown to improve anxiety, depression, and mood in populations both with and without mood disorders (175). In particular, low to moderate intensity aerobic exercise that utilizes large muscle groups have the greatest psychological benefits. However, overtraining or high-intensity exercise can result in central fatigue, negating the beneficial effects of exercise on mood (176).

There are various theories that explain the positive impact that exercise has on brain functioning. Moderate exercise stimulates monoamine systems (responsible for the release of dopamine, serotonin, and norepinephrine), which may explain mood enhancement (175, 176). Brain-derived neurotrophic factor, which modulates depression, is released by exercise. In addition, IGF-1 is increased by exercise, particularly strength training, which supports cognitive functioning (177). The increase of cerebral blood flow may also impact stress management by regulating the hypothalamic-pituitary-adrenal (HPA) axis (175).

Given that women with PCOS are more likely to experience poor psychological health and lower QoL, moderate exercise could have several benefits for this population beyond weight loss, metabolic improvements and regulating ovulation (178). Further controlled studies with larger sample sizes are needed so that appropriate regression modeling can be used to study the relative importance that exercise has on PCOS. In addition, future studies need to assess the benefits of exercise not only in women with PCOS and obesity but also in women with lean PCOS.

1.10.3 Mind-Body Medicine

Mindfulness is the practice of purposefully paying attention to one's thoughts, emotions, and body. Small, nonrandomized studies in non-PCOS populations have shown that mindfulness-based stress reduction programs reduce blood pressure, glucose levels and inflammation due to changes in brain activity that positively affect the autonomic nervous system and HPA axis (179-181). These changes could be very beneficial for women struggling with physical symptoms associated with PCOS, such as obesity, anovulation, and fertility, as well as psychological symptoms, such as depression, anxiety, and stress. In 2015, a small randomized controlled trial in women with PCOS demonstrated that an eight-week mindfulness-based intervention improved symptoms of depression, anxiety, stress, QoL, and reduced salivary cortisol (182). In addition, a randomized controlled trial involving 86 women who were overweight or obese, 31 of which had PCOS, found that 8 weeks of mindfulness-based stress reduction decreased perceived stress and fasting glucose (183). Further randomized controlled trials of larger sample sizes are necessary to elucidate the range of benefits that may be possible for women with PCOS.

1.10.4 Complementary and Alternative Medicine

Research evaluating the effectiveness of complementary and alternative medicine (CAM) for managing PCOS is limited. However, women's interest in CAM is high. The results of a large survey completed by 657 women with PCOS demonstrated that 99% of respondents would rather use CAM than treatments usually recommended by clinicians (184). It has also been reported that 70% of women with PCOS are already using CAM in the form of vitamin, mineral and herbal supplements. Some reasons women reported for using CAM were "to treat PCOS," "to treat infertility," "to improve general well-being," and "to reduce depression" (185).

1.10.4.1 Acupuncture

In traditional Chinese medicine, acupuncture is a treatment method that has been used for over 3000 years (133). In a randomized controlled trial of women with PCOS, acupuncture was shown to improve menstrual frequency and to lower testosterone, AMH, and ovarian volume when compared to an exercise intervention (186, 187). There is also evidence that acupuncture can improve ovulation frequency (188). The mechanism may be its ability to suppress adrenal cortisol secretion and manage central B-endorphin secretion both of which impact the release of gonadotropin-releasing hormone (GnRH) (133). While literature shows some promise, there is currently weak evidence supporting the use of acupuncture to treat infertility in women with PCOS (133).

1.10.4.2 Herbal Medicine

Several herbs have been used to manage PCOS, however, there is limited high-quality evidence supporting their effectiveness. The three herbs with the highest quality of data are Cimicifuga racemose, Cinnamomum cassia and Vitex agnus-castus (189). The following herbs discussed have been assessed by at least one randomized controlled trial in women with PCOS and are cited accordingly.

Cimicifuga racemosa (black cohosh), a phytoestrogen-producing plant, is hypothesized to act on the hypothalamus by reducing GnRH and, therefore, LH secretion (190). Black cohosh has demonstrated beneficial effects on fertility in three randomized controlled trials in women with PCOS (189, 191-193). In two of these trials, black cohosh was combined with CC and this resulted in significantly higher clinical pregnancy rates than with CC alone (CC with black cohosh: 34.8% and 36.7%, versus CC alone: 17.2 % and 13.6%) (191, 193). The sample sizes of

these trials were 194 and 119, respectively (191, 193). In the third trial, black cohosh alone outperformed CC with a greater pregnancy rate by 6% in a sample of 100 women with PCOS (although this was not statistically significant) (192). Across all three trials, similar hormonal changes were seen including a decrease in serum LH, and an increase in mid-luteal estradiol, mid-cycle estradiol, and mid-luteal progesterone levels (191-193). Endometrial thickness was significantly increased in women using black cohosh thus supporting implantation (191-193).

Cinnamomum cassia (cinnamon) may be effective at improving metabolic health due to its ability to stimulate insulin receptor autophosphorylation and inhibit protein tyrosine phosphatase I (194). One randomized controlled trial including 15 women demonstrated that daily oral cinnamon intake reduced IR in overweight women with PCOS (195). These clinical results agreed with previous animal studies where cinnamon was as effective as metformin (and also had an additive effect) at decreasing levels of testosterone, LH and HOMA-IR in rats with PCOS (196).

Vitex agnus-castus (chaste tree berry) acts on the anterior pituitary gland regulating LH release. Chaste tree berry has been shown to improve menstrual regularity in three randomized controlled trials, however, these studies had small sample sizes and baseline characteristics of groups were not reported (189, 197-199). In addition, chaste tree berry has been shown to lower serum prolactin as effectively as bromocriptine, but without nausea and vomiting associated with such dopamine agonists, in women with mild hyperprolactinemia (200).

Glycyrrhiza glabra (licorice root) taken in combination with spironolactone has been shown to reduce side effects associated with spironolactone, such as water loss (201). Licorice root is hypothesized to block 17-hydroxysteroid dehydrogenase and 17-20 lyase (202). It has

been shown to reduce testosterone levels in two clinical trials (one of which included women with PCOS) and in a third study when it was combined with Paeonia lactiflora (Chinese peony) (202).

Mentha spicata Labiatae (spearmint) has been shown to have anti-androgen effects in women with PCOS. A randomized controlled trial in 41 women with PCOS compared drinking two cups of spearmint tea a day to a placebo herbal tea (203). After the 30-day period, free and total testosterone levels were significantly decreased in the group drinking spearmint tea. Spearmint oil has been tested on rats with PCOS and, when compared to sesame oil, it reduced body weight, testosterone level, ovarian cysts, atretic follicles and increased Graafian follicles (204).

In conclusion, there is currently weak evidence to recommend herbs to manage symptoms or treat infertility in women with PCOS (133). It is noteworthy that no adverse effects have been reported by clinical investigations so far (189).

1.10.4.3 Inositol

As per recent guidelines, inositol is considered an experimental therapy for PCOS as the evidence to date is limited and weak (10). Humans consume inositols in foods such as fruits and beans. Inositols are incorporated into cell membranes as phosphatidyl-myo-inositol which is a precursor of inositol triphosphate (205). Inositol triphosphate is a second messenger for hormones such as insulin and FSH which makes inositol potentially useful for managing IR in PCOS.

Inositol has nine cis/trans-isomers. Two stereoisomers of inositol of therapeutic interest for women with PCOS are myo-inositol (MI) and D-chiro-inositol (DCI). MI is the most

abundant isomer of inositol in nature and the human body. It is involved in the regulation of thyroid-stimulating hormone (TSH), FSH and insulin (206). MI is converted to DCI by epimerase. Most body tissues have a MI:DCI ratio of 40:1 (205, 207). Some literature has examined the role of these two stereoisomers in improving symptoms of PCOS separately and synergistically.

1.10.4.3.1 Myo-inositol

MI is synthesized by glucose-6-phosphate in the human body (mostly in the kidneys). MI has been shown to induce menstruation, increase the occurrence of ovulation, improve oocyte quality, decrease acne and hirsutism, and assist with weight management in women with PCOS (206, 208). Small randomized controlled trials have compared two to four grams of MI a day to folic acid and have demonstrated that MI can increase rates of ovulation (70% versus 21%), decrease time to ovulation, increase HDL-cholesterol and result in weight loss (209-211). Another single-arm study indicated that four grams of MI a day can restore ovulation in 72% of women with PCOS (212). Beyond fertility therapy, a meta-analysis including only randomized controlled trials concluded that MI decreased fasting serum insulin levels and HOMA-IR in women with PCOS (206). While total testosterone levels also seemed to decrease, this effect was inconclusive due to the high heterogeneity of the study populations (206). Large doses of MI have been considered to help treat depression, but trials are limited, and results are inconclusive (213). Further research is necessary to determine if inositol has a therapeutic use in depression.

1.10.4.3.2 D-chiro-inositol

DCI administration has had positive effects on endocrine, reproductive and metabolic factors in small studies including women with PCOS however few randomized controlled trials have been conducted (205, 214). In one study, 22 women with obesity and PCOS took 500

milligrams of DCI a day for three months. After treatment, there were significant reductions in levels of LH, estradiol, androstenedione, testosterone, fasting insulin, and BMI (215). Another small study treated 47 women with DCI and found that menstrual cycles regulated (216).

One randomized controlled trial compared oocyte quality in women with PCOS undergoing gonadotrophin stimulation and taking either DCI or metformin (217). Another randomized controlled trial compared DCI to a placebo and found significantly higher ovulation rates and lower testosterone levels in the DCI group (218).

1.10.4.3.3 Myo-inositol + D-chiro-inositol

While MI appears to be more effective for women with PCOS due to its ability to regulate menstruation and improve oocyte quality, the combination of MI and DCI may have the additional benefit (219). A large randomized controlled trial of 100 women with PCOS undergoing IVF treatment compared a combination of MI and DCI (1100 mg MI combined with 27.6 mg DCI) to 500 mg DCI alone (219). This trial found that the combined therapy improved oocyte quality, embryo quality, and increased pregnancy rates compared to DCI alone. Further research is required to determine the best ratio of MI to DCI to administer to women with PCOS.

1.11 Nutritional Methods

1.11.1 Dietary Assessment Methods

1.11.1.1 Food Record

Food records are open-ended diaries that allow participants to record their dietary intake throughout a chosen number of days. Food records are considered the gold standard of dietary methods and are often used as a reference in validation studies (220). Some reasons for their high validity and precision are that the participants do not need to rely on memory, they can weigh or measure their food while recording, and they can describe portion sizes in detail (or through

additional photographs). When choosing the number of days, research indicates that food records of greater than four days can result in participant fatigue and therefore, under-reporting of intake (220). Special considerations should be made such as how many working versus weekend days participants are recording and repeating records in different seasons. Some disadvantages of food records are that they put a high burden on participants and therefore, may result in a high drop-out rate. The act of recording dietary intake may also influence behavior and is often even used as a weight loss technique (221). In addition, they give a snapshot in time of dietary intake and cannot be assumed to be long-term intake. Manually recorded records are also highly laborious for the researcher (220, 221). They require a large amount of time and effort by the researcher in training the participants on how to record and measure their food, following-up with participants after the food record is completed to verify intake, entry of data, conversions of portion sizes and analyses. For these reasons, a food record is not advantageous for large population studies (220).

1.11.1.2 Food Frequency Questionnaire

Food frequency questionnaires are validated instruments that are provided to participants who are asked to account for, from a list, how often they have eaten specific foods over a set amount of time (ranging from the past week to year). While much information can be collected, it is of limited detail. This method is inexpensive and easily implementable in large population studies. However, drawbacks include a large amount of measurement error particularly in terms of portion sizes. When a three-day food record was compared to a food frequency questionnaire, the three-day food record had higher correlations with nine-day food records. However, relative validities of both methods were determined acceptable for assessing dietary intake (222).

1.11.1.3 24-Hour Recall

In 24-hour recalls, the participant is usually interviewed in-person or over the phone and asked to list all food consumed over the past 24 hours (221). The protocol used in the United States is the Automated Multiple-Pass Method which goes through five steps for researchers to follow with the participant (223). Some benefits of this method are that the immediate recall requires limited memory (although still more memory than a food record requires), and the interview is generally a low burden to the participant. In addition, since the interview is retrospective, food choices are not influenced by the study. However, some disadvantages are that memory loss can occur, and the interview situation may be uncomfortable leading to under-reporting. In addition, if the habitual intake is being assessed, multiple days of recalls are necessary.

1.11.2 Energy Adjustment

Since the intakes of most specific nutrients are correlated with total energy intake, epidemiologic studies must adjust each nutrient for total energy intake (224). Potential associations between the prevalence of disease and specific nutrient intakes can be overlooked if variations in total energy intake are not adjusted (224). A couple of methods can be used to adjust for overall caloric intake, including the residual method and the nutrient density model (224). The residual method utilizes a regression model where the independent variable is the total energy intake and the dependent variable is the individual nutrient intake. Thus, the adjusted nutrient intake is the residual for the subject in addition to the expected nutrient intake given the mean energy intake. The nutrient density model follows a simple equation where the reported specific nutrient intake is divided by reported overall caloric intake essentially providing a percentage of intake from that nutrient. Similarly, when macronutrients are reported, they are

commonly described in percentage of total caloric intake (protein, carbohydrates, and fats), and therefore, already adjust for total energy intake.

1.11.3 Under-Reporting

Under-reporting is an issue in all forms of dietary assessment due to their self-assessment nature (225). Further, specific groups of people have been shown to be more likely to under-report energy intake (226). Dieting in the form of energy restriction is a source of under-reporting. Studies have indicated that women who are overweight may under-report energy intake due to being on hypocaloric diets (227, 228). Under-reporting is also associated with high socio-professional class and having dieted at least once before (228).

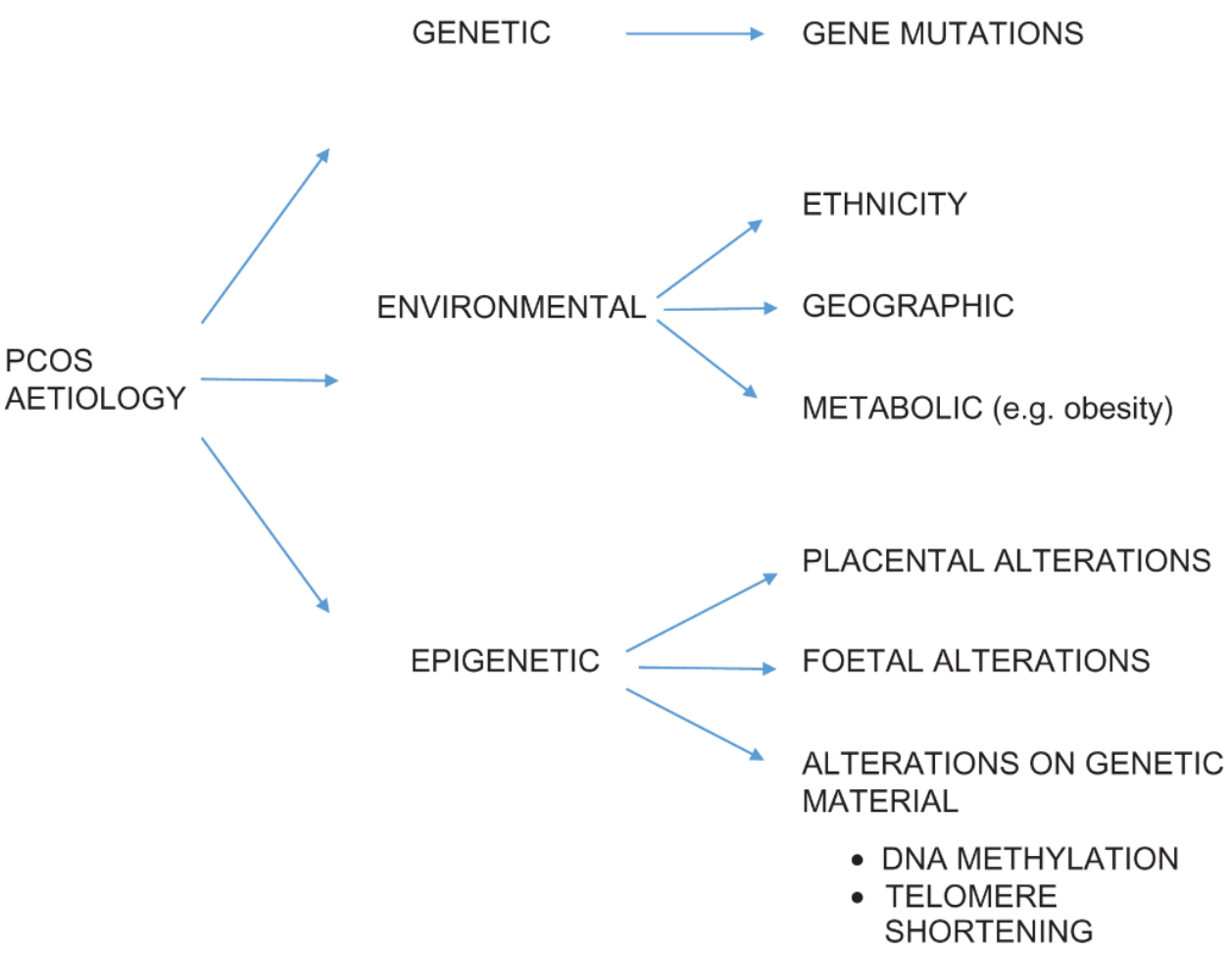
To determine under-reporters the Goldberg Cutoff method is frequently used (229). This method uses principles of energy physiology to determine the lowest possible energy intake of an individual to survive when taking into account their biological sex, age, body weight and height (approximating a basal metabolic rate (BMR)). Goldberg et al provided two cut-off limits. The first is stricter as it requires BMR to be measured rather than predicted and identifies if the reported dietary intake is sustainable over a long period of time. The second determines if the amount of food reported is plausible over the short duration of time reporting. To predict BMR in this dissertation, the Schofield equation was used (230). If an individual's intake fell below this cut-off, it was presumed that under-reporting occurred.

Table 1.1 Typical sub-types of PCOS based on clinical presentation.

Sub-Type	Androgen Excess	Ovulatory Dysfunction	Polycystic Ovarian Morphology
1	+	+	+
2	+	+	-
3	+	-	+
4	-	+	+

Note: The concept for this table was adapted from "Lujan ME, Chizen DR, Pierson RA. Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. *J Obstet Gynaecol Can*. 2008;30:671-679.".

Figure 1.1 Proposed factors in the aetiology of PCOS.



Note: Filippou, P & Homburg, R. Is foetal hyperexposure to androgens a cause of PCOS? Human Reproduction Update, 2017, Vol 23, No. 4, p. 423, by permission of Oxford University Press.

Chapter 2: Associations between Dietary Intake and Phenotypic Differences in PCOS

2.1 Assessing caloric and macronutrient intake and its associations with obesity, insulin resistance and hyperandrogenism in PCOS - *a cohort study*

2.1.1 Introduction

A review by Lin and Lujan noted large discrepancies in the current research assessing the lifestyles of women with PCOS (136). Some studies have reported that women with PCOS consume a higher caloric intake compared to women without PCOS, while others showed no significant differences in either dietary intake or physical activity (139, 144, 172, 231, 232). While caloric surplus (excess calories and/or inactivity) is a common cause of obesity, previous studies have indicated that women with PCOS may have a slower basal metabolic rate and/or reduced postprandial thermogenesis (the rate at which food is broken down) (233, 234). In addition, inconsistent findings have been reported in studies assessing the intakes of fiber and glycemic load in women with PCOS, two important dietary components for managing weight, insulin levels, and dyslipidemia (139, 144, 235, 236). Fiber consumption has been shown to increase satiety, reduce glucose, and decrease both total and LDL cholesterol levels (237). Reducing glycemic load has been shown to assist in weight management, improve glycemic control in diabetics, reduce dyslipidemia and increase HDL cholesterol levels (238). One study provided evidence that when 29 women with PCOS followed a low glycemic diet for 12 months, they showed a significant increase in sensitivity to insulin, improved menstrual regularity and improved QoL scores in comparison to the 21 women with PCOS that consumed a healthy diet of normal glycemic load (141).

In clinical practice, women with PCOS are often frustrated that, despite lifestyle modification, they have difficulty controlling weight and improving IR. Further, reduced fiber intake and increased glycemic load have been associated with an increased prevalence of T2D and metabolic syndrome (239). We, therefore, hypothesized that these dietary factors may also be linked to IR in PCOS.

Our main objective was to investigate overall caloric intake, physical activity, and obesity in women with and without PCOS. Our second objective was to assess intakes of main dietary components (protein, carbohydrate, fat, fiber, and glycemic load) to identify associations between these dietary components and obesity, IR and hyperandrogenism in women with PCOS.

2.1.2 Methods

Study Design, Setting and Participants

This observational, cohort study took place from May 2014 to December 2016. Women between the ages 20-44 years were recruited by me (DA Cutler) at Grace Fertility and Reproductive Medicine ("Grace Fertility Centre") in Vancouver, Canada (n = 137) following their initial consultation with a reproductive endocrinologist. There were 87 women with PCOS, according to the Rotterdam criteria, and 50 sub-fertile women without PCOS for comparison. Of the 87 women with PCOS, 49 were categorized as "Hyperandrogenic PCOS (HA)" when all three Rotterdam criteria were present or when hyperandrogenism and oligo-/anovulation were present without PCOM on ultrasound. Hyperandrogenism was defined as the presence of biochemical and/or clinical androgen excess. Biochemical androgen excess was identified if at least one of the following three androgens were elevated according to local laboratory references (total testosterone ≥ 1.8 nmol/L, androstenedione ≥ 7.48 nmol/L, or DHEA-S ≥

10.8 umol/L). In clinical practice at Grace Fertility Centre, all patients with PCOS are routinely asked to self-report for the presence of hirsutism using the modified Ferriman-Gallway (mFG) score in addition to clinical assessment by the physician. Participants' corresponding mFG scores were compiled for the study. For those of European, Aboriginal and South American descent, a hirsutism score of 8 or higher constituted hyperandrogenism while a score of 6 or higher for East Asian participants, and 10 or higher for South Asian participants sufficed (240-242). The remaining 38 women, with both oligo-/anovulation and PCO on ultrasound, were categorized as "non-hyperandrogenic PCOS (Non-HA)". "PCO on ultrasound" was defined by the Rotterdam criteria as (a) having twelve or more follicles measuring 2–9 millimeters in diameter in one or both ovaries, or (b) the ovarian volume exceeding 10 centimeters^3. It should be noted that many of our participants had greater than 12 follicles, some with 25 or more follicles per ovary, which was proposed recently as a better criterion for PCOM (243). The infertility diagnoses of the control group were unexplained (41%), male factor (17%), diminished ovarian reserve (19%), prior history of oligo-ovulation (8%), endometriosis (2%), and mixed (13%). Women recently diagnosed with or still recovering from an ED were excluded, as well as women who had recently begun following a diet or exercise regimen with a professional.

Dietary and Activity Assessment

Participants completed a three-day food and activity record, which consisted of two weekdays and one weekend day (Appendix B) (222, 244, 245). They were instructed to provide detailed accounts of their daily food and drink intake. This included the amount (using measuring utensils, scales, or food labels), brand names, flavors, condiments, cooking methods, and time of eating. Participants were provided with a list of common objects to compare to their food portions if food could not be measured (ie. dining out). For example, participants were

instructed to record that they ate either '¾ cup brown rice, cooked' or its equivalent which is a 'tennis ball size portion of brown rice, cooked'. Food records were accompanied by electronic photographs of meals. Once food records were returned to the researchers, food items, quantities, details, and commonly forgotten items were verified with each participant. Pedometers (SM-2000 Step Pedometer by Heart Rate Monitors USA) were provided to quantify participants' daily steps, in addition to their physical activity record. Participants were encouraged to maintain their typical dietary and activity regimens for the duration of the study.

Dietary intake, comprising total caloric intake, protein, carbohydrates, fat, glycemic load, and fiber, was determined using a nutrition and fitness software program suitable for research and clinical purposes (ESHA Food Processor 10.12, 2013) (246). This software accesses data on nutritional components, including calories, protein, carbohydrates, fat, and fiber from Health Canada's Canadian Nutrient File. Every food item from each participant's food record was assessed by trained researchers and manually matched to its equivalent food item in Health Canada's Canadian Nutrient File. To calculate the glycemic load, the glycemic index of each food containing carbohydrates was identified based on published literature and manually input into our software (244).

Anthropometrics

All patients have their height, weight, waist and hip circumference recorded, and BMI and WHR calculated as a routine part of clinical practice at Grace Fertility Centre. Corresponding data for participants were compiled for the study.

Biochemical Assays

Similarly, as a routine part of clinical practice at Grace Fertility Centre, all patients presented with PCOS or infertility have a baseline transvaginal ultrasound assessment by one

physician (APC) using the EC9-5/10 endovaginal transducer (SonixTouch, Ultrasonix) and hormone measurements for FSH, estradiol (E_2), prolactin (PRL), and TSH (Abbott Architect Immunoassay) and C-reactive protein (CRP). Women with PCOS have additional hormone and metabolic measurements which include: LH, progesterone (P), androstenedione (A_4), and 17-OHP (Agilent 6410, LCMS methodology); testosterone (T), DHEA-S and fasting insulin (Roche Cobas e602 Immunoassay); blood glucose levels after a 12-hour fast (FBG) and at two hours after a 75 gram oral glucose tolerance test (2 hour glucose level) (Roche Cobas c701 Roche Diagnostics, hexokinase/G6P-DH method); total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TG) (Roche Cobas c701 Roche Diagnostics enzymatic colorimetric method, and with polyethylene glycol-modified enzymes and dextran sulfate for HDL-C). Corresponding data for participants were compiled for the study.

The HOMA-IR was calculated using the formula: HOMA-IR = fasting blood glucose (mmol/L) x fasting insulin (μIU/mL)/22.5 (247). HOMA-IR is a reliable clinical tool for measuring insulin sensitivity with a strong correlation to the more laborious glucose clamp measurements (246, 248). IR was defined as a HOMA-IR greater than or equal to 3.8, as used in previous studies evaluating women with PCOS (249).

Statistical Analyses

Since nutrient intakes can be affected by total food quantity consumed, crude nutrient intakes for fiber and glycemic load were adjusted for total energy intake using the residual method (224). Protein, carbohydrates, and fats were recorded as percentages of total energy intake, and therefore, were already adjusted for total caloric intake. Dietary under-reporters were identified according to the Goldberg Cutoff method and analysis was conducted with and without the data of the under-reporters (250). The statistical significance found in our analysis

was unchanged when under-reporters were removed, so they were included in all reported results (230, 250).

Baseline characteristics and outcome parameters were assessed for statistical normality and compared with either a 2-sample t-test or its non-parametric equivalent, Mann-Whitney U test for two groups; or, one-way ANOVA (with post-hoc pairwise comparison according to Tukey) or its non-parametric equivalent, Kruskal-Wallis test when groups were stratified by BMI or fiber intake. Relationships between continuous variables were determined by Pearson and Spearman's Rank correlations, as appropriate. Stepwise multiple linear regression was used to identify independent predictors of HOMA-IR. Analyses were executed using R software. A P-value of <0.05 was considered statistically significant.

2.1.3 Results

All Participants

The ethnicity of study participants was 42% East Asian, 40% European, 15% South Asian, 2% Aboriginal and 1% South American. Women with PCOS had significantly higher BMI ($P < 0.001$), higher WHR ($P < 0.01$) and were younger ($P < 0.001$) than women without PCOS (Table 2.1). No significant differences were found between the caloric intake or physical activity levels in women with or without PCOS (Table 2.1). Macronutrient composition of dietary intake as percentages of protein, carbohydrate, and fat did not differ, nor did the crude or adjusted glycemic loads of the two groups. However, women with PCOS consumed significantly less fiber than women without PCOS (crude: $P < 0.01$, adjusted: $P < 0.01$, Table 2.1).Participants with PCOS

The characteristics of women with PCOS were summarized and compared according to BMI grouping (normal weight, overweight and obese) and IR (HOMA-IR < 3.8 vs. HOMA-IR ≥ 3.8) (Table 2.2). There was a strong correlation between BMI and HOMA-IR (rho = 0.72, P < 0.001), but no correlation between age and BMI nor HOMA-IR in our PCOS cohort.

When women with PCOS were compared by BMI, no differences were found in dietary intake or activity level (Table 2.3). However, BMI was significantly greater in women with IR-PCOS than non-IR-PCOS (P < 0.001). Fiber intake was also significantly less in women with IR-PCOS after adjusting for caloric intake (P < 0.05). When adjusted fiber intake was categorized by tertiles, HOMA-IR differed significantly (P = 0.01), and women with the least fiber intake had significantly higher HOMA-IR than women with the greatest fiber intake (P < 0.01, Figure 2.1). Furthermore, fiber intake was negatively correlated with HOMA-IR (rho = -0.35, P < 0.005), fasting insulin (rho = -0.37, P < 0.005), 2 hr glucose level (rho = -0.23, P < 0.05), triglycerides (rho = -0.27, P = 0.02), total cholesterol/HDL-C ratio (rho = -0.29, P < 0.01), and positively correlated with HDL-C (rho = 0.28, P = 0.01). Fiber intake was not correlated with fasting blood glucose (FBG), total cholesterol or LDL-C.

Glycemic load was significantly greater for women with IR-PCOS, both before (P < 0.01) and after (P = 0.03) adjusting for caloric intake (Table 2.3, Figure 2.2). Glycemic load, however, was not correlated with other metabolic or lipid parameters.

The effects of adjusted fiber intake, adjusted glycemic load, BMI, age, and PCOS phenotype on HOMA-IR were further assessed by multiple linear regression analysis. Fiber intake and BMI were independent predictors of HOMA-IR explaining 54.0% of the variance in the predictive model (P < 0.0001).

When the dietary intakes of women with PCOS were compared by phenotype (HA versus Non-HA) there were no significant differences in total caloric intake, activity or macronutrient intake. However, fiber intake was negatively correlated with testosterone (rho = -0.35, $P < 0.005$, Figure 2.3) and DHEA-S (rho = -0.27, P = 0.02, Figure 2.4). There were no correlations between androstenedione and any dietary components. When fiber intakes were grouped in tertiles, women with lower fiber intake had significantly higher testosterone (P = 0.01, Figure 2.5) and DHEA-S (P = 0.02, Figure 2.6) levels than those with higher fiber intake.

2.1.4 Discussion

Our study indicated that, despite significant differences in BMI and WHR, overall caloric intake and physical activity did not differ between women with and without PCOS, as have been observed in previous studies conducted in North America (172, 231). These findings demonstrate that obese women with PCOS are not in an energy surplus state supporting previous studies that suggest women with PCOS could indeed have an altered metabolism contributing to their obesity and IR (113, 233, 234).

This is the first study to identify low fiber intake as a significant factor in women with IR-PCOS. Women with IR-PCOS (by HOMA-IR score) consumed less fiber than women non-IR-PCOS; and, fiber intake was an independent predictor of HOMA-IR. Other metabolic markers were also inversely associated with fiber intake as in the 2-hour glucose, fasting insulin, triglyceride levels, total cholesterol/HDL-C ratio, and directly associated with HDL-C. Although others have reported that dietary composition, including fiber intake, was not associated with IR in their PCOS cohorts, these previous studies had smaller sample sizes than ours (231, 251). More importantly, fiber intake was not adjusted for total calories consumed

as in our study. Similarly, while it was found that neither caloric or macronutrient intake were associated with IR in women with PCOS, the use of 24-hour dietary recall, for only one day had a greater likelihood for error due to reliance on memory compared to a food record (251). Furthermore, by obtaining three days of data, we accounted for day-to-day variability, without compromising participants' attention to detail (which may occur with more than four days of records) (222). Lastly, we found that fiber intake was significantly reduced in women with PCOS when compared to women without PCOS which agrees with findings by Wild et al, but in contrast to those of Moran et al (144, 236). Although Moran et al performed a large, longitudinal study, one major limitation was that PCOS diagnoses were self-reported, and therefore, the group with PCOS might have been underrepresented especially since PCOS is frequently undiagnosed (144, 252).

In the general population, dietary fiber intake has been inversely associated with T2D and CVD, two conditions that share similar risk factors of metabolic syndrome as seen in PCOS (237, 253, 254). Dietary fiber can help regulate blood glucose by slowing its absorption into the circulation thereby improving glucose tolerance. Soluble fiber lowers the postprandial glucose response, while insoluble fiber increases insulin sensitivity (237). The consumption of fiber has also been shown to help manage weight potentially through increased post-prandial satiety resulting in reduced overall caloric consumption (237). Indeed, women who consumed less dietary fiber had been shown to gain more weight over time (255). Even in populations with normal BMI, reduced fiber intake has been associated with T2D and metabolic syndrome (256). The daily recommended fiber intake for Canadian women is 25 grams per day, but in our study, women with PCOS consumed only an average of 19.6 grams per day. A small difference

of 5 to 10 grams of dietary soluble fiber daily has been shown to reduce LDL-cholesterol by 5% (257).

Women with PCOS have been reported to consume greater glycemic loads than women without PCOS (231, 258). However, we found higher glycemic loads in women with IR-PCOS like findings reported by Graff et al (139). Intake of carbohydrates with a low glycemic index reduces the rate of glucose absorption. In turn, duodenal enterocyte hormone secretion of incretins stimulates insulin secretion to lower glucose levels. Reduction in the glucose load over an extended period suppresses free fatty acid levels and improves insulin sensitivity and glucose levels (259). In non-PCOS populations, meta-analyses of randomized controlled trials have shown that low glycemic diets can reduce fasting insulin levels, pro-inflammatory markers, total and LDL-cholesterol (260-262). Meals with increased fiber can also alter the glycemic response and reduce glucose absorption by either hindering glucose absorption in the small intestine, and/or inhibiting α-amylase action (263). Therefore, we suggest both glycemic load and fiber be reported in studies as they are interrelated. Examining total glycemic load alone without a fiber analysis would fail to account for the effect of mixed meals (246). Our results support implementing high fiber, low glycemic meals in the management of IR in patients with PCOS (140, 141).

PCOS is a spectrum disorder resulting in phenotypic differences. Hyperandrogenism can increase the severity and associated risks of PCOS, and also contribute to anxiety, low self-esteem, poor body image and loss of female identity (102, 264, 265). Our analysis comparing women with HA PCOS to Non-HA PCOS did not show any differences in dietary intake, but when fiber intake was categorized by tertiles, testosterone and DHEA-S were

increased in those who consumed less fiber. It is known that higher BMI, as well as IR with compensatory hyperinsulinemia (IR/HI), can exacerbate hyperandrogenism (54, 266). While we did not find an association between BMI and fiber, our findings were consistent with the well-documented association between IR/HI and hyperandrogenism and identified a potential dietary target to improve IR/HI and hyperandrogenism. Katcher et al demonstrated that differences in acute postprandial testosterone and DHEA-S levels were dependent on meal composition (a high fiber, low fat meal was compared to a high fat, low fiber meal) (267). Our study further demonstrated that regular dietary fiber intake over a long time period correlates not only with IR/HI, but with testosterone and DHEA-S levels. While our study utilized a standard androgen profile, recent studies have shown that adrenal 11-oxygenated androgens are substantial contributors to the total circulating androgen pool in PCOS and correlate with IR (268). Nonetheless, our results indicate that increasing fiber is an important dietary target in the management of hyperandrogenic PCOS.

A strength of our study was our adjustment of nutrient intakes, using the residual method, to control for the total amount of food consumed. In epidemiologic studies, potential associations between the prevalence of disease and specific nutrient intakes can be overlooked if variations in total energy intake are not adjusted (224). Other strengths include our analysis of dietary differences between PCOS phenotypes and the use of an objective method (a pedometer) to evaluate physical activity, both recommended in a recent literature review (136). Additionally, PCOS as a diagnosis was strictly defined according to the Rotterdam criteria and assessed through consistent methods by one reproductive endocrinologist (APC) as part of routine clinical practice. Some of these factors may contribute to varied findings from the nutritional studies

available on PCOS. Finally, our study is the first to assess dietary intake and physical activity in women with PCOS residing in Canada (13).

While the women with PCOS participating in this study were younger, the difference of five years in the reproductive age group would be expected to have little impact on metabolic health or dietary intake. Under-reporting can be a limitation when assessing dietary intake through self-report methods, especially in groups more likely to under-report (ex. those with higher BMI) (225). However, removing under-reporters, according to the Goldberg Cutoff method, did not affect the statistical significance found (230, 250). Miscommunication of portion size is a common limitation in self-report food records, but this was addressed by encouraging the use of measuring tools, the addition of food photographs, and providing participants with a list of common objects to compare to their portion sizes. Finally, our findings apply to women with PCOS whose primary reason for seeking medical care is infertility and may not be generalizable to all women with PCOS.

In conclusion, we found that women with PCOS and obesity were not in a caloric surplus state. However, dietary components, specifically low fiber, and high glycemic load, may contribute to IR/HI and obesity. In addition, low fiber intake may contribute to hyperandrogenemia. Future randomized controlled trials are required to determine the benefit of high fiber, low glycemic diets in improving glucose tolerance, and preventing metabolic complications in women with PCOS.

2.2 Evaluating micronutrient intake and its associations with obesity, insulin resistance and dyslipidemia in PCOS – *a cohort study*

2.2.1 Introduction

Specific micronutrients have been identified for managing PCOS, as discussed in Chapter 1, Section 1.10.1.7. Magnesium is critical for maintaining glucose and insulin homeostasis (269). In a large study including 1485 women and 1223 men without T2D, intakes of magnesium were inversely associated with the following T2D risk factors: fasting insulin, post-glucose challenge plasma insulin and HOMA-IR (270). Serum magnesium levels are often decreased in patients with T2D (165). While magnesium status has been inversely associated with metabolic syndrome and BMI in the general population, its role in PCOS is unclear. When magnesium intake was evaluated in women with PCOS, it was found that their intake was no different than women without PCOS and did not correlate with fasting insulin or insulin-to-glucose ratio (231). However, more recent evidence concluded that micronutrient intake, such as magnesium and iron, was greater for women with PCOS (144). In addition, there is evidence that magnesium deficiency is not related to IR in PCOS (271). Therefore, it is uncertain if specific dietary micronutrient intakes differ between women with and without PCOS. Furthermore, it is ambiguous whether micronutrient intakes contribute to higher rates of obesity, IR and dyslipidemia in PCOS.

Previously, in Section 2.1, data were presented from a cohort study assessing caloric and macronutrient intake of women with PCOS and non-PCOS. It was observed that the women with PCOS had overall higher BMIs and yet were not in a caloric surplus, and had no differences in percent intake of carbohydrates, fat, or protein. Therefore, the following observational study

assesses if inadequate micronutrient intake, such as magnesium, contributes to IR and dyslipidemia in PCOS.

2.2.2 Methods

The Methods used were identical as those discussed in Section 2.1.2, including the same population, except the outcome measurements were dietary micronutrient intake. The micronutrients assessed were vitamins A, C, E, folate, calcium, iron, sodium, zinc, magnesium and cholesterol. All micronutrient intakes were adjusted for caloric intake using the residual method and reported as both crude and adjusted amounts.

2.2.3 Results

All Participants

The ethnicity of study participants was 42% East Asian, 40% European, 15% South Asian, 2% Aboriginal and 1% South American. Women with PCOS had significantly higher BMI ($P < 0.001$), higher WHR ($P < 0.01$) and were younger ($P < 0.001$) than women without PCOS (Table 2.1).

Women with PCOS consumed less magnesium (adjusted: 238.9 vs. 273.9 mg, $P < 0.05$) and less vitamin A (adjusted: 6245.3 vs. 8366.1 IU, $P < 0.01$) than women without PCOS (Table 2.4).

Participants with PCOS

Women with IR-PCOS consumed significantly less magnesium than women with non-IR-PCOS (adjusted: 208.4 mg vs. 264.5, $P = 0.04$) (Table 2.5, Figure 2.7). IR was negatively correlated with magnesium intake (rho = -0.32, $P < 0.01$), and positively correlated with cholesterol intake (rho = 0.25, $P = 0.04$).

In women with PCOS, magnesium intake was less in women with obesity, but this was not statistically significant (adjusted: 210.5 vs. 247.0 mg, P = ns) (Table 2.5).

Magnesium intake was also negatively correlated with CRP (rho = -0.47, P < 0.001, Figure 2.8), testosterone (rho = -0.30, P < 0.01, Figure 2.9), and positively correlated with HDL cholesterol (rho = 0.29, P = 0.01). All other vitamin and mineral intakes assessed were not related to BMI, WHR or PCOS phenotype.

2.2.4 Discussion

While serum magnesium deficiencies have been previously reported in women with PCOS, this is the first study in women with PCOS to find associations between habitual dietary magnesium intake and hormonal and metabolic outcomes. Not only were women with PCOS, as a group, consuming less magnesium, but this insufficiency may have contributed to their increase in IR and obesity. A similar finding was discovered in a large population study in Newfoundland. The authors reported decreased magnesium intake in people with IR, particularly for those who were obese (272).

In addition, magnesium intake may be related to dyslipidemia given its positive correlation with HDL cholesterol. A rat study demonstrated that magnesium supplementation can improve lipid levels, however, a recent meta-analysis concluded that magnesium supplementation had no effect on both diabetic and non-diabetic patients (273, 274).

Systemic low-grade inflammation is one possible factor in the progression and manifestation of PCOS. Our results indicated that magnesium intake and CRP, a marker of inflammation, were negatively correlated. This same observation has been made in the general population through meta-analyses and systematic review (275). In women with PCOS, a

randomized controlled trial was published in 2018 identifying magnesium and zinc co-supplementation to be beneficial for decreasing inflammation (276).

Another micronutrient that was significantly decreased in the diets of women with PCOS was vitamin A. Vitamin A has been implicated in animal studies as essential for fetal pancreatic beta cell growth and development. Its deficiency is associated with diabetes in these animals (277). A more recent study further implicates an important role for the vitamin A metabolite, all-trans retinoic acid, on human pancreatic beta islet cell function and thereby insulin output via the GPRC5C receptor (278).

Our results associating cholesterol intake with IR were consistent with the biological role of cholesterol and emphasizes the equal importance of refining dietary components in advising lifestyle changes in PCOS. Serum levels of magnesium have been found to be positively correlated with HDL-cholesterol, although not statistically significant (279).

In conclusion, we found that dietary micronutrient intake, particularly magnesium, may contribute to IR, obesity, dyslipidemia, and hyperandrogenism in women with PCOS. Supplementation of magnesium may prove beneficial for this population in future randomized controlled trials.

Table 2.1 Characteristics and daily dietary intake in women with and without PCOS.

Characteristics	PCOS (n=87)	Non-PCOS (n=50)
Age (years)	30.7 (4.6)	35.7 (5.2)[a]
BMI (kg/m^2)	29.0 (7.1)	24.1 (5.1)[a]
WHR	0.84 (0.08)	0.79 (0.07)[b]
FSH (IU/L)	5.4 (1.7)	6.2 (2.9)
E_2 (pmol/L)	165.9 (70.5)	201.9 (176.9)
P (μg/L)	12.1 (8.7)	10.6 (4.8)
TSH (mIU/L)	2.4 (2.3)	2.0 (0.9)
BMR (kcal)	1439 (1353-1604)	1362 (1301-1414)
Under-reporters (%)	23.0	12.5
Dietary Intake		
Energy (kcal)	1783 (1516–1966)	1815 (1578–2083)
Step Count	6554 (4918-9173)	7234 (5558-8663)
Protein (%)	16.8 (14.2-19.8)	16.4 (14.4-18.8)
Carbohydrate (%)	46.2 (42.4-50.8)	49.0 (42.7-52.2)
Fat (%)	36.0 (32.3-39.2)	34.0 (30.1-38.7)
Fiber (g): Crude	19.6 (15.9-23.9)	23.3 (19.4-31.8)[b]
Adjusted	19.6 (15.7-24.0)	24.7 (19.7-30.7)[b]
Glycemic Load: Crude	84.1 (58.9-106.2)	86.0 (68.2-105.1)
Adjusted	83.7 (66.8-105.7)	83.0 (69.0-107.5)

Note: Statistical significance where 'a' denotes P≤0.001, 'b' denotes P≤0.01 and 'c' denotes P<0.05. Values are expressed as mean (SD) or median (interquartile range). While glycemic load and glycemic index are related, glycemic load accounts for both the amount and quality of carbohydrate while glycemic index refers only to the quality. Fiber and glycemic load are presented as both raw (crude) and adjusted data. The adjusted amount accounts for overall energy intake using the residual method (227). BMI: body mass index; WHR: waist to hip ratio; FSH: follicle-stimulating hormone; E_2: estradiol; P: prolactin; TSH: thyroid-stimulating hormone; BMR: calculated basal metabolic rate according to Schofield equation (230).

Table 2.2 Characteristics of women with PCOS by BMI and insulin resistance.

Characteristics	BMI Category			Insulin Resistance	
	Normal (n=25)	Overweight (n=31)	Obese (n=31)	HOMA-IR < 3.8 (n=30)	HOMA-IR ≥ 3.8 (n=42)
BMI (kg/m^2)	22.2 (1.8)	27.0 (1.4)	36.6 (6.3)[a]	26.0 (4.4)	32.7 (7.6)[a]
WHR	0.81 (0.08)	0.85 (0.05)	0.86 (0.09)[c]	0.83 (0.1)	0.86 (0.1)
FBG (mmol/L)	4.9 (0.4)	5.1 (0.6)	5.4 (0.7)[b]	4.8 (0.4)	5.4 (0.7)[a]
2hr gluc (mmol/L)	5.3 (1.6)	6.6 (1.9)	8.6 (2.8)[a]	5.7 (1.6)	8.2 (2.7)[a]
Insulin (pmol/L)	59.3 (38.7)	132.4 (65.9)	222.0 (147.9)[a]	56.5 (24.2)	218.9 (118.7)[a]
HOMA-IR	1.9 (1.4)	4.5 (2.5)	8.0 (6.4)[a]	1.8 (0.8)	7.8 (5.1)[a]
LH (IU/L)	6.9 (5.3)	8.2 (4.6)	7.6 (4.1)	6.3 (4.5)	8.3 (4.0)
FSH (IU/L)	5.4 (1.6)	5.4 (1.5)	5.5 (1.9)	5.0 (1.8)	5.8 (1.6)[c]
LH:FSH	1.2 (0.7)	1.5 (0.7)	1.4 (0.7)	1.3 (0.8)	1.4 (0.6)
E_2 (pmol/L)	159.3 (86.4)	159.5 (55.5)	177.9 (66.5)	168.0 (84.1)	164.4 (59.3)
P (nmol/L)	1.9 (0.8)	2.5 (1.9)	2.1 (1.9)	2.8 (1.8)	2.7 (0.5)
17-OHP (nmol/L)	1.9 (1.4)	1.5 (0.5)	1.4 (1.5)	1.5 (1.1)	1.6 (1.4)
A_4 (nmol/L)	5.5 (2.7)	6.2 (3.8)	4.7 (2.5)	4.8 (2.6)	5.5 (3.0)
T (nmol/L)	1.2 (0.5)	1.4 (0.6)	1.4 (0.7)	1.2 (0.5)	1.5 (0.7)
DHEAS (μmol/L)	6.3 (1.9)	8.3 (3.4)	6.1 (2.4)[b]	6.9 (2.7)	6.9 (2.9)
TC (mmol/L)	4.9 (0.8)	4.9 (0.8)	4.9 (1.1)	4.7 (0.9)	5.0 (0.9)
LDL-C (mmol/L)	2.7 (0.7)	3.0 (0.8)	2.9 (0.8)	2.8 (0.8)	3.0 (0.8)
HDL-C (mmol/L)	1.7 (0.6)	1.4 (0.7)	1.2 (0.2)[b]	1.5 (0.4)	1.3 (0.7)
TG (mmol/L)	1.0 (0.6)	1.5 (0.9)	1.6 (0.6)[c]	0.9 (0.4)	1.8 (0.7)[a]
TC:HDL-C	3.1 (0.9)	4.0 (1.2)	4.1 (0.9)[b]	3.3 (0.8)	4.3 (1.1)[a]

Note: Statistical significance where 'a' denotes P≤0.001, 'b' denotes P≤0.01, 'c' denotes P<0.05. Values are expressed as mean (SD). BMI is categorized as normal (between 18.5 and 24.9 kg/m^2), overweight (between 25 and 29.9 kg/m^2) and obese (over 30 kg/m^2). A HOMA-IR of 3.8 or higher defines insulin resistance. Statistical tests were performed comparing anthropometric characteristics among women in normal, overweight and obese BMI categories. In addition, statistical tests were performed comparing anthropometric characteristics between women with insulin resistance and women without insulin resistance. BMI: body mass index, WHR: waist to hip ratio, FBG: fasting blood glucose; HOMA-IR: homeostasis model of assessment for insulin resistance; LH: luteinizing hormone; FSH: follicle stimulating hormone; E_2: estradiol; P: prolactin; 17-OHP: 17-hydroxyprogesterone; A_4: androstenedione; T: total testosterone; DHEAS: dehydroepiandrosterone sulfate; TC: total cholesterol; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TG: triglycerides.

Table 2.3 Daily dietary intake of women with PCOS by BMI and insulin resistance.

Dietary Intake	BMI Category			Insulin Resistance	
	Normal (n=25)	Overweight (n=31)	Obese (n=31)	HOMA-IR < 3.8 (n=30)	HOMA-IR ≥ 3.8 (n=42)
Energy (kcal)	1713 (1380-1943)	1853 (1628-1979)	1783 (1512-1928)	1792 (1346-1976)	1858 (1660-1992)
Steps (/day)	6813 (5138-11360)	6796 (4902-9402)	5967 (4605-8123)	5985 (4930-8337)	6167 (4664-8297)
Protein (%)	16.7 (14.1-18.2)	16.7 (14.4-20.0)	17.6 (14.6-21.0)	16.7 (14.1-19.2)	17.4 (14.4-20.6)
Carbohydrate (%)	48.3 (41.1-53.9)	46.7 (43.6-50.6)	45.2 (41.4-48.2)	45.5 (42.1-51.8)	46.3 (43.3-50.3)
Fat (%)	34.9 (32.0-41.2)	36.0 (32.3-38.0)	36.0 (33.0-42.2)	36.1 (34.1-37.9)	35.1 (30.0-38.8)
Fiber (g):					
Crude	20.9 (17.1-26.5)	20.0 (15.1-23.8)	17.5 (15.7-22.1)	22.2 (15.6-26.5)	17.9 (16.0-22.6)
Adjusted	22.1 (18.5-26.2)	19.3 (15.9-23.2)	18.2 (14.4-23.6)	22.1 (17.6-26.2)	18.6 (14.0-23.4)[c]
Glycemic Load:					
Crude	69.3 (55.3-93.1)	93.4 (59.6-108.4)	84.6 (63.7-105.8)	69.3 (56.3-96.0)	93.2 (78.0-111.5)[b]
Adjusted	83.6 (67.0-96.3)	82.7 (60.8-104.3)	94.7 (68.4-108.2)	83.5 (61.4-95.9)	93.5 (73.6-109.4)[c]

Note: Statistical significance where 'a' denotes P≤0.001, 'b' denotes P≤0.01, 'c' denotes P<0.05. Values are expressed as median (interquartile range). Fiber and glycemic load are presented in crude and adjusted intakes. BMI is categorized as normal (between 18.5 and 24.9 kg/m^2), overweight (between 25 and 29.9 kg/m^2) and obese (over 30 kg/m^2). A HOMA-IR of 3.8 or higher defines insulin resistance. Statistical tests were performed comparing dietary intake among women in normal, overweight and obese BMI categories. In addition, statistical tests were performed comparing dietary intakes between women with insulin resistance and women without insulin resistance. BMI: body mass index; HOMA-IR: homeostasis model of assessment for insulin resistance.

Table 2.4 Daily micronutrient intake in women with and without PCOS.

Dietary Intake	PCOS (n=87)	Non-PCOS (n=50)
Vitamin A (IU): Crude	6738.0 (2177.7-10139.3)	8591.7 (5002.5-14214.9)[b]
Adjusted	6245.3 (2533.1-9401.8)	8366.1 (4940.7-14954.4)[b]
Vitamin C (mg): Crude	96.5 (60.5-126.4)	112.8 (60.5-181.7)
Adjusted	100.8 (52.9-176.0)	111.2 (66.7-167.4)
Vitamin D (mcg): Crude	2.53 (1.40-4.72)	2.21 (1.31-3.67)
Adjusted	2.83 (1.64-4.69)	2.17 (1.29-3.80)
Folate (mcg): Crude	256.5 (168.9-369.9)	283.5 (204.2-355.9)
Adjusted	255.6 (173.4-358.3)	276.1 (211.6-353.9)
Calcium (mg): Crude	606.5 (428.2-807.8)	637.5 (465.8-797.7)
Adjusted	658.3 (474.1-799.2)	606.9 (504.6-791.0)
Iron (mg): Crude	11.6 (9.0-14.7)	13.3 (11.4-15.0)[c]
Adjusted	11.8 (9.4-14.0)	12.2 (10.7-14.9)
Sodium (mg): Crude	2188.0 (1517.6-2874.5)	2182.1 (1718.4-2898.7)
Adjusted	2242.2 (1632.5-2825.1)	2065.1 (1601.3-2873.6)
Zinc (mg): Crude	7.85 (5.7-10.6)	8.7 (6.6-10.8)
Adjusted	8.12 (6.48-10.5)	9.10 (7.15-10.1)
Magnesium (mg): Crude	236.7 (157.9-326.9)	292.7 (209.6-417.5)[c]
Adjusted	238.9 (185.3-312.6)	273.9 (213.7-402.9)[c]
Cholesterol (mg): Crude	284.1 (179.9-430.3)	260.8 (146.0-340.6)
Adjusted	297.5 (183.9-439.8)	254.9 (146.0-338.6)

Note: Statistical significance where 'a' denotes $P \leq 0.001$, 'b' denotes $P \leq 0.01$ and 'c' denotes $P < 0.05$. Values are expressed as median (interquartile range). Micronutrients are presented as both raw (crude) and adjusted data. The adjusted amount accounts for overall energy intake using the residual method (227).

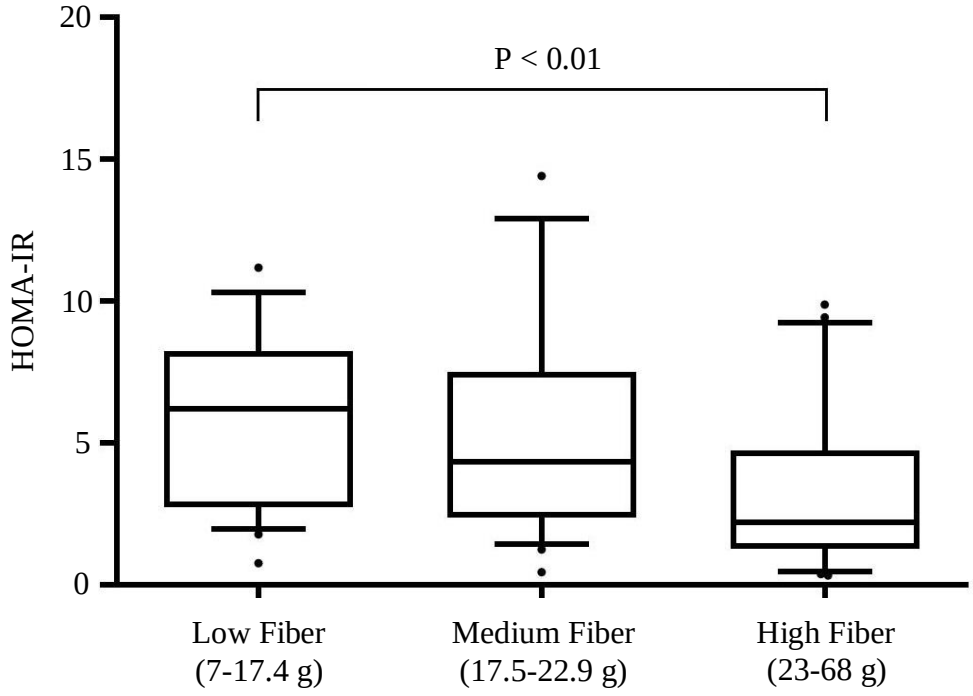
Table 2.5 Daily micronutrient intake of women with PCOS by BMI and insulin resistance.

Dietary Intake	BMI Category			Insulin Resistance	
	Normal (n=25)	Overweight (n=31)	Obese (n=31)	HOMA-IR < 3.8 (n=30)	HOMA-IR ≥ 3.8 (n=42)
Vitamin A (IU):					
Crude	7883.0 (2365.1-9279.1)	6985.76 (3755.7-11350.2)	3055.8 (1997.6-7061.2)	6863.28 (3070.3-11407.9)	4737.14 (2089.9-7281.7)
Adjusted	7807.5 (2838.7-9618.4)	6979.9 (3824.4-11419.1)	3296.1 (1877.6-6869.6)	7162.02 (3214.3-11872.4)	4433.22 (2267.26-7277.4)
Vitamin C (mg):					
Crude	103.7 (68.8-177.9)	107.8 (78.5-150.8)	63.8 (40.4-108.7)	100.4 (67.9-120.9)	91.33 (52.7-124.8)
Adjusted	103.2 (73.2-170.0)	106.1 (76.8-153.6)	66.8 (41.4-111.4)	98.9 (68.4-119.8)	87.8 (51.5-131.4)
Vitamin D (mcg):					
Crude	2.17 (1.18-4.71)	2.74 (1.61-5.15)	2.76 (1.77-3.88)	2.51 (1.21-4.71)	2.88 (1.58-4.17)
Adjusted	2.29 (1.43-4.00)	2.85 (1.68-5.38)	2.97 (1.63-3.89)	2.38 (1.63-4.46)	2.93 (1.63-4.11
Folate (mcg):					
Crude	227.2 (155.1-421.7)	264.7 (172.3-363.9)	268.8 (182.0-300.6)	237.8 (158.7-365.3)	267.9 (167.0-295.7)
Adjusted	242.6 (167.6-404.8)	266.2 (174.6-358.7)	254.9 (185.7-309.5)	231.5 (192.5-355.3)	252.8 (164.8-301.7)
Calcium (mg):					
Crude	710.8 (455.3-818.5)	552.6 (470.1-771.0)	580.6 (345.7-782.4)	687.5 (431.5-849.7)	569.5 (406.0-796.1)
Adjusted	748.6 (593.2-818.6)	576.3 (420.8-747.4)	616.1 (436.8-759.6)	686.6 (530.6-845.5)	604.2 (414.3-708.8)
Iron (mg):					
Crude	11.4 (9.0-14.8)	11.6 (9.5-14.3)	11.6 (8.7-13.1)	10.78 (8.88-14.3)	11.65 (9.65-14.0)
Adjusted	10.8 (9.5-14.7)	11.5 (9.4-13.0)	11.8 (8.7-13.1)	10.67 (9.35-13.83)	11.47 (9.03-13.13)
Sodium (mg):					
Crude	2115.1 (1886.9-2932.5)	2108.9 (1379.3-2824.5)	2374.3 (1601.6-3092.2)	2050.34 (1522.4-2710.3)	2342.32 (1590.0-3553.4)
Adjusted	2195.5 (1889.4-2915.8)	2024.6 (1607.3-2480.1)	2396.4 (1888.1-2972.2)	2126.65 (1691.7-2508.1)	2388.13 (1612.6-3116.0)
Zinc (mg):					
Crude	8.12 (6.06-10.0)	8.27 (6.04-10.56)	7.32 (5.30-10.24)	7.65 (5.55-9.15)	8.28 (5.97-11.16)
Adjusted	7.51 (6.23-9.14)	8.36 (7.30-10.5)	7.51 (5.28-10.33)	7.93 (6.37-10.43)	7.94 (6.78-10.31)

Dietary Intake	BMI Category			Insulin Resistance	
	Normal (n=25)	Overweight (n=31)	Obese (n=31)	HOMA-IR < 3.8 (n=30)	HOMA-IR ≥ 3.8 (n=42)
Magnesium (mg):	251.0	241.5	200.4	240.7	212.3
Crude	(157.7-385.2)	(164.9-332.2)	(161.8-260.3)	(147.4-398.4)	(156.7-289.0)
Adjusted	247.0	243.5	210.5	264.5	208.4
	(201.4-359.8)	(188.1-340.8)	(156.2-275.1)	(195.1-348.1)	(153.1-278.5)[c]
Cholesterol (mg):					
Crude	248.4	220.3	416.4	228.2	300.6
	(120.8-349.7)	(173.6-352.5)	(225.4-568.3)	(171.2-368.6)	(183.4-507.0)
Adjusted	261.9	214.9	432.9	213.45	302.29
	(126.4-350.3)	(183.5-368.9)	(221.2-559.0)	(178.8-374.9)	(178.8-504.1)

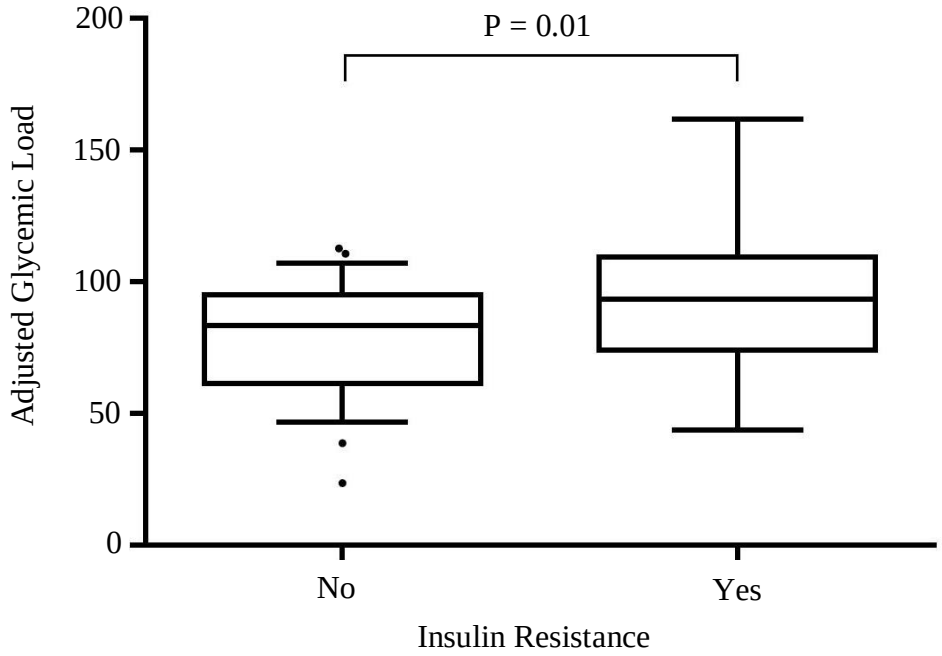
Note: Statistical significance where 'a' denotes P≤0.001, 'b' denotes P≤0.01, 'c' denotes P<0.05. Values are expressed as median (interquartile range). Micronutrients are presented in crude and adjusted intakes. BMI is categorized as normal (between 18.5 and 24.9 kg/m^2), overweight (between 25 and 29.9 kg/m^2) and obese (over 30 kg/m^2). A HOMA-IR of 3.8 or higher defines insulin resistance. Statistical tests were performed comparing dietary intake among women in normal, overweight and obese BMI categories. In addition, statistical tests were performed comparing dietary intakes between women with insulin resistance and women without insulin resistance.

Figure 2.1 Daily fiber intake and HOMA-IR of women with PCOS.



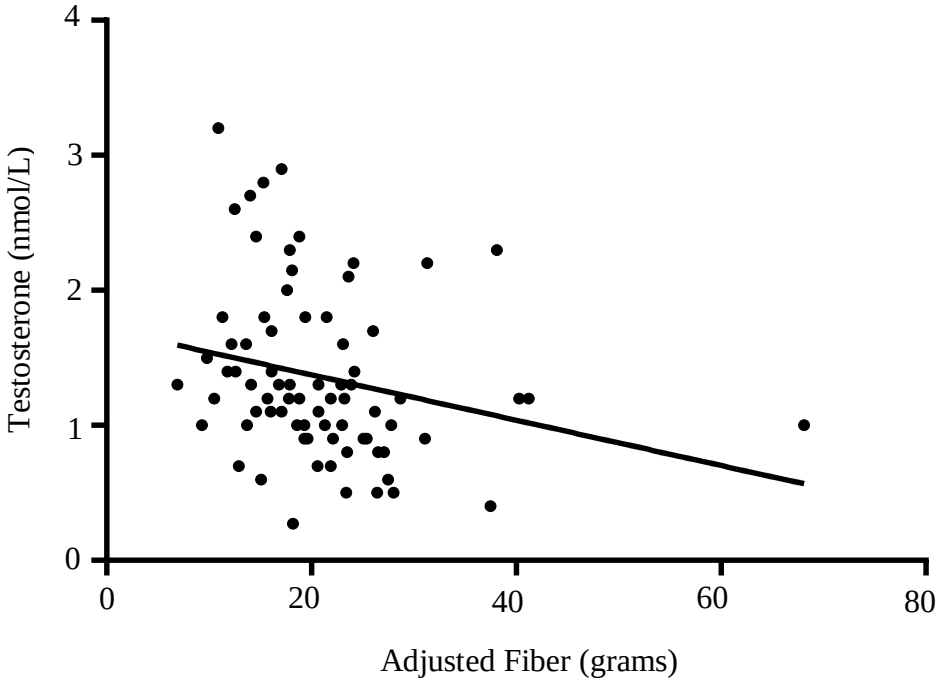
Note: HOMA-IR was compared according to tertiles of adjusted fiber intake (n = 72). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.2 Daily intake of glycemic load in women with PCOS and insulin resistance.



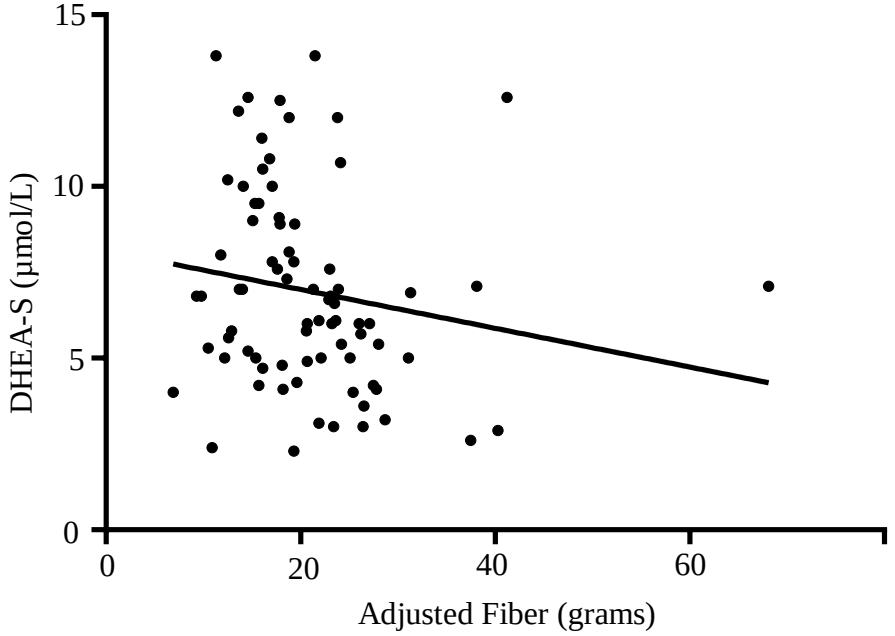
Note: Adjusted intake of glycemic load was compared according to the HOMA-IR cut-off of 3.8 (n = 72). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.3 Serum testosterone levels and daily dietary fiber intake in women with PCOS.



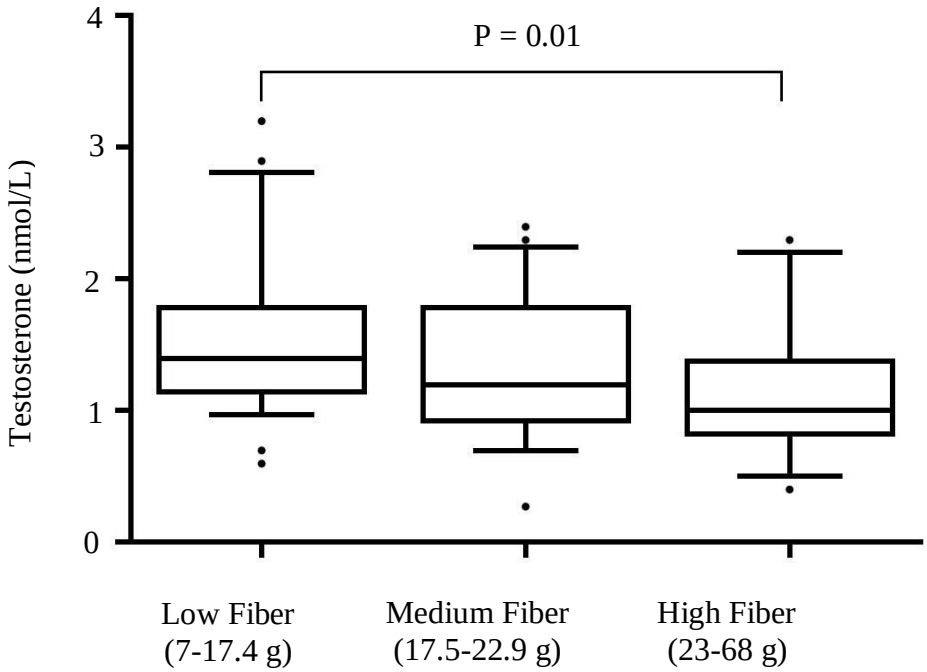
Note: Serum testosterone was negatively correlated with intake of adjusted fiber (n = 78). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.4 Serum DHEA-S levels and daily dietary fiber intake in women with PCOS.



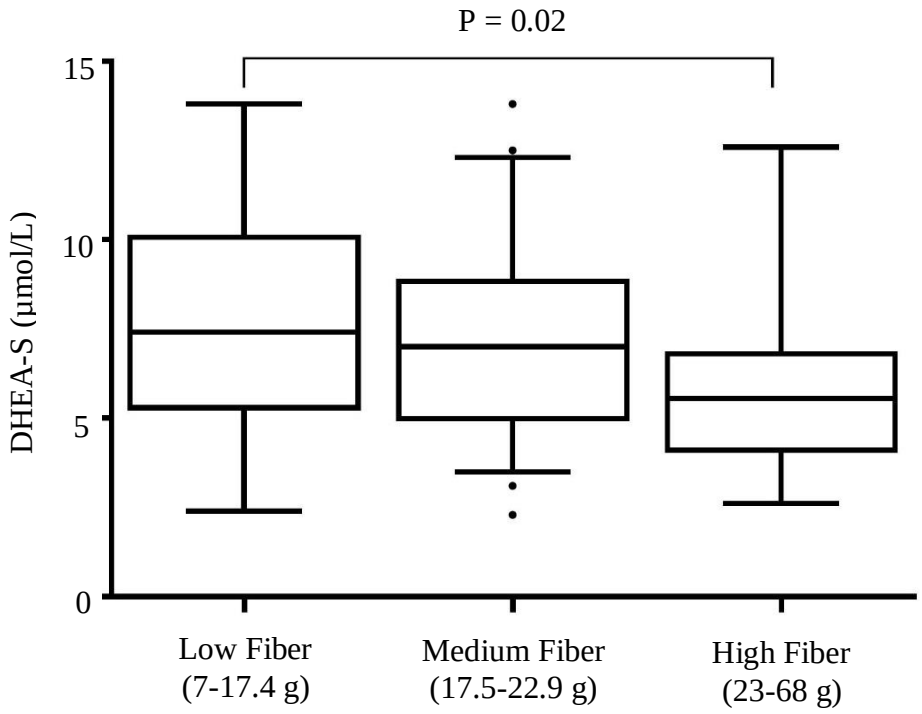
Note: Serum DHEA-S was negatively correlated with intake of adjusted fiber (n = 79). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.5 Serum testosterone levels and daily dietary fiber intake in women with PCOS.



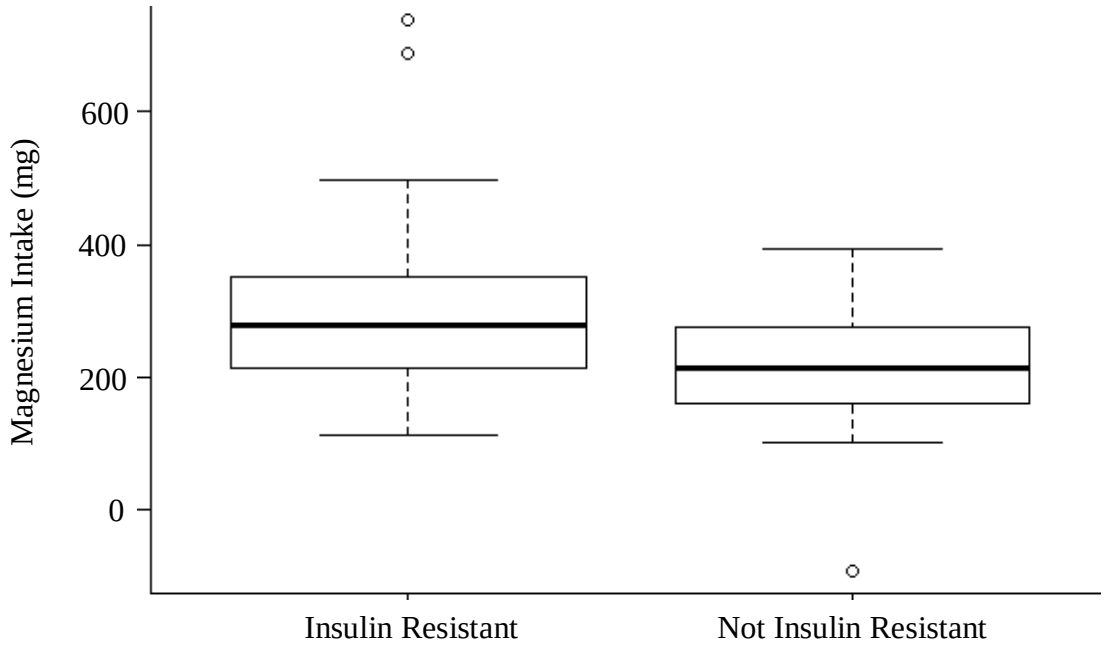
Note: Serum testosterone was compared according to tertile of adjusted fiber intake (n = 78). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.6 Serum DHEA-S levels and daily dietary fiber intake in women with PCOS.



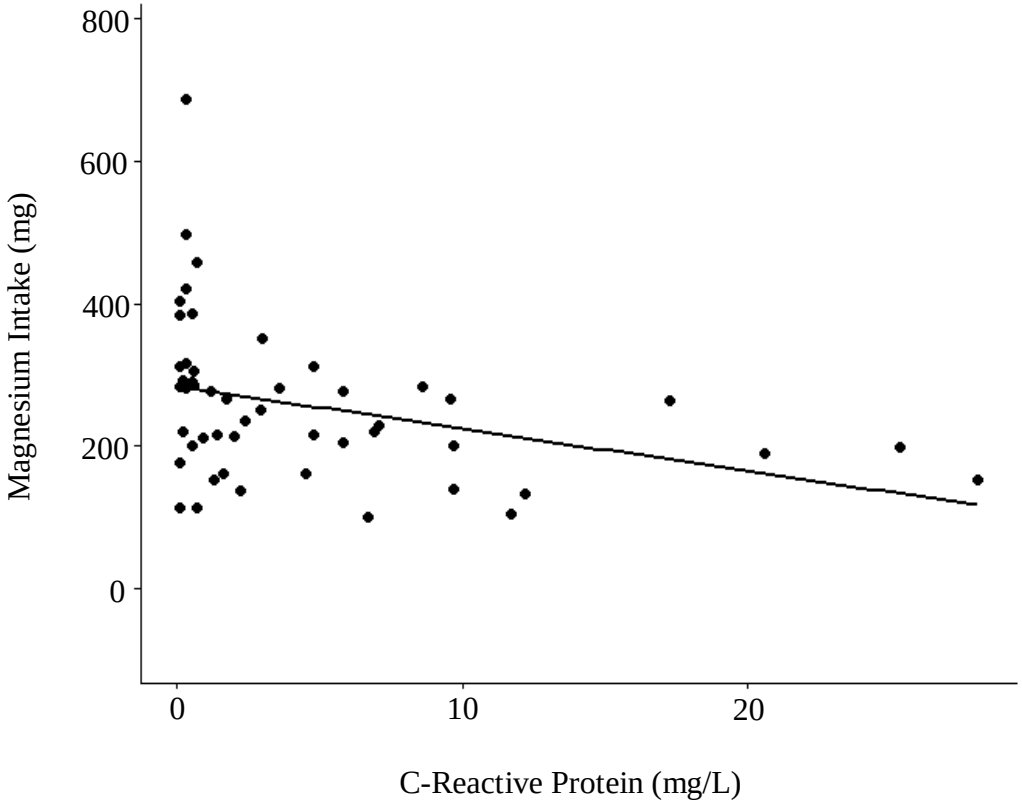
Note: Serum DHEA-S was compared according to tertile of adjusted fiber intake (n = 79). Figure previously published in "Cutler et al. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12."

Figure 2.7 Daily magnesium intake and insulin resistance in women with PCOS.



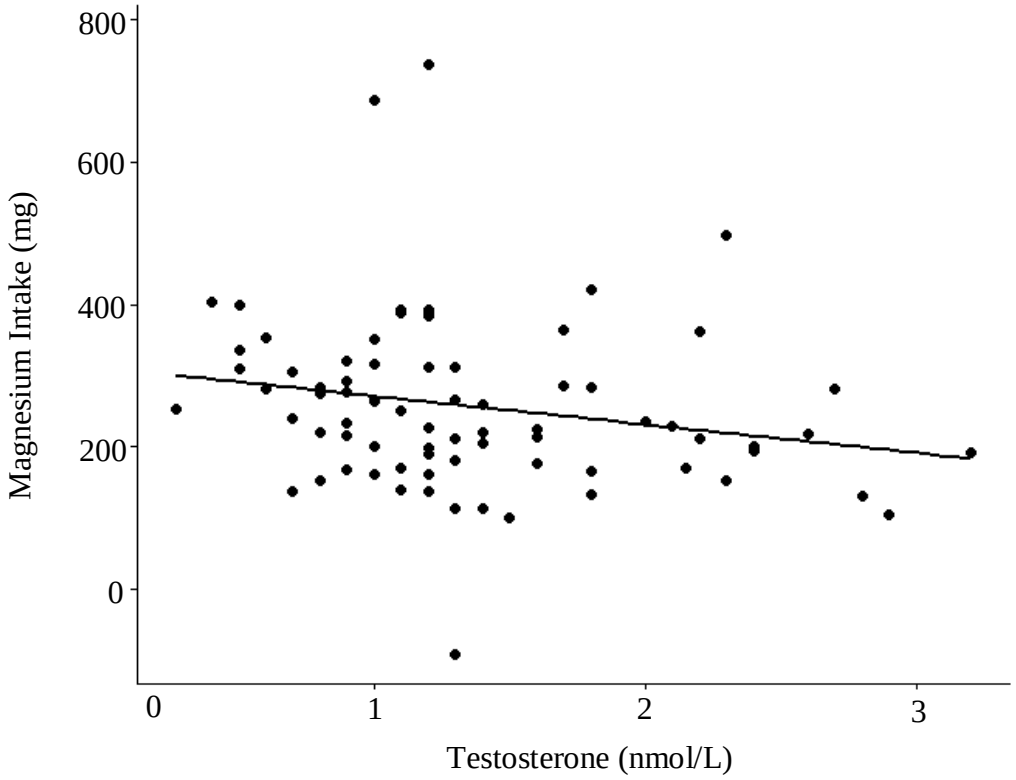
Note: Adjusted magnesium intake was compared according to the HOMA-IR cut-off of 3.8 (n = 72).

Figure 2.8 Daily magnesium intake and C-reactive protein in women with PCOS.



Note: Adjusted magnesium intake was negatively correlated with C-reactive protein (n = 51).

Figure 2.9 Daily magnesium intake and total testosterone in women with PCOS.



Note: Adjusted magnesium intake was negatively correlated with serum testosterone (n = 78).

Chapter 3: Determinants of Psychological Health in PCOS

3.1 Determining the associations between levels of depression, anxiety, stress, and quality of life with symptoms of PCOS – *a cohort study*

3.1.1 Introduction

Previous literature has reported that women with PCOS are at a significantly increased risk for developing psychiatric co-morbidities, including depressive and anxiety disorders, when compared to women without PCOS (5, 92). However, limited high-quality studies and conflicting reports have resulted in inconclusive findings to explain this increased distress, as discussed in Chapter 1, Section 1.8.8. Poor body image due to obesity, hirsutism, and acne have been proposed as potential links to decreased psychological well-being in women with PCOS (90, 93-95, 97, 99).

The first aim of this study was to investigate symptoms of depression, anxiety, stress and fertility-related QoL in women with and without PCOS. Our second aim was to determine how psychological distress relates to the physical, biochemical and ethnic differences among women with PCOS. Lastly, we aimed to identify whether particular phenotypes of PCOS are at greater risk of decreased psychological well-being.

3.1.2 Methods

Study Design, Setting and Participants

A cohort study was performed between May 2014 and April 2017 at Grace Fertility Centre in Vancouver, Canada. Women of reproductive age (18 to 45 years) with sub-fertility were given the opportunity to participate in the study. Of these women, 302 completed the study including 152 women diagnosed with PCOS (Rotterdam criteria), and 150 sub-fertile women without PCOS. Of the 152 women with PCOS, 85 were categorized as ‘Hyperandrogenic (HA)

PCOS' when all three Rotterdam criteria were present or if hyperandrogenism and oligo-anovulation were present without PCOM on ultrasound. The remaining 67 women presented with both oligo-/anovulation and PCOM on ultrasound, and therefore were categorized as 'Non-androgenic (Non-HA) PCOS'. There were no women in the PCOS population diagnosed with 'Ovulatory PCOS'. The infertility diagnoses of the control group were unexplained (40%), diminished ovarian reserve (19%), male factor (15%), prior history of oligo-ovulation (10%), tubal factor (3%), and mixed (13%).

Psychological Well-being Assessment

Participants completed the Depression, Anxiety and Stress Scale (DASS) and the Fertility Quality of Life Questionnaire (FertiQoL) to quantify symptoms of depression, anxiety, stress and fertility-related QoL (including QoL subscales: emotional, relational, mind-body and social) (Appendices C and D). These self-report methods have both been validated and used widely (280, 281).

The DASS reports a single numerical score for each of the following: depression, anxiety, and stress. A higher score implies greater symptoms of depression, anxiety, or stress, accordingly. In addition, these scores are ranked on a scale of 1-5 with 1 being "normal", 2 being "mild", 3 being "moderate", 4 being "extreme" and 5 being "severely extreme" (Appendix C).

The FertiQoL provides a score for total QoL out of possible 100, the higher score implying a higher quality of living. In addition, four sub-categories are also each ranked out of 100: Emotional, Mind-Body, Relational and Social (Appendix D).

Anthropometrics and Physical Characteristics

All patients have their height, weight, waist and hip circumference recorded, and BMI and WHR calculated as a routine part of clinical practice at Grace Fertility Centre. Patients with

PCOS are routinely asked to self-report for the presence of hirsutism using the modified Ferriman-Gallway (mFG) score in addition to clinical assessment by the physician. Corresponding data for participants were compiled for the study.

Hirsutism and acne were also obtained as a self-reported categorical variable (yes/no) in addition.

Ultrasound Assessment, Biochemical and Hormonal Assays

Similarly, as a routine part of clinical practice at Grace Fertility Centre, all patients presented with PCOS or infertility have a baseline transvaginal ultrasound assessment by one physician (APC) using the EC9-5/10 endovaginal transducer (SonixTouch, Ultrasonix), and hormone measurements for FSH, estradiol (E_2), prolactin (P), and TSH (Abbott Architect Immunoassay) and C-reactive protein (CRP). Women with PCOS have additional hormone and metabolic measurements which include: LH, progesterone, androstenedione (A_4), and 17-OHP (Agilent 6410, LCMS methodology); testosterone (T), DHEA-S and fasting insulin (Roche Cobas e602 Immunoassay); blood glucose levels after a 12-hour fast (FBG) and at two hours after a 75 gram oral glucose tolerance test (2 hour glucose level) (Roche Cobas c701 Roche Diagnostics, hexokinase/G6P-DH method); total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TG) (Roche Cobas c701 Roche Diagnostics enzymatic colorimetric method, and with polyethylene glycol-modified enzymes and dextran sulfate for HDL-C). The corresponding data for participants were compiled for the study.

Statistical analyses

Calculated depression, anxiety, stress, and QoL scores were assessed for “statistical” normality by histogram visualizations and Shapiro-Wilk tests. In our sample, age was the only variable normally distributed. Multiple groups were compared by analysis of variance (ANOVA)

or its non-parametric equivalents and post-hoc analysis was assessed by Tukey and Kramer (Nemenyi) tests. The analysis included Student's t tests for normally distributed data and Mann-Whitney and Spearman's Rank tests for nonparametric data. Analysis of covariance was used to adjust for age and BMI when comparing women with PCOS to women without PCOS. OGTT was also adjusted for when necessary. All analyses were executed in R software. Data of statistical significance was considered as $P < 0.05$.

3.1.3 Results

Aim 1: To investigate symptoms of depression, anxiety, stress and fertility-related QoL in women with and without PCOS.

Baseline Characteristics

Women with PCOS were significantly younger than women without PCOS and had significantly greater BMI and WHR. Women with PCOS had significantly lower FSH levels, higher LH, and higher CRP levels, but after adjusting for BMI and age these differences were not statistically significant (Table 3.1). Participants reported their ethnicity resulting with 44% East Asian, 35% European, 15% South Asian, and the remaining 6% included women of Aboriginal, Iranian, Russian and/or South American descent.

Psychological Outcomes

Women with PCOS had significantly higher scores for symptoms of depression ($P = 0.02$), anxiety ($P < 0.001$), and stress ($P = 0.03$) than women without PCOS, however, after adjusting for BMI and age, only symptoms of anxiety remained significantly different ($P = 0.03$) (Table 3.3). Total QoL scores, as well as all QoL sub-categories, were no different between the two groups both before and after adjustments (Table 3.3).

Ethnic Differences

Psychological outcomes were compared between the four main ethnic groups in this study (Table 3.4). Women of South Asian descent and women grouped as "Others" (Aboriginal, Iranian, Russian and/or South American) had higher scores for symptoms of depression, anxiety and stress than women of East Asian and European descent, which did not reach statistical significance. Total QoL and sub-categories of QoL scores did not differ significantly between ethnic groups with the exception that East Asian women had greater emotional QoL than women grouped as "Others" ($P = 0.02$) (Table 3.4).

Aim 2: To determine how psychological distress relates to physical, biochemical and ethnic differences of women with PCOS.

BMI

The baseline characteristics of women with PCOS were compared between women with BMIs considered "normal" (between 18.5 and 24.9 kg/m^2), "overweight" (between 25 and 29.9 kg/m^2), and "obese" (over 30 kg/m^2). There were no significant differences between age, FSH, LH, estradiol, progesterone, 17-OHP, androstenedione, testosterone, DHEA-S, OGTT, fasting insulin or HOMA-IR based on BMI grouping. Women with "obese" or "overweight" BMI had higher fasting glucose levels than women who had "normal" BMI. After post-hoc analysis, women with "normal" BMI had significantly lower fasting glucose levels than women with "obese" BMI ($P < 0.001$). Symptoms of depression, anxiety, stress, QoL, and sub-categories of QoL did not differ significantly between women based on BMI grouping (Table 3.6). BMI was not significantly associated with symptoms of depression, anxiety, stress, total QoL or any QoL sub-categories.

WHR

The baseline characteristics of women with PCOS were compared among those with WHRs considered “normal” (under 0.80), “overweight” (between 0.80 and 0.84), and “obese” (equal to or over 0.85). There were no significant differences between age, FSH, LH, estradiol, progesterone, 17-OHP, androstenedione, testosterone, DHEA-S, OGTT, fasting insulin or HOMA-IR based on WHR grouping. Fasting glucose was significantly lower in women with a “normal WHR” compared to “overweight” or “obese” WHRs (P = 0.036). The post-hoc analysis found that women with “normal” WHR had significantly lower fasting glucose levels than women with “overweight” WHR (P = 0.017), but not women with “obese” WHR (P = 0.067). Symptoms of depression, anxiety, stress, QoL, and sub-categories of QoL did not differ significantly among women according to their WHR grouping (Table 3.7). WHR was not significantly associated with symptoms of depression, anxiety, stress, total QoL or any QoL sub-categories.

Insulin Resistance

The baseline characteristics of women with IR-PCOS were compared to women with non-IR-PCOS. There were no significant differences between age, FSH, LH, estradiol, progesterone, 17-OHP, androstenedione, testosterone or DHEA-S. Women with IR-PCOS had significantly higher levels of fasting glucose, OGTT, fasting insulin in addition to HOMA-IR scores. Symptoms of depression, anxiety, stress, QoL, and sub-categories of QoL did not differ significantly between IR-PCOS and non-IR-PCOS (Table 3.8). HOMA and OGTT were not significantly associated with symptoms of depression, anxiety, stress, total QoL or any QoL sub-categories.

Ethnic Differences

The baseline characteristics of women with PCOS were compared between the four ethnic groups and there were no significant differences between BMI, WHR, age, activity level, years trying to conceive, hirsutism, reproductive hormone levels, or metabolic health. In addition, there were no significant differences between symptoms of depression, anxiety, stress, QoL, or sub-categories of QoL (Table 3.5).

Aim 3: To identify which phenotypes of PCOS are at greater risk of decreased psychological well-being.

Hyperandrogenism

Women with HA PCOS had significantly higher levels of LH, estradiol, 17-OHP, androstenedione, and testosterone than women with Non-HA PCOS. Women with HA PCOS had significantly higher symptoms of anxiety and lower social QoL scores than women with Non-HA PCOS (Table 3.9). There were no correlations between LH, 17-OHP, androstenedione, testosterone, or DHEA-S with symptoms of depression, anxiety, stress, total QoL or any QoL sub-categories with the exception that androstenedione levels were negatively correlated with relational QoL (rho = -0.25, P = 0.02, Figure 3.1). Women with increased symptoms of anxiety (ranging from mild to extremely severe) had significantly greater amounts of hirsutism than women with no anxiety (P = 0.04). The severity of anxiety symptoms was positively associated with levels of self-reported hirsutism, but not to statistical significance (Figure 3.2). Estradiol was negatively correlated with symptoms of depression (rho = -0.19, P = 0.02), anxiety (rho = -0.18, P = 0.03, Figure 3.3), and stress (rho = -0.22, P = 0.01). There were no significant correlations between estradiol and total QoL or sub-categories of QoL. Women with PCOS and

acne had similar symptoms of depression, anxiety, stress, total QoL, emotional QoL, relational QoL, social QoL, and mind-body QoL as those without acne.

Infertility

Women with PCOS and primary infertility had similar symptoms of depression, anxiety, stress, and relational QoL as women with PCOS and secondary infertility. Women with PCOS and primary infertility had significantly lower levels of total QoL (P = 0.04, Figure 3.4), social QoL (P = 0.03), mind-body QoL (P = 0.03) and emotional QoL (P = 0.02) than women with PCOS and secondary infertility. There were no significant correlations between time to conceive measured in years and symptoms of depression, anxiety, stress, total QoL or any sub-categories of QoL in women with PCOS.

Explanatory Model for Anxiety

Multiple linear regression analysis was performed to determine key factors impacting increased anxiety symptoms in women with PCOS. The following variables were included in the regression model: reproductive hormones (estradiol, testosterone, androstenedione, DHEA-S), metabolic markers (HOMA, OGTT) and physical presentation (hirsutism, BMI, WHR). Backwards stepwise regression determined that androstenedione, estradiol, WHR, and hirsutism were the strongest predictors of anxiety symptoms in women with PCOS accounting for 26% of the variance ($r^2 = 0.26$, P = 0.034). Decreased estradiol was the most significant predictor of increased anxiety symptoms (P = 0.026).

3.1.4 Discussion

This study indicates that women with PCOS have increased anxiety symptoms when compared to other women with an infertility diagnosis. Further, the analysis demonstrates that this increase in anxiety may be related to hyperandrogenism. In particular, the symptom of PCOS

most associated with increased anxiety was hirsutism. This finding supports one previous study where hirsutism was associated with anxiety and an additional study where hirsutism was shown to negatively influence women's body image and QoL (98, 282). Women with PCOS and excessive hair growth have described themselves as a "freak", "disgrace", and have expressed feelings of living in a prison in their body (98). Methods to escape such distress range from removing the hair, covering the body and even contemplating suicide (98). Another study found that suicide attempts are seven times more common in women with PCOS than other women (283). Expectations of female beauty are rooted in Western societies and being hairless is one major social norm of femininity (98, 284, 285). Hirsutism is a more distressing symptom than may be perceived by those who do not suffer from this symptom.

Symptoms of depression and stress were also significantly greater for women with PCOS, but only before adjusting for BMI and age differences. This suggests that women with PCOS, particularly those of higher BMI and younger age, may be under higher psychological distress and at risk for developing depressive and stress symptoms.

When multiple linear regression was performed to examine the correlation between reproductive hormones and psychological well-being, estradiol and androstenedione were significant predictors of anxiety symptoms. In addition, estradiol was also a significant predictor of stress symptoms. Estradiol has been shown to be protective against anxiety and depressive symptoms (286).

This is one of the first studies to compare psychological well-being between various ethnic groups of women with PCOS. Our findings suggest that symptoms of depression, anxiety, stress, and overall QoL do not discriminate and that the psychological experience of PCOS appears to affect women of all ethnic backgrounds.

A major strength of this study was the large sample size which allowed for comparison between PCOS phenotypes and ethnic groups. In addition, previous literature of this magnitude often used a self-reported diagnosis of PCOS, while in this study PCOS was strictly defined by one clinician, and according to the widely accepted Rotterdam criteria. Also, the control group resulted in an accurate representation of the range of women being treated at a fertility centre. Thus, infertility was a unifying characteristic among both groups of women effectively removing it as a factor in the causes of psychological distress.

One limitation of this study is that it pertains to a specific population of higher socioeconomic status who are in generally good health, and therefore, results cannot be generalized across all women with PCOS.

In conclusion, psychological distress in the form of symptoms of anxiety, depression, and stress is increased in some women with PCOS. This observational study sheds light on the hyperandrogenic phenotype being most at risk for experiencing symptoms of anxiety and poorer QoL.

3.2 Evaluating the relationship between lifestyle and psychological health in PCOS – *a cohort study*

3.2.1 Introduction

The impact that lifestyle, such as dietary patterns and physical activity, has on the development and management of poor psychological health, such as anxiety and depression, is largely unknown. A systematic review published in 2010 summarized the data from 34 observational studies and concluded that dietary nutrients were not associated with symptoms of depression (287). However, more recently, further observational studies have linked high intakes of fruit, vegetables, fish, and whole grains to a reduced risk of depression while high intakes of sugar and processed foods have been associated with an increased risk of depression, as well as anxiety (288-290).

The results from the data presented in Chapter 2, Section 2 identified lower intakes of magnesium in women with PCOS. Magnesium is a critical mineral for regulating the hypothalamic-pituitary-adrenocortical axis. Magnesium status has been inversely associated with anxiety (290). A recent systematic review concluded that supplementation of magnesium can be beneficial for alleviating mild anxiety (291). In addition, the results of Chapter 3, Section 1 identified decreased levels of psychological well-being in women with PCOS, particularly greater anxiety and lower quality of life. Therefore, the first aim of this study was to determine if dietary intake, particularly micronutrients such as magnesium, can explain poor psychological well-being outcomes in women with PCOS.

As discussed in Chapter 1, Section 1.10.2, physical activity can have a positive effect on mood, particularly low to moderate intensity aerobic activity. For example, reports indicate that when women walk more than 7500 steps a day, the prevalence of depression is reduced by half

(292). Another study showed that walking 10,000 steps a day could lower depression, as well as anxiety and anger, in overweight individuals (293). Therefore, the second aim was to determine if the amount of daily physical activity can explain poor psychological health outcomes in women with PCOS.

3.2.2 Methods

This was a cohort study which took place at Grace Fertility Centre in Vancouver, Canada. The study included 87 women diagnosed with PCOS. This is the same cohort of women with PCOS who completed the three-day dietary intakes described in Chapter 2.

Lifestyle and Psychological Assessment

Dietary intake was assessed using a 3-day food record while physical activity was measured using a pedometer, as described in Chapter 2, Section 2.1.2. Psychological well-being was evaluated by the Depression, Anxiety and Stress Scale (DASS) and the Fertility Quality of Life Questionnaire (FertiQoL), as described in Chapter 3, Section 3.1.2.

Anthropometrics and Physical Characteristics

Data were collected pertaining to participants' height, weight, waist, and hip circumference, BMI, WHR, hirsutism (both yes/no and modified Ferriman-Gallway scores), and acne (yes/no).

Biochemical and Hormonal Assays

Data on women's metabolic status were collected including fasting blood glucose (FBS), a 2-hour oral glucose tolerance test (OGTT) and fasting insulin. Hyperandrogenism was assessed with the following bloodwork: total testosterone, DHEA-S, and androstenedione.

Statistical analyses

All outcome variables for dietary intake and psychological well-being were assessed for "statistical" normality by histogram visualizations and Shapiro-Wilk tests. Outcome variables were not normally distributed with the exception of age. Multiple groups were compared by analysis of variance (ANOVA) or its non-parametric equivalents and post-hoc analysis was assessed by Tukey and Kramer (Nemenyi) tests. The analysis included Student's t tests for normally distributed data and Mann-Whitney and Spearman's Rank tests for nonparametric data. All analyses were performed in R software. Significance tests where $P < 0.05$ were statistically significant.

3.2.3 Results

Depression

Women with PCOS and scores in the severe to extremely severe range of depressive symptoms consumed significantly less vitamin D than women in the normal to moderate range of depressive symptoms ($P = 0.018$, Figure 3.5). Specifically, women in the severe and extremely severe range of depressive symptoms consumed significantly less vitamin D than women in the mild and moderate range of depressive symptoms ($P = 0.003$) and no symptoms of depression ($P = 0.036$). Overall caloric, vitamin and mineral intake, and physical activity did not differ between women grouped by various degrees of depressive symptoms. Numerical scores for depressive symptoms were not significantly correlated with caloric intake, carbohydrates, protein, fat, fiber, glycemic load, sugar, folate, calcium, iron, zinc, sodium, cholesterol, magnesium, manganese, vitamins A, B1, B2, B6, B12, C, D, or daily number of steps taken.

Anxiety

Overall caloric, vitamin and mineral intake, and physical activity did not differ between women grouped by various degrees of anxiety symptoms (ranging from normal to severe).

Numerical anxiety symptom scores were not significantly correlated with intake of calories, carbohydrates, protein, fat, fiber, glycemic load, sugar, folate, calcium, iron, zinc, sodium, cholesterol, magnesium, manganese, vitamins A, B1, B2, B6, B12, C, D, or daily number of steps taken.

Stress

Overall caloric, vitamin and mineral intake, and physical activity did not differ between women grouped by various degrees of stress symptoms (ranging from normal to severe). Numerical stress symptom scores were not significantly correlated with intake of calories, carbohydrates, protein, fat, fiber, glycemic load, sugar, folate, calcium, iron, zinc, sodium, cholesterol, magnesium, manganese, vitamins A, B1, B2, B6, B12, C, D, or daily number of steps taken.

Quality of Life

The overall quality of life was negatively correlated with the total caloric intake (rho = -0.32, P = 0.014, Figure 3.6). Quality of life scores were not significantly correlated with intake of carbohydrates, protein, fat, fiber, glycemic load, sugar, folate, calcium, iron, zinc, sodium, cholesterol, magnesium, manganese, vitamin A, B1, B2, B6, B12, C, D or daily number of steps taken.

Social quality of life was negatively correlated with total caloric intake (rho = -0.44, P = 0.0005). Emotional and mind-body quality of life scores were negatively correlated with manganese intake (rho = -0.30, P = 0.018, and rho = -0.26, P = 0.043).

3.2.4 Discussion

This is the first study to examine the dietary intakes of women with PCOS in relation to their psychological well-being. With the recent emergence of evidence identifying micronutrient

deficiencies and increased psychological health issues for women with PCOS, as discussed in Chapter 1, this research recognizes an important and underappreciated relationship.

The finding of lower vitamin D intake in women with greater depressive symptoms supports a report concerning the role of vitamin D in mood disorders in the general population (294). A population-based cohort study from the Netherlands assessed serum 25-hydroxyvitamin D levels of 1,200 people over the age of 64 and found that people with both minor and major depression had 14% lower serum 25-hydroxyvitamin D levels compared to those without depression (295). In women with PCOS, levels of serum vitamin D have been positively associated with central obesity, IR, infertility, and hirsutism (296). Moreover, a few trials have shown that supplementation of vitamin D may improve insulin sensitivity and HOMA-IR in women with PCOS (297). Whether vitamin D supplementation can reduce depression in women with PCOS is yet to be examined.

While our results did not show an association between the majority of nutrients assessed and poor psychological health, the relationship between diet and mental health may be a function of dietary intake as a whole rather than as parts. For example, a meta-analysis published in Clinical Nutrition in 2018 reviewed the results of 11 cross-sectional and longitudinal studies assessing the potential link between an anti-inflammatory diet and depression (298). This review found that a pro-inflammatory diet could increase the risk of depression by 1.4-fold. Women with PCOS often present with an increase in inflammatory markers, and therefore, an anti-inflammatory diet could potentially target and ameliorate a variety of their symptoms, such as depression, metabolic and hormonal imbalances. A randomized controlled trial demonstrated that a Mediterranean diet (typically anti-inflammatory) supplemented with nuts (walnuts, hazelnuts, and almonds) could decrease the incidence of depression in adults with type 2 diabetes (299,

300). The specific nuts supplemented were also considered anti-inflammatory due to their high polyunsaturated fats, monounsaturated fats, vitamin E, and low in saturated fat.

To conclude, in general, the habitual dietary intake and physical activity of women with PCOS were not associated with symptoms of depression, anxiety, stress, or quality of life. Future work in this area should examine dietary intake with regard to overall dietary patterns rather than individual food groups or nutrients and perhaps provide further insight into the dietary impact on the psychological well-being of women with PCOS. The finding that vitamin D intake was associated with greater depressive symptoms adds to the literature on a role vitamin D might play in PCOS.

Table 3.1. Anthropometric characteristics of women with and without PCOS.

	PCOS (n=152)	Control (n=150)	P	P (adj for BMI and/or Age)
BMI (kg/m²)	27.5 (23.8-31.8)	23.6 (21.0-26.4)	<0.001*	<0.001*
WHR	0.83 (0.78-0.90)	0.79 (0.73-0.83)	<0.001*	<0.001*
Age	31.2 ± 5.2	35.6 ± 5.2	<0.001*	<0.001*
FSH (IU/L)	5.4 (4.1-6.4)	6.0 (4.6-7.9)	<0.01*	0.09
LH (IU/L)	7.25 (4.35-10.9)	3.8 (2.1-6.8)	<0.001*	0.47
Estradiol (pmol/L)	158.0 (114.0-218.0)	137.5 (100.0-202.8)	0.11	0.58
Prolactin (nmol/L)	9.3 (6.9-14.5)	10.0 (8.0-15.5)	0.08	0.26
TSH (mIU/L)	1.7 (1.2-2.5)	1.6 (1.2-2.3)	0.57	0.69
CRP (mg/L)	2.3 (0.5-5.8)	0.7 (0.3-2.85)	<0.01*	0.20

Note: Values are expressed as median (IQR) or mean ± SD. P values are marked with an asterisk when statistically significant. BMI: body mass index; WHR: waist to hip ratio; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TSH: thyroid-stimulating hormone; CRP: C-reactive protein.

Table 3.2. Scoring ranges for the Depression Anxiety Stress Scales.

	Normal	Mild	Moderate	Severe	Extremely Severe
Depression	0-9	10-13	14-20	21-27	28+
Anxiety	0-7	8-9	10-14	15-19	20+
Stress	0-14	15-18	19-25	26-33	34+

(281) Lovibond SH, Lovibond PF. Manual for the Depression Anxiety Stress Scales. 2nd ed. Sydney: Psychology Foundation; 1995.

Table 3.3 Psychological outcomes of women with and without PCOS.

	PCOS (n=152)	Control (n=150)	P	P (adj for BMI, Age)
Depression	4.0 (1.5-9.5)	3.0 (1.0-7.75)	0.02*	0.18
Anxiety	6.0 (2.0-11.0)	3.5 (1.0-7.0)	<0.001*	0.03*
Stress	11.0 (6.0-18.0)	8.0 (4.0-14.0)	0.03*	0.31
QoL	66.3 (56.3-72.9)	66.7 (55.2-74.0)	0.93	0.66
Emotional QoL	66.7 (50.0-70.8)	62.5 (47.9-70.8)	0.55	0.68
Mind-Body QoL	70.8 (54.2-83.3)	75.0 (54.2-87.5)	0.16	0.93
Relational QoL	50.0 (42.9-60.7)	50.0 (42.9-53.6)	0.56	0.26
Social QoL	70.8 (62.5-79.2)	70.8 (60.4-79.2)	0.75	0.32

Note: Values are expressed as median (IQR). P values are marked with an asterisk when statistically significant. See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100. Most participants completed both questionnaires (DASS: n = 297, FertiQol: n = 234).

Table 3.4 Psychological outcomes among ethnic groups in all women.

	East Asian (n=127)	European (n=102)	South Asian (n=43)	Others (n=20)	P
Depression	3.0 (1.0-8.0)	3.0 (1.0-9.0)	4.5 (1.0-12.8)	4.5 (1.8-7.8)	0.759
Anxiety	4.0 (2.0-10.0)	3.5 (1.0-8.0)	6.0 (2.8-14.3)	6.0 (4.8-9.0)	0.071
Stress	8.0 (5.0-14.0)	8.5 (4.3-17.8)	12.0 (5.8-18.3)	13.0 (8.0-16.8)	0.146
QoL	67.71 (55.43-75.0)	67.39 (58.85-73.26)	65.05 (51.04-70.31)	61.46 (53.75-63.54)	0.730
Emotional QoL	66.67 (50.0-75.0)	62.50 (45.83-70.83)	62.50 (41.67-67.71)	52.08 (37.5-61.46)	0.118
Mind Body QoL	75.00 (54.17-87.5)	75.00 (62.5-83.3)	60.42 (44.79-76.04)	68.75 (48.96-78.13)	0.150
Relational QoL	50.00 (42.86-57.14)	50.00 (42.86-57.14)	50.00 (42.86-60.18)	46.43 (40.18-50.00)	0.246
Social QoL	70.83 (54.17-79.17)	70.83 (66.67-83.33)	68.75 (62.5-76.04)	75.00 (63.54-78.13)	0.397

Note: Values reported as median (IQR). See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100.

Table 3.5 Psychological outcomes of women with PCOS by ethnicity.

	East Asian (n=64)	European (n=45)	South Asian (n=28)	Others (n=9)	P
Depression	5 (2-11)	3 (1-7)	3 (1-10)	5 (2-7)	0.434
Anxiety	6.5 (2-11)	5 (2-9)	8 (3-16)	6 (4-9)	0.389
Stress	9 (6-16.8)	10 (5-19)	13 (6-19)	11 (8-15)	0.579
QoL	65.96 (55.99-73.18)	68.23 (63.8-72.11)	62.5 (52.6-69.7)	61.46 (57.3-71.1)	0.902
Emotional QoL	66.67 (50.0-71.88)	66.67 (58.33-70.83)	62.50 (41.67-72.92)	60.42 (52.08-62.50)	0.216
Mind Body QoL	70.83 (54.17-83.33)	75.00 (66.67-79.17)	58.33 (50.00-70.83)	68.75 (60.42-73.96)	0.580
Relational QoL	46.43 (42.86-60.71)	53.57 (46.43-61.6)	50.00 (46.43-60.36)	44.64 (40.18-49.11)	0.657
Social QoL	70.83 (57.29-79.17)	72.92 (66.67-76.04)	70.83 (62.5-75.0)	70.83 (60.42-75.0)	0.287

Note: Values are expressed as median (IQR). See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100.

Table 3.6 Psychological outcomes of women with PCOS by BMI category.

BMI	Normal (n=45)	Overweight (n=45)	Obese (n=55)	P
Depression	3.0 (1.0-8.0)	4.5 (1.75-9.25)	4.0 (2.0-9.25)	0.3743
Anxiety	6.0 (2.0-11.0)	7.5 (3.0-14.0)	7.0 (2.0-10.25)	0.6883
Stress	11.0 (6.0-16.0)	10.5 (6.0-19.5)	11.0 (5.75-18.25)	0.5734
QoL	65.42 (56.25-73.70)	63.54 (51.04-72.03)	68.75 (61.46-75.00)	0.5908
Emotional QoL	58.33 (50.00-69.79)	60.42 (41.67-67.71)	66.67 (60.42-72.92)	0.3179
Mind Body QoL	70.83 (54.17-86.46)	66.67 (53.13-79.17)	75.00 (58.33-83.33)	0.3687
Relational QoL	46.43 (42.86-50.00)	50.00 (42.86-67.50)	50.00 (42.86-60.71)	0.9156
Social QoL	72.92 (66.67-83.33)	66.67 (54.17-76.04)	75.00 (62.5-77.08)	0.3255

Note: Values are expressed as median (IQR). See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100. BMI: body mass index.

Table 3.7 Psychological outcomes of women with PCOS by WHR.

WHR	Normal (n=75)	Overweight (n=17)	Obese (n=25)	P
Depression	5.0 (1.0-9.0)	5.0 (3.0-11.0)	3.0 (2.0-7.0)	0.9554
Anxiety	6.0 (2.0-10.25)	5.0 (2.0-13.0)	7.0 (2.0-13.0)	0.2091
Stress	10.0 (6.0-19.0)	8.0 (6.0-17.0)	13.0 (3.0-18.0)	0.5511
QoL	64.58 (54.69-73.44)	65.63 (48.96-72.92)	67.71 (64.06-72.87)	0.5883
Emotional QoL	58.33 (43.75-70.83)	62.50 (41.67-75.00)	70.83 (60.42-75.00)	0.6996
Mind Body QoL	70.83 (54.17-79.17)	58.33 (54.17-83.33)	66.67 (62.50-83.33)	0.3598
Relational QoL	46.43 (42.86-55.36)	42.86 (42.86-46.43)	53.57 (46.43-63.93)	0.5525
Social QoL	70.83 (62.5-79.17)	75.00 (62.5-79.17)	75.00 (66.67-81.25)	0.5609

Note: Values are expressed as median (IQR). See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100. WHR: waist to hip ratio.

Table 3.8 Psychological outcomes of women with PCOS with and without insulin resistance.

	IR (n=64)	No-IR (n=62)	P
Depression	3.0 (1.0-8.25)	5.0 (2.0-10.0)	0.6558
Anxiety	6.0 (2.75-11.50)	7.5 (2.25-13.75)	0.9668
Stress	11.0 (5.5-20.0)	10.5 (6.0-19.0)	0.6383
QoL	65.63 (60.42-72.92)	68.75 (51.04-72.92)	0.8357
Emotional QoL	62.50 (50.00-70.83)	66.67 (50.00-75.00)	0.1811
Mind Body QoL	75.00 (58.33-83.33)	70.83 (50.00-79.17)	0.4283
Relational QoL	50.00 (46.43-57.14)	46.43 (42.86-60.71)	0.5912
Social QoL	70.83 (66.67-75.00)	70.83 (54.17-79.17)	0.7848

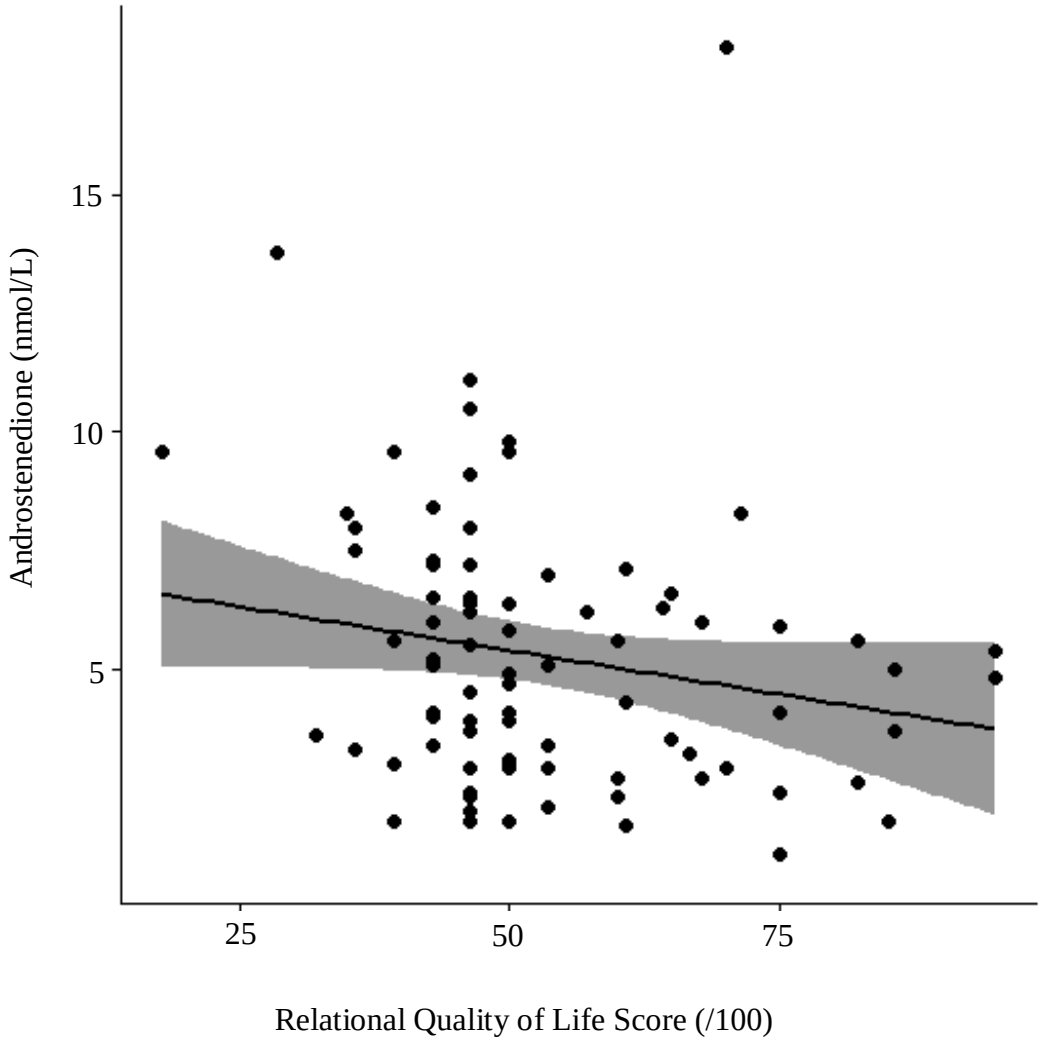
Note: Values are expressed as median (IQR). P values are marked with an asterisk when statistically significant. See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100.

Table 3.9 Characteristics and psychological outcomes in hyperandrogenic vs non-hyperandrogenic phenotypes of women with PCOS.

	HA (n=85)	Non-HA (n=67)	P
Characteristics			
17-OHP (nmol/L)	1.7 (1.1-2.0)	1.0 (0.8-1.3)	<0.001*
A_4 (nmol/L)	6.0 (4.5-8.0)	3.8 (2.9-5.6)	<0.001*
T (nmol/L)	1.6 (1.1-1.9)	1.1 (0.9-1.3)	<0.001*
DHEA-S (μmol/L)	6.9 (5.0-10.0)	6.0 (4.8-7.8)	0.10
Ferriman-Gallwey Score	13.5 (9.0-20.1)	2.0 (0.75-5.3)	<0.001*
PCO Morphology (%)	72	28	<0.001*
Psychological Outcomes			
Depression	5.5 (1.0-11.0)	3.0 (2.0-8.0)	0.380
Anxiety	8.0 (3.0-12.25)	4.0 (2.0-9.5)	<0.01*
Stress	12.5 (6.0-19.0)	8.0 (4.5-16.5)	0.188
QoL	65.63 (56.25-72.92)	67.71 (60.42-73.96)	0.302
Emotional QoL	64.58 (41.67-70.83)	66.67 (50.00-75.00)	0.230
Mind Body QoL	75.00 (54.17-83.33)	70.83 (58.33-83.33)	0.850
Relational QoL	46.43 (46.43-60.71)	50.00 (42.86-60.71)	0.434
Social QoL	68.75 (58.33-75.00)	75.00 (66.67-83.33)	0.029*

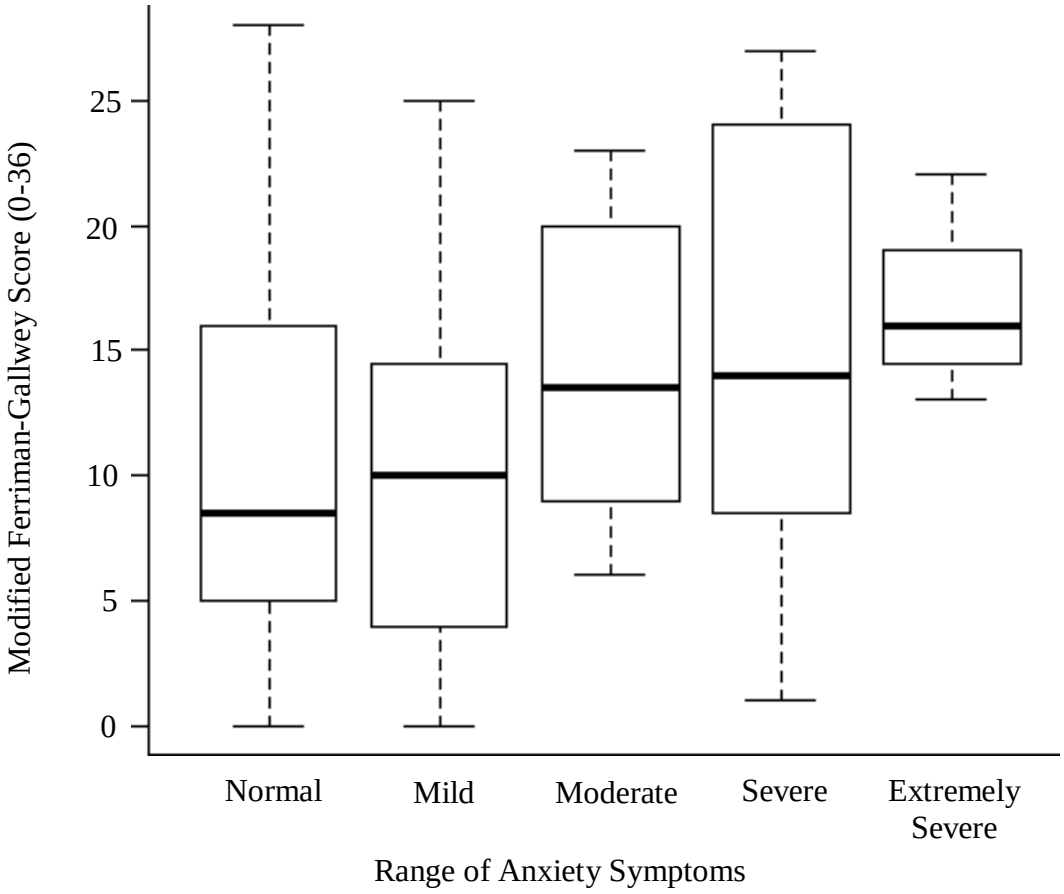
Note: Values are expressed as median (IQR) or percentages for categorical variables. P values are marked with an asterisk when statistically significant. See Table 3.2 for depression, anxiety, and stress scoring ranges. All QoL scores are out of a total of 100. 17-OHP: 17-hydroxyprogesterone; A_4: androstenedione; T: total testosterone; DHEA-S: dehydroepiandrosterone sulfate.

Figure 3.1 Correlation between androstenedione and relational quality of life in women with PCOS.



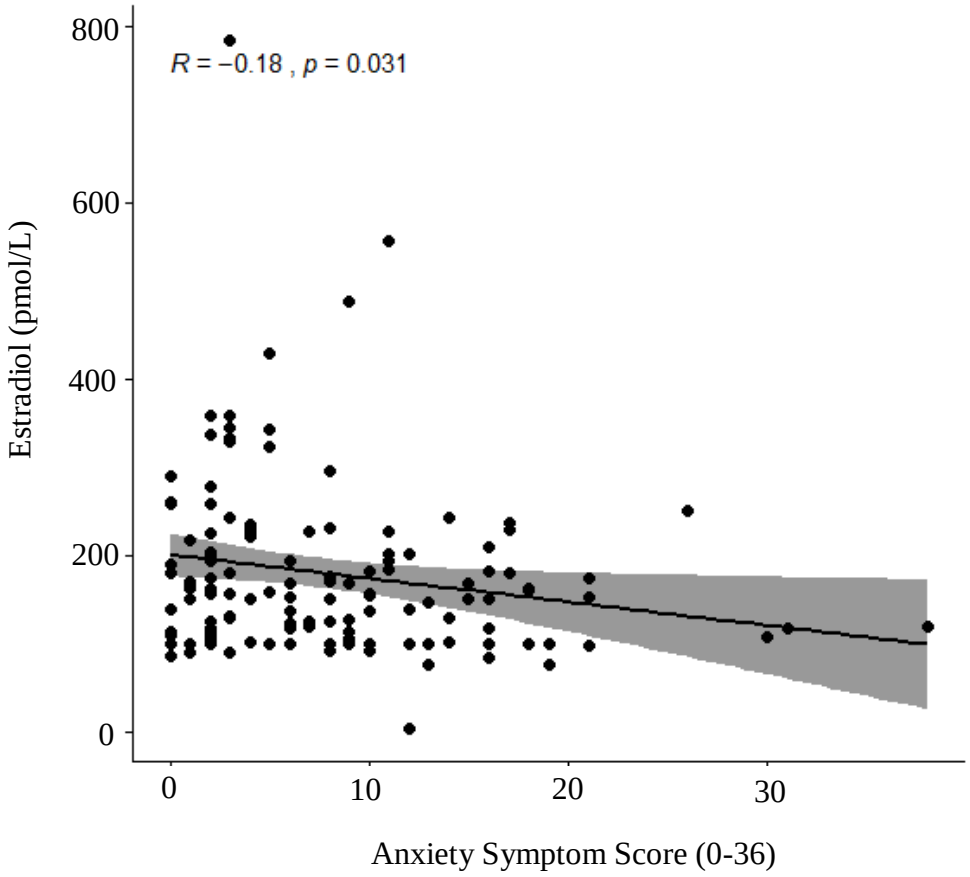
Note: Serum androstenedione was negatively correlated with relational quality of life (n = 88).

Figure 3.2 Self-reported levels of anxiety and hirsutism in women with PCO



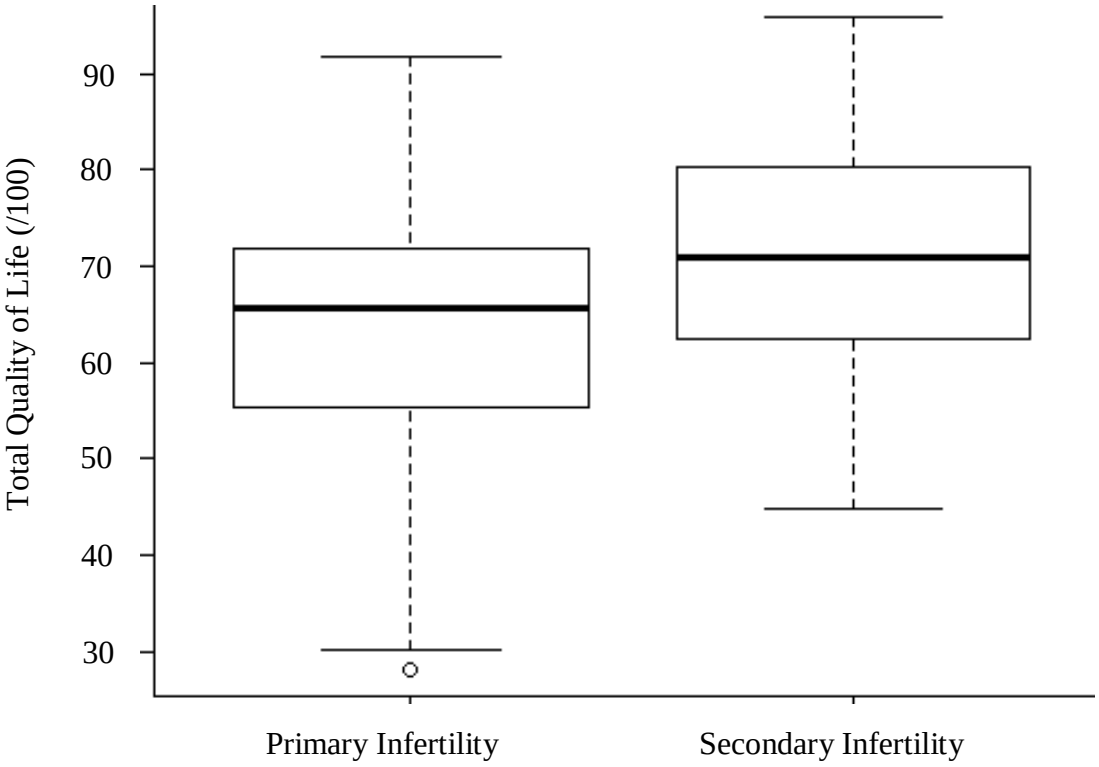
Note: Scores assessing hirsutism were compared according to level of symptoms of anxiety (n = 57).

Figure 3.3 Anxiety scores and estradiol levels in women with PCOS.



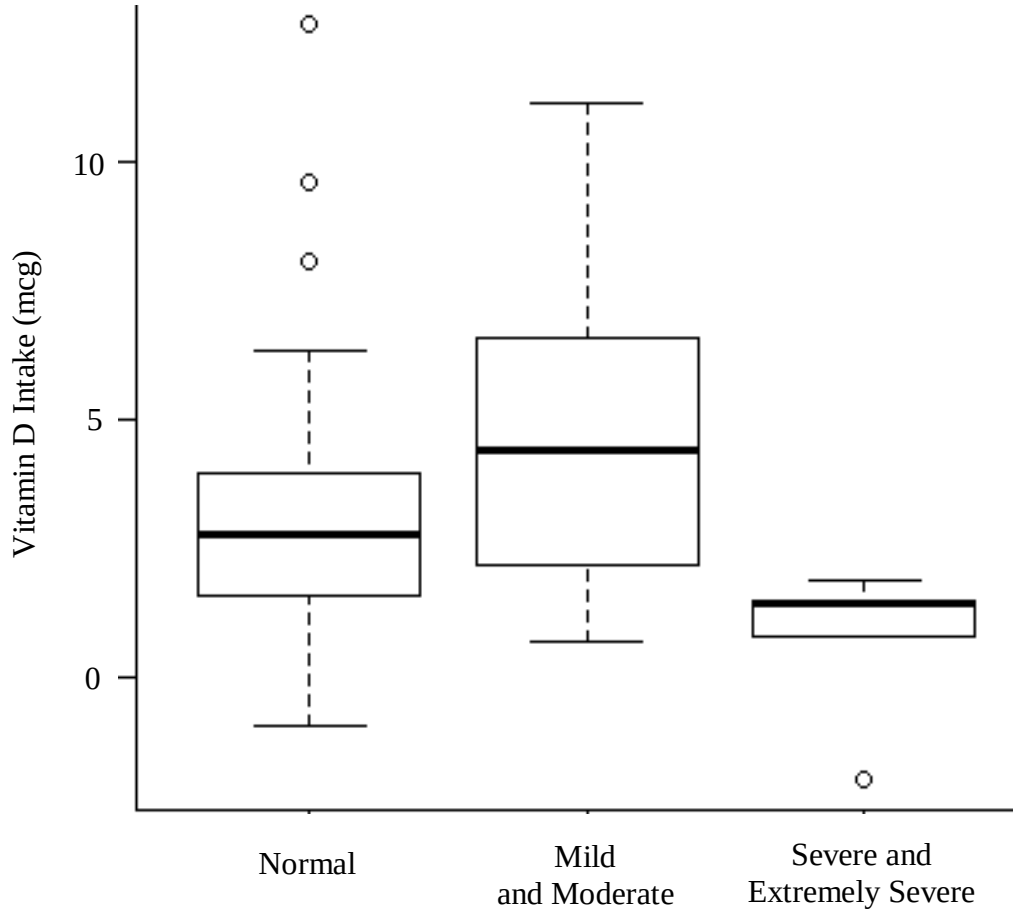
Note: Serum estradiol was negatively correlated with symptoms of anxiety (n = 137).

Figure 3.4 Quality of life in women with PCOS and infertility.



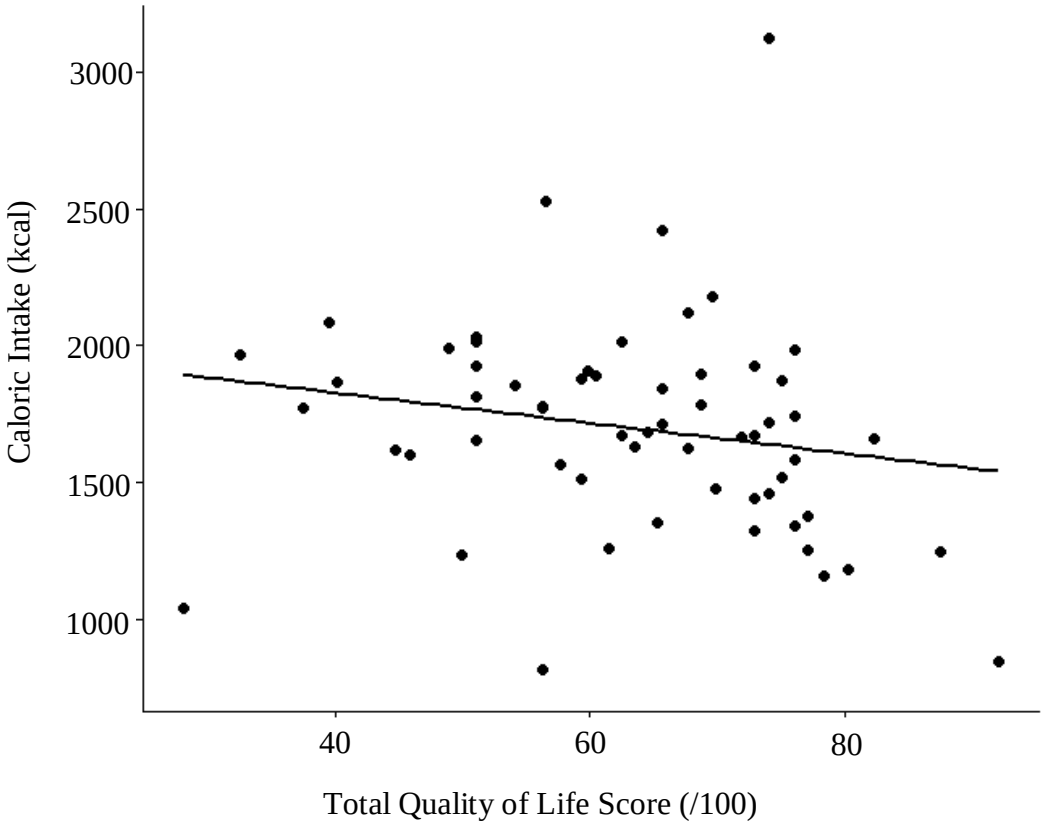
Note: Total quality of life score was compared according to type of infertility (n = 94).

Figure 3.5 Daily vitamin D intake and level of depression in women with PCOS.



Note: Daily average adjusted vitamin D intake was compared according to level of depressive symptoms (n = 84).

Figure 3.6 Overall quality of life and caloric intake in women with PCOS.



Note: Total daily caloric intake was negatively correlated with total quality of life (n = 60).

Chapter 4: Lifestyle-Based Intervention for PCOS

4.1 A randomized controlled trial comparing lifestyle intervention to letrozole for ovulation in women with PCOS – a study protocol

4.1.1 Introduction

Between 70 to 80% of women with PCOS have difficulty conceiving due to oligo- or anovulation. Despite ovulation rates of 75 to 80% achieved with the use of ovulation induction agents and a conception rate per cycle of 22% compared to that of a healthy woman trying to conceive naturally in their first year, some women with PCOS remain unresponsive (81, 301). In contrast, evidence suggests that lifestyle (diet, activity and stress management) influences ovulation and the effectiveness of fertility treatments (302-304). Despite the indication that women with PCOS have expressed the need for alternative fertility treatments, lifestyle interventions incorporating a nutritional plan with supplementation, increased physical activity, and techniques for stress management have not been combined as a program and studied in this population. The literature presented in Chapter 1, Section 1.10, suggests that each of these individual components has the potential to positively influence reproductive hormones, metabolic markers, and psychological well-being.

While lifestyle intervention is recommended as a first-line treatment for women with PCOS and obesity, 45% of women with PCOS reported they have never been provided information on lifestyle management and only 61 to 76% of REI-ObGyn and 46 to 61% ObGyn recommended lifestyle modifications to their patients for fertility or nonfertility reasons (48, 95). Even with efforts to improve dietary and exercise habits, women with PCOS often experience difficulty managing their weight. In addition, 62% of women with PCOS reported never

receiving information about emotional support or counselling (48). Attention to general physical and psychological health appears to be inconsistent at the clinical level yet recognized as an important component in the effective management of women receiving the life-altering diagnosis of PCOS and/or infertility (5, 102).

There have been no studies to date evaluating the reproductive outcomes of a comprehensive four-component intervention program incorporating structured nutritional and physical activity components, combined with techniques for stress management in women with PCOS trying to conceive (125). Literature shows that women with PCOS can significantly benefit from lifestyle changes, specifically, eating a low glycemic diet, incorporating nutritional supplements, increasing their activity level, and managing stress (115, 141, 182, 305). However, prior lifestyle intervention studies have limitations including high drop-out rates, lack of defining specific PCOS phenotypes, and small cohort sizes. In addition, most studies have focused on only one lifestyle-related change, such as diet, as opposed to a more synergistic approach wherein diet, exercise and stress reduction are combined. In addition, the implementation of a mindfulness program to help reduce stress which could potentially increase ovulation and conception has been studied in a few PCOS cohorts. Cognitive behavioural therapy has been implemented in infertile populations for over thirty years (87). These programs teach a variety of coping skills such as learning relaxation techniques, stress management, and provide group support. The relaxation response is a powerful tool proven effective in stress-related diseases such as cancer, CVD and mental disorders. Since women with PCOS have a higher prevalence of mental health conditions, inducing the relaxation response through mindfulness training could prove an effective stress management strategy for our study cohort (306).

In addition to a low glycemic diet, physical activity, and stress reduction, a diet supplemented with myo-inositol (MI) may provide further hormonal homeostasis and improve metabolic functioning (307). Several studies have shown that IR with its compensatory hyperinsulinemia plays a pivotal role in both the metabolic and ovarian hormone dysfunction observed in PCOS women. There is evidence that women with PCOS and IR could be deficient in MI which would impact glucose metabolism. In addition, MI levels in follicular fluid are lower in PCOS women with hyperinsulinemia compared to healthy women (308, 309). One study described that higher levels of MI in follicular fluid correlated with oocyte quality and maturity (310). When supplemented with MI, women with PCOS have experienced reduced serum insulin, testosterone and increased rates of ovulation (211, 311). MI has also been shown to improve oocyte number and quality (208).

We hypothesize that implementing a four-tier comprehensive program of lifestyle changes in women with PCOS will help restore ovulation by weight loss, reducing serum androgen levels and increasing sensitivity to insulin. A lifestyle change program may also ameliorate hirsutism. Such changes should increase overall psychological well-being and QoL. Finally, we expect that the distinct phenotypes of PCOS will respond differently to lifestyle intervention. This information will allow us to better define those phenotypes best served by this comprehensive approach, and to identify factors affecting the outcome in the less responsive phenotype.

The objectives of this study are: to determine whether the comprehensive lifestyle interventions described are effective in restoring ovulation and compare this to letrozole; to

determine if the addition of MI improves ovulation rates, IR, metabolic parameters; and finally, to evaluate any differences in the responses of PCOS phenotypes.

4.1.2 Methods

This is a randomised controlled trial which will include 240 women diagnosed with PCOS, according to the Rotterdam criteria, who are trying to conceive. Participants will be randomised to either a comprehensive lifestyle intervention program or prescribed oral fertility medication (letrozole). These two groups will be further randomised to consume MI or a placebo. Participants will be between the ages of 18 and 37. Exclusion criteria include women who have already begun fertility treatment, who are taking MI or have taken it in the past three months, or who are currently being treated for or have a past history of an ED. The primary outcome will be the ovulation rate, while the secondary outcome will be conception. Other outcomes include miscarriage rates, validated rating measures of overall QoL (including social, relational, mind/body and emotional sub-categories) and psychological well-being scores (depression, anxiety, and stress).

Overall study design

This study follows a factorial design. Women with PCOS will be randomised with a 1:1 allocation into two groups: the first group participating in a comprehensive lifestyle intervention program ("Graceful Lifestyle Changes" (GLC) program) while the second group will be prescribed letrozole to induce ovulation, with the standard clinician counselling. Within each group, participants will be further randomised with a 1:1 allocation to either be given oral MI or a placebo. Block randomisation will be performed at both stages using a computer-generated random numbers table and the software program REDCap. All clinicians, researchers, and

participants involved in the study will be blinded to block sizes and allocation of MI/placebo. A researcher not involved in the study will be responsible for the randomisation procedures.

Lifestyle Intervention Group (GLC)

The GLC group will have weekly check-ins with physicians and educators for 12 consecutive weeks. A wellness booklet designed and provided by clinicians will outline the main concepts being taught. Each week will consist of an educational portion guiding women on how to follow a low glycemic meal plan, incorporate walking 10,000 steps a day, and induce the relaxation response through cognitive behavioural therapy techniques. The low glycemic meal plan requires participants to consume 45% of carbohydrates in their diet and no more than 55 grams of glycemic load a day, which is similar to previous low glycemic diet intervention studies. Lists will be provided outlining foods that are low, medium, or high in their glycemic load. Hence, within their overall meal plan, participants will be advised to eat predominantly low glycemic foods and to limit high glycemic foods. They will also be provided with a pedometer to record the number of steps taken each day. These steps can be achieved by any form of exercise that participants can sustain. Lastly, participants will practice relaxation response exercises for twenty minutes daily on their own, in combination with six weeks of mindfulness training provided within the 12-week intervention program.

Data Collection

Participants will complete a three-day food record report during the baseline, 4th, 8th, and 12th weeks (See Appendix A). Participants will also receive a phone call from a trained researcher to complete a 24-hour diet recall at the end of the 2nd, 6th, and 10th weeks. These combined methods will aim to assess overall compliance to the low glycemic diet, provide

researchers with information on the length of time participants take to comply, evaluate any fluctuations in eating habits, and compare these two types of nutritional assessments.

Participants will also record their physical activity during the baseline, 4th, 8th, and 12th weeks based on their pedometer recordings and the meditation and relaxation exercises performed daily.

Compliance to the physical activity portion will be measured through daily pedometer readings recorded by the participant. Compliance will be ensured additionally through weekly check-ins/weigh-ins and frequent motivational email reminders.

Psychological well-being (depression, anxiety, stress, and QoL) will be assessed before and after the 12-week intervention by two validated self-report questionnaires: the DASS and the FertiQoL (280, 281).

Letrozole Group

The use of CC to treat anovulatory infertility is common. However, in agreement with what we have observed in clinical practice, recent evidence has suggested that letrozole is more effective than CC in achieving ovulation in this population (129). Additionally, letrozole does not negatively affect the endometrial thickness. The initial dose will be 5 milligrams daily for five days and increased to a total daily dose of 7.5 milligrams, if necessary, depending on the ovulatory response, as in clinical practice at Grace Fertility Centre in Vancouver, Canada. Letrozole will be taken for five days with the day of the first dose defined as “Cycle Day 3” which can correspond to day 3 of spontaneous menses or induced bleeding after progestogen-withdrawal. This treatment regimen will continue for three cycles or until pregnancy is achieved. It will be recommended for participants to have intercourse every other day in the expected

ovulatory period (starting on cycle day 12, for a week, and using urinary ovulation prediction kits).

Myo-inositol Group

Participants will be instructed to consume six grams of MI (or its placebo) in water every morning for 12 weeks. While most previous literature on MI for PCOS is based on four grams of MI a day, increasing the dose may provide more efficient effects. A dose of six grams a day is still safe as MI has been used in doses up to 18 grams a day to treat panic disorder (312).

Outcomes

Ovulation is the primary outcome (categorical "yes" or "no") and frequency of ovulation (nominal "0", "1", "2, or "3") during the 12-week (84-day) study. The study length allows the observation of three potential ovulatory cycles. The upper limit of a normal ovulatory cycle length is 35 days, hence, to document ovulation using progesterone levels in the expected luteal phase, the study may extend to the end of week 14. Ovulation will be identified based on progesterone level checks on day 22, and if appropriate, one week later for evidence of ovulation, defined as a progesterone level greater than 10 nmol/L. The secondary outcome is conception (categorical "yes" or "no"). Other outcomes that will be evaluated are miscarriage rates, changes in numerical scores of symptoms of depression, anxiety, stress, and QoL based on the DASS and the FertiQoL.

Statistical Methods

The participants' biochemical and hormonal profiles, physical attributes (BMI, hirsutism), ovulatory response, pregnancy status, scores for depression, anxiety, stress, and QoL will be assessed for "statistical" normality. Data from all four groups will be compared by analysis of variance (ANOVA) or its non-parametric equivalents. Categorical data will be

compared by Chi-square statistics. Differences in dietary intake and its compositions over time will be compared by ANOVA or its non-parametric equivalent with time as the repetitive measure. The primary comparison will be between GLC and letrozole as the observed effects of MI alone is small in clinical experience. There are no reported interactions between MI and ovulation-induction medications or lifestyle change in the literature. Hence, our power calculations have been based on the two main effects acting independently. Nevertheless, a factorial design will allow for the exploration of potential interactions, acknowledging the sample size required in this situation may be different (313, 314). Regression analysis will also be performed to determine the individual impacts of diet, activity, and mindfulness on ovulation. In particular, the ovulation rates of four main sub-groups of PCOS will be explored as potential predictors using regression analysis and adjusting for BMI and age.

Sample Size and Power Calculations

The target sample size will be 240 participants (120 participants in the GLC group and 120 participants on oral medication). This is based on: a power calculation using a 5% significance level, 80% power comparing two proportions, and current knowledge of the average ovulation rates of lifestyle interventions and letrozole, our main effect. We explored several combinations of the ovulation rates from letrozole and lifestyle interventions and chose the most conservative estimate to ensure that our sample size was adequate, i.e., 91 in each group. By enrolling 120 participants in each group, we have allowed for a drop-out rate of 32%. Table 4.1 describes the lowest and highest reported ovulation rates for letrozole and lifestyle interventions, the sample size required in each scenario comparing the two proportions, and the three highest sample sizes required from our calculations, considering the length of lifestyle intervention and the start dose of letrozole.

When calculating the sample size necessary to assess the effect of MI on ovulation rates, we reviewed the few studies already published. One randomized controlled trial reported an ovulation rate of 70% when MI was given to women with PCOS versus a 21% ovulation rate for in controls (211). Similarly, another randomized controlled trial reported an ovulation rate of 65% using MI in women with PCOS (315). Using these rates in a comparison of two proportions, a total sample size of 26 is needed and easily covered by our original calculation above.

4.1.3 Discussion

This trial will determine the effectiveness of a structured comprehensive lifestyle-based intervention program for women with PCOS experiencing infertility. In addition, it will determine whether supplementing with MI provides any further benefit. The objective of this study is to assess a possible non-pharmacological solution to ovulatory dysfunction in these patients and perhaps improve other associated facets of their lives.

While previous lifestyle-related management programs can improve ovulation rates in some women with PCOS, they have generally focused on weight reduction alone. Legro et al compared three preconception interventions for women with PCOS prior to undergoing ovulation induction therapy. The first was lifestyle modification which consisted of caloric restriction, weight loss medication, and exercise. The second was oral contraceptive pills (OCPs) alone, and the third was lifestyle modification in combination with OCPs. They found that the two groups that incorporated lifestyle modification achieved greater weight loss, higher rates of ovulation and managed to avoid the onset of metabolic syndrome in comparison to the OCP group (302).

However, restrictive diets resulting in weight cycling is associated with ED (as well as depression) (316). This is concerning as women with PCOS seem to be at greater risk of developing ED than the general population (317). Furthermore, long-term weight loss medication is not a sustainable solution for ameliorating symptoms of PCOS.

The intended outcome of this research is the development of a successful comprehensive 12-week program for women with PCOS wishing to conceive. It is evident that women are seeking alternative fertility options that are natural, safe and effective (181). While fertility medications can be effective, they are costly and increase the risk of complications such as twin pregnancy. On the other hand, a lifestyle-based program can provide benefits such as weight loss, decreased symptoms of PCOS (decreased hair growth, more regular cycles), improved energy, reduced stress, and better QoL. Patients frequently drop out of infertility treatment due to factors such as financial and emotional stress and disappointment from failure to conceive despite repeated treatments (87). Hence, the management of PCOS should be more comprehensive and address the multiple factors at play in this condition. This includes not only the endocrine and metabolic perturbations, and anovulatory infertility but also the psychological and emotional impacts of PCOS (306).

Potential Biases or Limitations

The drop-out rate is often high in lifestyle-based interventions. To mitigate a high drop-out rate we have taken steps such as providing daily email contacts and weekly check-ins between participants and health care providers throughout the duration of the study (12 weeks). A lack of compliance can often become another limitation for intervention studies. To ensure compliance of participants, especially with the lifestyle modification group, we will provide

weekly in-person education sessions, as well as periodic check-ins over the phone, in order to explain and reinforce the importance and significance of the recommendations provided and how the women in the study will directly benefit from these comprehensive lifestyle changes. These weekly sessions will also serve as an opportunity for the participants to ask questions and receive support from both clinicians and other women struggling with similar issues. After each session, the participant's weight will be recorded (in a private setting) to help them remain motivated in adhering to dietary changes and increased physical activity. Several dietary recall methods will be used to measure compliance to the low glycemic diet, such as a 24-hour recall and a food record, recognizing these tools also have their own limitations.

Although the main goal of this intervention is to achieve a pregnancy, improving overall health and QoL for women with PCOS is an important component in achieving this primary goal. Infertility per se has a large psychological impact on women, as does the diagnosis of PCOS. Failed repetitive cycles of hormone therapy can add to stress and decrease QoL further in women with PCOS, providing a rationale for a comprehensive supportive program implementing sustainable lifestyle changes. Hopefully, this will not only improve women's chances of achieving a pregnancy but also enhance long-term physical and mental health.

Table 4.1 Sample size calculation based on previous literature.

Range of Reported Ovulation Rates	Letrozole Ovulation Rate	Lifestyle Ovulation Rate	Total Sample Size Needed
Lowest	62% *(129)*	38% *(318)*	130
Highest	86% *(319)*	67% *(320)*	150
Median	70%	50%	182

Note: Table previously published in "Cutler DA, et al. A randomized controlled trial comparing lifestyle intervention to letrozole for ovulation in women with polycystic ovary syndrome: a study protocol. *Trials*. 2018 Dec;19(1):632."

Chapter 5: Conclusion

This dissertation illuminates the significance of lifestyle in the varied presentations of PCOS. Original data drew several conclusions, which were either yet to be studied in PCOS populations or had been previously reported with contradictory results.

Firstly, it was found that obesity in PCOS cannot be explained by a surplus of energy intake. In other words, overweight women with PCOS are not eating more or exercising less than women of "normal" weight. This implies that an underlying metabolic dysregulation is likely and could compound the difficulty in managing weight and normalizing insulin levels. Increased awareness among clinicians regarding these difficulties that women with PCOS may have could help better manage their weight and insulin resistance, in addition to limiting the weight stigmatization experienced, and the perpetual dieting cycles which have proven largely ineffective (113).

Secondly, it was found that fiber intake was associated with IR and hyperandrogenism in women with PCOS (321). While causation cannot be drawn, increasing fiber intake can be easily implemented with little to no side-effects. Clinicians should advise women with PCOS and IR to consume high-fiber foods (ex. vegetables, fruits, and whole grains). In addition, magnesium intake was also associated with IR, dyslipidemia, and inflammation. Likewise, increasing dietary magnesium intake by specific foods and supplementation could be beneficial with little harm. Good sources of magnesium include spinach, avocado, edamame, black beans, quinoa, nuts, and tofu. In addition, for the first time in a PCOS cohort, this dissertation examined the relationship between dietary intake and mood. It was found that insufficient vitamin D intake may be associated with depression. Thus, perhaps nutritional management in PCOS is best summarized as "quality over quantity" as we found that macro- and micronutrient intakes (such as fiber,

magnesium, and vitamin D) were more significantly associated with PCOS, and phenotypic differences, than overall caloric intake.

Thirdly, this dissertation advances nutritional epidemiology for PCOS research. Few previous studies have accounted for overall caloric intake when assessing macronutrient and micronutrient intake in women with PCOS. This is crucial for obtaining accurate food intakes and, therefore, previous evidence may be unreliable.

Fourthly, it was found that anxiety is increased in women with PCOS, and in particular, within the hyperandrogenic phenotype. Both hirsutism and high levels of androstenedione were related to increased anxiety. This draws attention to the impact that the physical presentation of PCOS symptoms may be having on the psychological well-being of women with PCOS. Clinicians should be cognizant of the potential for hyperandrogenic women to be distressed about their symptoms and consider providing resources for coping with anxiety.

Finally, a study protocol for the first multi-component lifestyle intervention aimed at increasing ovulation, conception, and overall QoL for women with PCOS was designed and presented. This 12-week intervention incorporated the four main tiers of PCOS management discussed in this dissertation: nutrition, exercise, stress management, and supplementation. The publication of this protocol in 2018 of *Trials* allows for research groups worldwide to examine its effectiveness at treating infertility in women with PCOS without the use of ovulation induction therapy (322).

5.1 Strengths

One main strength throughout the original studies presented in this dissertation was the consistent diagnosis of PCOS using the Rotterdam criteria. Much of the literature to date is heterogeneous in regard to how PCOS is diagnosed. Studies use a range of criteria from the

broad Rotterdam criteria to the strict AE-PCOSS criteria, and even unreliable self-diagnoses in some cases, which impedes scientific understanding of this syndrome.

The nutritional assessments conducted in Chapters 2 and 3 were well-designed using a three-day food record, which was the most reliable method for the goals of these studies. In addition, the appropriate adjustments were made to account for overall energy intake and potential under-reporters.

The inclusion of a control group allowed for nutritional intake, physical activity, and psychological scores to be compared to other women as opposed to comparing to, for example, national recommendations for intake and activity or average levels of psychological distress in the general population (as seen in previous studies).

A final strength in this work was the inclusion of over 300 women, presented in Section 1 of Chapter 3. This large sample size allowed for an in-depth analysis of psychological well-being among PCOS phenotypes and ethnic groups.

5.2 Limitations

The use of self-reported dietary intakes can lead to misreporting and under-reporting. With the limited options available to assess such a complex topic as human nutritional intake, there is a need to develop further methods of dietary assessment, such as dietary biomarkers (323). Self-reported dietary intakes are still valuable for informing dietary guidance and public health policy as long as several recommendations for methodology, made by Subar et al in the Journal of Nutrition, are followed, such as energy adjustment (323).

In addition, the data collected in Chapters 2 and 3 were from patients attending a private fertility centre, located in Vancouver, who are typically of higher socioeconomic status, and

generally in satisfactory health. Accordingly, these results cannot be assumed to be the case for women of different socioeconomic status with limited access to healthcare.

Lastly, even though HOMA-IR is a strong predictor of IR, diagnosing IR using HOMA-IR is problematic as there is no HOMA-IR cut-off established for women with PCOS.

5.3 Future Directions

There is plenty of opportunity for future work in the field of lifestyle and PCOS. In terms of nutritional management, there is a need for more randomized controlled trials on specific patterns of eating. The evidence that this dissertation presents is supportive of an anti-inflammatory, high fiber diet, similar to the well-known Mediterranean diet. For example, the Mediterranean diet has had positive outcomes for women going through IVF treatment (324). A focus on the anti-inflammatory aspects of this diet combined with an increase in high-fiber foods could help reduce the risk for T2D and CVD in women with PCOS. In addition, the supplementation of specific micronutrients, such as magnesium and vitamin D, need to be further examined.

While the amount of physical activity was assessed using a pedometer, it may be worthwhile to assess the well-being and anthropometrics of women with PCOS who exercise outdoors as opposed to indoors. Recent literature has found that walking in green spaces can positively impact mental health, in addition to weight management (325).

One area this dissertation did not assess was the sleep patterns of women with PCOS. Sleep is crucial for overall well-being and evidence has identified sleep disturbances as independent risk factors of IR (326). Recent research suggests women with PCOS may be more likely to have sleep apnea and this could be exacerbating metabolic dysfunction (327). Future studies in the identification of sleep patterns and how this may associate with hormonal and

metabolic imbalances in women with PCOS may provide another avenue for lifestyle management (328).

5.4 Knowledge Translation

Given academic journals are not widely accessible to some of those most in need of the information, such as patients, knowledge translation is important for scientists and clinicians. To make the findings of this dissertation accessible to the public, I have been active in the online dissemination of information (329). I have built a community using social media platforms, with a following of close to 10,000, where I provide evidence-based information in accessible language to the public and have accepted invitations to speak in non-academic settings (wellness shows, television, etc). In addition, I have co-founded an online support group for women with PCOS seeking information and social support in making lifestyle changes.

In conclusion, the material presented in this dissertation highlights the importance of considering several aspects of lifestyle in the presentations and management of PCOS. Future research with an emphasis on these lifestyle factors would benefit our knowledge and ability to better assist those with PCOS.

Bibliography

1. Fauser BC, Tarlatzis BC, Rebar RW, et al. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril*. 2012;97:28-38. e25.
2. March, WA, Moore, VM, Willson, KJ, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod*. 2010;25:544-51.
3. The Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*. 2004;81:19-25.
4. Shroff R, Syrop CH, Davis W, Van Voorhis BJ, Dokras A. Risk of metabolic complications in the new PCOS phenotypes based on the Rotterdam criteria. *Fertil Steril*. 2007;88:1389-1395.
5. Veltman-Verhulst SM, Boivin J, Eijkemans MJ, Fauser BJ. Emotional distress is a common risk in women with polycystic ovary syndrome: a systematic review and meta-analysis of 28 studies. *Hum Reprod Update*. 2012;18:638-651.
6. Daniilidis A, Dinas K. Long term health consequences of polycystic ovarian syndrome: a review analysis. *Hippokratia*. 2009;13(2):90.
7. Farquhar C. Introduction and history of polycystic ovary syndrome. In: Kovacs GT, Norman R, eds. *Polycystic Ovary Syndrome.* 2nd ed. Cambridge: Cambridge University Press; 2007:4-24.

8. Júnior JM, Baracat MCP, Maciel GAR, Baracat EC. Polycystic ovary syndrome: controversies and challenges. *Revista da Associação Médica Brasileira*. 2015;61:485-487.
9. Boyle J, Teede HJ. Polycystic ovary syndrome: an update. *Aust Fam Physician*. 2012;41:752.
10. International evidence-based guideline for the assessment and management of polycystic ovary syndrome. Monash University, Melbourne, Australia 2018.
11. Teede HJ, Misso ML, Costello MF, et al. Recommendations from the international evidence-based guideline for the assessment and management of polycystic ovary syndrome. *Hum Reprod*. 2018;33:1602-1618.
12. Stein IF. Amenorrhea associated with bilateral polycystic ovaries. *Obstet Gynecol*. 1935;29:181-191.
13. Lujan ME, Chizen DR, Pierson RA. Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. *J Obstet Gynaecol Can*. 2008;30:671-679.
14. Homburg R. Polycystic ovary syndrome: from gynaecological curiosity to multisystem endocrinopathy. *Hum Reprod*. 1996;11:29–39.
15. Kovacs GT, Norman R, eds. *Polycystic Ovary Syndrome.* 2nd ed. Cambridge: Cambridge University Press; 2007.
16. Stein IF, Cohen MR, Elson R. Results of bilateral ovarian wedge resection in 47 cases of sterility: Twenty-year end results: 75 cases of bilateral polycystic ovaries. *Am J Obstet Gynecol.* 1949;58(2):267-74.
17. Insler V, Lunesfeld B. Polycystic ovarian disease: A challenge and controversy. *Gynecol Endocrinol*. 1990;4:51-69.

18. Vallisneri A, 1721. Cited in Insler V, Lunesfeld B. Polycystic ovarian disease: A challenge and controversy. *Gynecol Endocrinol*. 1990;4:51-69.

19. Yen S, Vela P, Rankin J. Inappropriate secretion of follicle-stimulating hormone and luteinizing hormone in polycystic ovarian disease. *J Clin Endocrinol Metab*. 1970;30:435-442.

20. Rebar R, Judd HL, Yen SS, Rakoff J, Vandenberg G, Naftolin F. Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. *J Clin Invest*. 1976;57:1320-1329.

21. Swanson M, Sauerbrei EE, Cooperberg PL. Medical implications of ultrasonically detected polycystic ovaries. *J Clin Ultrasound*. 1981;9:219-222.

22. Adams J, Polson D, Abdulwahid N, et al. Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. *The Lancet*. 1985;326:1375-1379.

23. Szydlarska D, Machaj M, Jakimiuk A. History of discovery of polycystic ovary syndrome. *Adv Clin Exp Med*. 2017;26:555-558.

24. Zawadski JK, Dunaif A. Diagnostic criteria for polycystic ovary syndrome; towards a rational approach. In: Dunaif A, Givens JR, Haseltine F, eds. *Polycystic Ovary Syndrome*. Boston, MA: Black-well Scientific; 1992:377–84.

25. Balen AH, Conway GS, Kaltsas G, et al. Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. *Hum Reprod*. 1995;10(8):2107-11.

26. Welt CK, Duran JM. Genetics of polycystic ovary syndrome. *Semin Reprod Med*. 2014;32(3)177-182.

27. De Leo V, Musacchio MC, Cappelli V, Massaro MG, Morgante G, Petraglia F. Genetic, hormonal and metabolic aspects of PCOS: an update. *Reprod Biol Endocrinol*. 2016;14(1):38.

28. Filippou P, Homburg R. Is foetal hyperexposure to androgens a cause of PCOS? *Hum Reprod Update*. 2017;23:421-432.

29. Panda PK, Rane R, Ravichandran R, Singh S, Panchal H. Genetics of PCOS: A systematic bioinformatics approach to unveil the proteins responsible for PCOS. *Genom Data*. 2016;8:52-60.

30. Strauss JF, Modi BP, McAllister JM. The genetics of polycystic ovary syndrome: From genome-wide association to molecular mechanisms. In: *Reproductive Medicine for Clinical Practice*. Springer International; 2018:25-33.

31. McAllister JM, Legro RS, Modi BP, Strauss III JF. Functional genomics of PCOS: from GWAS to molecular mechanisms. *Trends Endocrinol Metab*. 2015;26(3):118-24.

32. Barker DJ. The fetal and infant origins of adult disease. *BMJ*. 1990;301:1111.

33. Franks S, McCarthy MI, Hardy K. Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl*. 2006;29:278-285.

34. Abbott D, Barnett D, Bruns C, Dumesic D. Androgen excess fetal programming of female reproduction: a developmental aetiology for polycystic ovary syndrome? *Hum Reprod Update*. 2005;11:357-374.

35. Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *J Clin Invest*. 2011;121(6):2126-32.

36. de Punder K, Pruimboom L. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front Immunol*. 2015;6:223.

37. González F. Inflammation in polycystic ovary syndrome: underpinning of insulin resistance and ovarian dysfunction. *Steroids*. 2012;77(4):300-5.

38. Zeng B, Lai Z, Sun L, Zhang Z, Yang J, Li Z, Lin J, Zhang Z. Structural and functional profiles of the gut microbial community in polycystic ovary syndrome with insulin resistance (IR-PCOS): a pilot study. *Res Microbiol*. 2019;170(1):43-52..

39. Torres PJ, Siakowska M, Banaszewska B, et al. Gut microbial diversity in women with polycystic ovary syndrome correlates with hyperandrogenism. *J Clin Endocrinol Metab*. 2018;103:1502-1511.

40. Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. *J Clin Endocrinol Metab*. 2004;89:2745-2749.

41. Ding T, Hardiman PJ, Petersen I, Wang F, Qu F, Baio G. The prevalence of polycystic ovary syndrome in reproductive-aged women of different ethnicity: a systematic review and meta-analysis. *Oncotarget*. 2017;8:96351.

42. Williamson K, Gunn AJ, Johnson N, Milsom SR. The impact of ethnicity on the presentation of polycystic ovarian syndrome. *Aust N Z J Obstet Gynaecol*. 2001;41:202-206.

43. Carmina E, Koyama T, Chang L, Stanczyk FZ, Lobo RA. Does ethnicity influence the prevalence of adrenal hyperandrogenism and insulin resistance in polycystic ovary syndrome? *Am J Obstet Gynecol.* 1992;167:1807-1812.

44. Carmina E, Legro RS, Stamets K, Lowell J, Lobo RA. Difference in body weight between American and Italian women with polycystic ovary syndrome: influence of the diet. *Hum Reprod*. 2003;18:2289-2293.

45. Sarkar M, Terrault N, Duwaerts CC, Tien P, Cedars MI, Huddleston H. The association of Hispanic ethnicity with nonalcoholic fatty liver disease in polycystic ovary syndrome. *Curr Opin Gynecol Obstet*. 2018;1(1):24.

46. Ladson G, Dodson WC, Sweet SD, et al. Racial influence on the polycystic ovary syndrome phenotype: a black and white case-control study. *Fertil Steril*. 2011;96(1):224-9.

47. Dokras A, Saini S, Gibson-Helm M, Schulkin J, Cooney L, Teede H. Gaps in knowledge among physicians regarding diagnostic criteria and management of polycystic ovary syndrome. *Fertil Steril*. 2017;107:1380-1386. e1.

48. Gibson-Helm ME, Lucas IM, Boyle JA, Teede HJ. Women's experiences of polycystic ovary syndrome diagnosis. *Family Practice*. 2014;31(5):545-9.

49. Dokras A, Witchel SF. Are young adult women with polycystic ovary syndrome slipping through the healthcare cracks? *J Clin Endocrinol Metab*. 2014;99(5):1583-5.

50. Carmina E, Oberfield SE, Lobo RA. The diagnosis of polycystic ovary syndrome in adolescents. *Obstet Gynecol*. 2010;203(3):201.e1-5.

51. Dunaif A, Fauser BC. Renaming PCOS—a two-state solution. *J Clin Endocrinol Metab*. 2013;98:4325-4328.

52. Ferriman D, Gallwey JD. Clinical assessment of body hair growth in women. *J Clin Endocrinol*. 1961; 21:1440-1447.

53. Hart R, Doherty DA. The potential implications of a PCOS diagnosis on a woman's long-term health using data linkage. *J Clin Endocrinol Metab*. 2015;100:911-919.

54. Lim, SS, Davies, MJ, Norman, RJ, et al. Overweight, obesity and central obesity in women with polycystic ovary syndrome: A systematic review and meta-analysis. *Hum Reprod Update*. 2012;18:618-37.

55. Hoeger K. Obesity and weight loss in polycystic ovary syndrome. *Obstet Gynecol Clin North Am*. 2001;28:85-97.

56. Dag ZO, Dilbaz B. Impact of obesity on infertility in women. *J Turk Ger Gynecol Assoc*. 2015;16:111-117.

57. Rich-Edwards JW, Goldman MB, Willett WC, et al. Adolescent body mass index and infertility caused by ovulatory disorder. *Am J Obstet Gynecol*. 1994;171:171-177.

58. Jungheim ES, Travieso JL, Hopeman MM. Weighing the impact of obesity on female reproductive function and fertility. *Nutr Rev*. 2013;71:S3-S8.

59. Bartelt A, Heeren J. Adipose tissue browning and metabolic health. *Nat Rev Endocrinol*. 2014;10(1):24.

60. Klein, S. Is visceral fat responsible for the metabolic abnormalities associated with obesity?: implications of omentectomy. *Diabetes Care*. 2010;33(7):1693-1694.

61. Goossens GH, Blaak EE. Adipose tissue dysfunction and impaired metabolic health in human obesity: a matter of oxygen? *Front Endocrinol (Lausanne*). 2015;6:55.

62. de Mutsert R, Gast K, Widya R, et al. Associations of abdominal subcutaneous and visceral fat with insulin resistance and secretion differ between men and women: the Netherlands epidemiology of obesity study. *Metab Syndr Relat Disord*. 2018;16(1):54-63.

63. Björntorp P. "Portal" adipose tissue as a generator of risk factors for cardiovascular disease and diabetes. *Arteriosclerosis*. 1990;10(4):493-6.

64. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes*. 1997;46(1):3-10.

65. Fabbrini E, Magkos F, Mohammed BS, et al. Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proc Natl Acad Sci USA*. 2009;106(36):15430-5.

66. Marshall JC, Dunaif A. Should all women with PCOS be treated for insulin resistance? *Fertil Steril*. 2012;97:18-22.

67. Kakoly N, Khomami M, Joham A, et al. Ethnicity, obesity and the prevalence of impaired glucose tolerance and type 2 diabetes in PCOS: A systematic review and meta-regression. *Hum Reprod Update*. 2018;24:455-467.

68. Gambarin–Gelwan M, Kinkhabwala SV, Schiano TD, Bodian C, Yeh H, Futterweit W. Prevalence of nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *Clin Gastroenterol Hepatol*. 2007;5:496-501.

69. Cerda C, Pérez-Ayuso RM, Riquelme A, et al. Nonalcoholic fatty liver disease in women with polycystic ovary syndrome. *J Hepatol*. 2007;47:412-417.

70. Rocha A, Faria L, Guimaraes T, et al. Non-alcoholic fatty liver disease in women with polycystic ovary syndrome: systematic review and meta-analysis. *J Endocrinol Invest*. 2017;40:1279-1288.

71. Scicchitano P, Dentamaro I, Carbonara R, et al. Cardiovascular risk in women with PCOS. *Int J Endocrinol Metab*. 2012;10:611-618.

72. Legro RS, Kunselman AR, Dunaif A. Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. *Am J Med*. 2001;111:607-613.

73. Carmina E. Is cardiovascular risk increased in women with PCOS? -Against. Presented at Society for Endocrinology BES 2017. Harrogate, UK. *Endocrine Abstracts*. 2017:49 D4.2.

74. Randeva H. Is cardiovascular risk increased in women with PCOS? -For. Presented at Society for Endocrinology BES 2017; Harrogate, UK. *Endocrine Abstracts*. 2017:49 D4.1.

75. Zhao L, Zhu Z, Lou H, et al. Polycystic ovary syndrome (PCOS) and the risk of coronary heart disease (CHD): a meta-analysis. *Oncotarget*. 2016;7:33715-33721.

76. Dumesic DA, Oberfield SE, Stener-Victorin E, Marshall JC, Laven JS, Legro RS. Scientific statement on the diagnostic criteria, epidemiology, pathophysiology, and molecular genetics of polycystic ovary syndrome. *Endocr Rev*. 2015;36:487-525.

77. Chittenden B, Fullerton G, Maheshwari A, Bhattacharya S. Polycystic ovary syndrome and the risk of gynaecological cancer: a systematic review. *Reprod Biomed Online*. 2009;19:398-405.

78. Onstad MA, Schmandt RE, Lu KH. Addressing the role of obesity in endometrial cancer risk, prevention, and treatment. *J Clin Oncol*. 2016;34:4225-4230.

79. Navaratnarajah R, Pillay OC, Hardiman P. Polycystic ovary syndrome and endometrial cancer. *Semin Reprod Med*. 2008;26:062-071.

80. Gottschau M, Kjaer SK, Jensen A, Munk C, Mellemkjaer L. Risk of cancer among women with polycystic ovary syndrome: a Danish cohort study. *Gynecol Oncol*. 2015;136:99-103.

81. Melo AS, Ferriani RA, Navarro PA. Treatment of infertility in women with polycystic ovary syndrome: approach to clinical practice. *Clinics*. 2015;70:765-769.

82. Cimino I, Casoni F, Liu X, et al. Novel role for anti-Müllerian hormone in the regulation of GnRH neuron excitability and hormone secretion. *Nat Commun*. 2016;7:10055.

83. Zegers-Hochschild F, Adamson GD, Dyer S, et al. The international glossary on infertility and fertility care, 2017. *Fertil Steril*. 2017;108(3):393-406.

84. Dennett CC, Simon J. The role of polycystic ovary syndrome in reproductive and metabolic health: overview and approaches for treatment. *Diabetes Spectr*. 2015;28:116-120.

85. Domar AD, Zuttermeister P, Friedman R. The psychological impact of infertility: a comparison with patients with other medical conditions. *J Psychosom Obstet Gynaecol*. 1993;14:45-45.

86. Cwikel J, Gidron Y, Sheiner E. Psychological interactions with infertility among women. *Eur J Obstet Gynecol Reprod Biol*. 2004;117:126-131.

87. Cousineau TM, Domar AD. Psychological impact of infertility. *Best Pract Res Clin Obstet Gynaecol*. 2007;21(2):293-308.

88. Boomsma CM, Fauser BC, Macklon NS. Pregnancy complications in women with polycystic ovary syndrome. *Hum Reprod Update*. 2008;26:072-084.

89. Damone AL, Joham AE, Loxton D, Earnest A, Teede HJ, Moran LJ. Depression, anxiety and perceived stress in women with and without PCOS: a community-based study. *Psychol Med*. 2018:1-11.

90. Kerchner A, Lester W, Stuart SP, Dokras A. Risk of depression and other mental health disorders in women with polycystic ovary syndrome: a longitudinal study. *Fertil Steril*. 2009;91:207-212.

91. Jones GL, Benes K, Clark TL, et al. The polycystic ovary syndrome health-related quality of life questionnaire (PCOSQ): A validation. *Hum Reprod*. 2004;19:371-377.

92. Cesta CE, Månsson M, Palm C, Lichtenstein P, Iliadou AN, Landén M. Polycystic ovary syndrome and psychiatric disorders: Co-morbidity and heritability in a nationwide Swedish cohort. *Psychoneuroendocrinology*. 2016;73:196-203.

93. Elsenbruch S, Benson S, Hahn S, et al. Determinants of emotional distress in women with polycystic ovary syndrome. *Hum Reprod*. 2006;21:1092-1099.

94. Barry JA, Kuczmierczyk AR, Hardiman PJ. Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod*. 2011;26:2442-2451.

95. Dokras A. Mood and anxiety disorders in women with PCOS. *Steroids*. 2012;77:338-341.

96. Klimczak D, Szlendak-Sauer K, Radowicki S. Depression in relation to biochemical parameters and age in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2015;184:43-47.

97. Bazarganipour, F, Ziaei, S, Montazeri, A, et al. Body image satisfaction and self-esteem status among the patients with polycystic ovary syndrome. *Iran J Reprod Med*. 2013;11:829-36.

98. Ekbäck M, Wijma K, Benzein E. "It is always on my mind": women's experiences of their bodies when living with hirsutism. *Health Care Women Int*. 2009;30:358-372.

99. Liao LM, Nesic J, Chadwick PM, Brooke-Wavell K, Prelevic GM. Exercise and body image distress in overweight and obese women with polycystic ovary syndrome: a pilot investigation. *Gynecological Endocrinology*. 2008;24:555-561.

100. Weiss TR, Bulmer SM. Young women's experiences living with polycystic ovary syndrome. *J Obstet Gynecol Neonatal Nurs*. 2011;40:709-718.

101. Nasiri Amiri F, Ramezani Tehrani F, Simbar M, Montazeri A, Mohammadpour Thamtan RA. The experience of women affected by polycystic ovary syndrome: a qualitative study from Iran. *Int J Endocrinol Metab*. 2014;12:e13612.

102. Kitzinger C, Willmott J. 'The thief of womanhood': Women's experience of polycystic ovarian syndrome. *Soc Sci Med*. 2002;54:349-361.

103. Harstock N. The Feminist Standpoint. In: Nicholson L, ed. *The Second Wave: A Reader in Feminist Theory*. New York, NY: Routledge; 1997:216-240.

104. Government of Canada. The human face of mental health and mental illness in Canada 2006. http://www.phac-aspc.gc.ca/publicat/human-humain06/pdf/human_face_e.pdf. Published 2006. Accessed February 26, 2019.

105. Canadian Mental Health Association British Columbia Division. Learn about eating disorders. http://www.heretohelp.bc.ca/sites/default/files/eating-disorders.pdf. Published 2014. Accessed February 26, 2019.

106. Field AE, Austin SB, Taylor CB, et al. Relation between dieting and weight change among preadolescents and adolescents. *Pediatrics*. 2003;112:900-906.

107. Bernadett M, Szemán-N A. Prevalence of eating disorders among women with polycystic ovary syndrome. *Psychiatr Hung*. 2016;31(2):136-45.

108. Sullivan P. Course and outcome of anorexia nervosa and bulimia nervosa. In: Fairburn CG, Brownell KD, eds. *Eating Disorders and Obesity*. New York, NY: Guilford; 2002:226-232.

109. McCluskey S, Evans C, Lacey JH, Pearce JM, Jacobs H. Polycystic ovary syndrome and bulimia. *Fertil Steril*. 1991;55:287-291.

110. Lacey JH. The bulimic syndrome at normal body weight: Reflections on pathogenesis and clinical features. *Int J Eat Disord*. 1982;2(1):59-66.

111. Hudson, JI, Hiripi E, Pope HG, Kessler RC. The prevalence and correlates of eating disorders in the national comorbidity survey replication. *Biological Psychiatry*. 2007;61(3), 348-358.

112. Mazzeo SE, Bulik CM. Environmental and genetic risk factors for eating disorders: what the clinician needs to know. *Child Adolesc Psychiatr Clin N Am*. 2009;18:67-82.

113. Huijgen, NA, Laven, JS, Labee, CT, et al. Are dieting and dietary inadequacy a second hit in the association with polycystic ovary syndrome severity? *PloS One*. 2015;10:e0142772.

114. de Melo AS, dos Reis RM, Ferriani RA, Vieira CS. Hormonal contraception in women with polycystic ovary syndrome: choices, challenges, and noncontraceptive benefits. *Open Access J Contracept*. 2017;8:13.

115. Harrison CL, Lombard CB, Moran LJ, Teede HJ. Exercise therapy in polycystic ovary syndrome: a systematic review. *Hum Reprod Update*. 2011;17:171-183.

116. Meyer C, McGrath BP, Teede HJ. Effects of medical therapy on insulin resistance and the cardiovascular system in polycystic ovary syndrome. *Diabetes Care*. 2007;30(3):471-8.

117. Soares GM, Vieira CS, de Paula Martins W, Dos Reis RM, De Sá MF, Ferriani RA. Metabolic and cardiovascular impact of oral contraceptives in polycystic ovary syndrome. *Int J Clin Pract*. 2009;63(1):160-9.

118. Teede HJ, Meyer C, Hutchison SK, Zoungas S, McGrath BP, Moran LJ. Endothelial function and insulin resistance in polycystic ovary syndrome: the effects of medical therapy. *Fertil Steril*. 2010;93(1):184-91.

119. Cortés ME, Alfaro AA. The effects of hormonal contraceptives on glycemic regulation. *Linacre Q*. 2014;81(3):209-18.

120. Cagnacci A, Paoletti AM, Renzi A, et al. Glucose metabolism and insulin resistance in women with polycystic ovary syndrome during therapy with oral contraceptives containing cyproterone acetate or desogestrel. *J Clin Endocrinol Metab*. 2003;88(8):3621-5.

121. Piltonen T, Puurunen J, Hedberg P, et al. Oral, transdermal and vaginal combined contraceptives induce an increase in markers of chronic inflammation and impair insulin

sensitivity in young healthy normal-weight women: a randomized study. *Hum Reprod*. 2012;27:3046-3056.

122. Simon D, Senan C, Gamier P, et al. Effects of oral contraceptives on carbohydrate and lipid metabolisms in a healthy population: the Telecom study. *Am J Obstet Gynecol*. 1990;163:382-387.

123. Mastorakos G, Koliopoulos C, Deligeoroglou E, Diamanti-Kandarakis E, Creatsas G. Effects of two forms of combined oral contraceptives on carbohydrate metabolism in adolescents with polycystic ovary syndrome. *Fertil Steril*. 2006;85:420-427.

124. Society TP. Consensus statement on the use of oral contraceptive pills in polycystic ovarian syndrome women in India. *J Hum Reprod Sci*. 2018;11:96.

125. Moran LJ, Hutchison SK, Norman RJ, Teede HJ. Lifestyle changes in women with polycystic ovary syndrome. *Cochrane Database Syst Rev*. 2011;(2).

126. Qin JZ, Pang LH, Li MJ, Fan XJ, Huang RD, Chen HY. Obstetric complications in women with polycystic ovary syndrome: a systematic review and meta-analysis. *Reprod Biol Endocrinol*. 2013;11(1):56.

127. Boomsma CM, Eijkemans MJ, Hughes EG, Visser GH, Fauser BC, Macklon NS. A meta-analysis of pregnancy outcomes in women with polycystic ovary syndrome. *Hum Reprod Update*. 2006;12(6):673-83.

128. Smithson DS, Vause TD, Cheung AP. No. 362-ovulation induction in polycystic ovary syndrome. *J Obstet Gynaecol Can*. 2018;40(7):978-87.

129. Legro RS, Brzyski RG, Diamond MP, et al. Letrozole versus clomiphene for infertility in the polycystic ovary syndrome. *N Engl J Med*. 2014;371(2):119–29.

130. Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. *Hum Reprod*. 2008;23:462-477.

131. Practice Committee of the American Society for Reproductive Medicine. Ovarian hyperstimulation syndrome. *Fertil Steril*. 2008;90(5):S188-93.

132. Dale PO, Tanbo T, Haug E, Abyholm T. The impact of insulin resistance on the outcome of ovulation induction with low-dose follicle stimulating hormone in women with polycystic ovary syndrome. *Hum Reprod*. 1998;13(3):567-70.

133. Balen AH, Morley LC, Misso M, et al. The management of anovulatory infertility in women with polycystic ovary syndrome: an analysis of the evidence to support the development of global WHO guidance. *Hum Reprod Update*. 2016;22(6):687-708.

134. Balen AH, Tan SL, Jacobs HS. Hypersecretion of luteinising hormone: a significant cause of infertility and miscarriage. *Br J Obstet Gynaecol*. 1993;100(12):1082-9.

135. Vause TD, Cheung AP, Sierra S, et al. Ovulation induction in polycystic ovary syndrome: No. 242, May 2010. *Int J Gynaecol Obstet*. 2010;111:95-100.

136. Lin AW, Lujan ME. Comparison of dietary intake and physical activity between women with and without polycystic ovary syndrome: a review. *Adv Nutr*. 2014;5:486-496.

137. Barrea L, Marzullo P, Muscogiuri G, et al. Source and amount of carbohydrate in the diet and inflammation in women with polycystic ovary syndrome. *Nutr Res Rev*. 2018;31:291-301.

138. Altieri P, Cavazza C, Pasqui F, Morselli AM, Gambineri A, Pasquali R. Dietary habits and their relationship with hormones and metabolism in overweight and obese women with polycystic ovary syndrome. *Clin Endocrinol (Oxf)*. 2013;78:52-59.

139. Graff, SK, Mário, FM, Alves, BC, et al. Dietary glycemic index is associated with less favorable anthropometric and metabolic profiles in polycystic ovary syndrome women with different phenotypes. *Fertil Steril*. 2013;100:1081-8.

140. Barr, S, Reeves, S, Sharp, K, et al. An isocaloric low glycemic index diet improves insulin sensitivity in women with polycystic ovary syndrome. *J Acad Nutr Diet*. 2013;113:1523-31.

141. Marsh KA, Steinbeck KS, Atkinson FS, Petocz P, Brand-Miller JC. Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. *Am J Clin Nutr*. 2010;92:83-92.

142. Moses RG, Luebcke M, Davis WS, et al. Effect of a low-glycemic-index diet during pregnancy on obstetric outcomes. *Am J Clin Nutr*. 2006;84:807-812.

143. McGrice M, Porter J. The effect of low carbohydrate diets on fertility hormones and outcomes in overweight and obese women: a systematic review. *Nutrients*. 2017;9:204.

144. Moran LJ, Ko H, Misso M, et al. Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutr Diet*. 2013;113:520-545.

145. Mavropoulos JC, Yancy WS, Hepburn J, Westman EC. The effects of a low-carbohydrate, ketogenic diet on the polycystic ovary syndrome: a pilot study. *Nutr Metab*. 2005;2(1):35.

146. Faghfoori Z, Fazelian S, Shadnoush M, Goodarzi R. Nutritional management in women with polycystic ovary syndrome: A review study. *Diabetes Metab Syndr*. 2017;11:S429-S432.

147. Eslamian G, Baghestani A, Eghtesad S, Hekmatdoost A. Dietary carbohydrate composition is associated with polycystic ovary syndrome: a case–control study. *J Hum Nutr Diet*. 2017;30(1):90-97.

148. Maskarinec G, Morimoto Y, Takata Y, Murphy SP, Stanczyk FZ. Alcohol and dietary fibre intakes affect circulating sex hormones among premenopausal women. *Public Health Nutr*. 2006;9:875-881.

149. Stamets K, Taylor DS, Kunselman A, Demers LM, Pelkman CL, Legro RS. A randomized trial of the effects of two types of short-term hypocaloric diets on weight loss in women with polycystic ovary syndrome. *Fertil Steril*. 2004;81:630-637.

150. Krebs M, Krssak M, Bernroider E, et al. Mechanism of amino acid-induced skeletal muscle insulin resistance in humans. *Diabetes*. 2002;51:599-605.

151. Linn T, Santosa B, Grönemeyer D, et al. Effect of long-term dietary protein intake on glucose metabolism in humans. *Diabetologia*. 2000;43:1257-1265.

152. Martin WF, Armstrong LE, Rodriguez NR. Dietary protein intake and renal function. *Nutr Metab*. 2005;2(1):25.

153. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. Protein intake and ovulatory infertility. *Obstet Gynecol*. 2008;198:210. e1-210. e7.

154. Nadjarzadeh A, Dehghani-Firouzabadi R, Daneshbodi H, Lotfi MH, Vaziri N, Mozaffari-Khosravi H. Effect of omega-3 supplementation on visfatin, adiponectin, and anthropometric indices in women with polycystic ovarian syndrome. *J Reprod Infertil*. 2015;16:212.

155. Nadjarzadeh A, Dehghani Firouzabadi R, Vaziri N, Daneshbodi H, Lotfi MH, Mozaffari-Khosravi H. The effect of omega-3 supplementation on androgen profile and menstrual

status in women with polycystic ovary syndrome: A randomized clinical trial. *Iran J Reprod Med*. 2013;11:665-672.

156. Cussons AJ, Watts GF, Mori TA, Stuckey BG. Omega-3 fatty acid supplementation decreases liver fat content in polycystic ovary syndrome: a randomized controlled trial employing proton magnetic resonance spectroscopy. *J Clin Endocrinol Metab*. 2009;94:3842-3848.

157. Brown AJ, Tendler DA, McMurray RG, Setji TL. Polycystic ovary syndrome and severe nonalcoholic steatohepatitis: beneficial effect of modest weight loss and exercise on liver biopsy findings. *Endocrine Practice*. 2005;11(5):319-24.

158. Yildizhan R, Kurdoglu M, Adali E, et al M. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet*. 2009;280(4):559.

159. Hahn S, Haselhorst U, Tan S, et al. Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes*. 2006;114:577-583.

160. Joham AE, Teede HJ, Cassar S, et al. Vitamin D in polycystic ovary syndrome: Relationship to obesity and insulin resistance. *Mol Nutr Food Res*. 2016;60:110-118.

161. Jamilian M, Razavi M, Fakhrie Kashan Z, Ghandi Y, Bagherian T, Asemi Z. Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Clin Endocrinol (Oxf)*. 2015;82:885-891.

162. Amooee S, Parsanezhad ME, Shirazi MR, Alborzi S, Samsami A. Metformin versus chromium picolinate in clomiphene citrate-resistant patients with PCOS: A double-blind randomized clinical trial. *Iran J Reprod Med*. 2013;11(8):611.

163. Arpaci D, Tocoglu AG, Ergenc H, Korkmaz S, Ucar A, Tamer A. Associations of serum Magnesium levels with diabetes mellitus and diabetic complications. *Hippokratia*. 2015;19:153-157.

164. Papavasiliou K, Papakonstantinou E. Nutritional support and dietary interventions for women with polycystic ovary syndrome. *Nutr Diet Suppl*. 2017;9:63-85.

165. Coskun A, Arikan T, Kilinc M, Arikan DC, Ekerbiçer HÇ. Plasma selenium levels in Turkish women with polycystic ovary syndrome. *Eur J Obst Gynecol Reprod Biol*. 2013;168:183-186.

166. Pasquali R, Casimirri F, Venturoli S, et al Body fat distribution has weight-independent effects on clinical, hormonal, and metabolic features of women with polycystic ovary syndrome. *Metabolism*. 1994;43(6):706-13.

167. Ross R, Janssen I, Dawson J, et al. Exercise-induced reduction in obesity and insulin resistance in women: a randomized controlled trial. *Obes Res*. 2004;12(5):789-98.

168. Hoeger KM. Exercise therapy in polycystic ovary syndrome. *Semin Reprod Med*. 2008;26(1), 93-100.

169. Thomson R, Buckley J, Brinkworth G. Exercise for the treatment and management of overweight women with polycystic ovary syndrome: a review of the literature. *Obes Rev*. 2011;12:e202-e210.

170. Randeva HS, Lewandowski KC, Drzewoski J, et al. Exercise decreases plasma total homocysteine in overweight young women with polycystic ovary syndrome. *Int J Clin Endocrinol Metab*. 2002;87(10):4496-501.

171. Crosignani PG, Colombo M, Vegetti W, Somigliana E, Gessati A, Ragni G. Overweight and obese anovulatory patients with polycystic ovaries: parallel improvements in anthropometric indices, ovarian physiology and fertility rate induced by diet. *Hum Reprod*. 2003;18(9):1928-32.

172. Wright C, Zborowski J, Talbott E, McHugh-Pemu K, Youk A. Dietary intake, physical activity, and obesity in women with polycystic ovary syndrome. *Int J Obes*. 2004;28:1026-1032.

173. Hutchison SK, Stepto NK, Harrison CL, Moran LJ, Strauss BJ, Teede HJ. Effects of exercise on insulin resistance and body composition in overweight and obese women with and without polycystic ovary syndrome. *Int J Clin Endocrinol Metab*. 2011;96(1):E48-56.

174. Daley A. The role of exercise in the treatment of menstrual disorders: the evidence. *Br J Gen Pract*. 2009;59(561):241-242.

175. Guszkowska M. Effects of exercise on anxiety, depression and mood. *Psychiatria polska*. 2004;38(4):611-20.

176. Lin TW, Kuo YM. Exercise benefits brain function: the monoamine connection. *Brain Sci*. 2013;3(1):39-53.

177. Tumati S, Burger H, Martens S, van der Schouw YT, Aleman A. Association between cognition and serum insulin-like growth factor-1 in middle-aged & older men: an 8 year follow-up study. *PLoS One*. 2016;11(4):e0154450.

178. Barnard L, Ferriday D, Guenther N, Strauss B, Balen AH, Dye L. Quality of life and psychological well-being in polycystic ovary syndrome. *Hum Reprod*. 2007;22(8):2279-86.

179. Ludwig DS, Kabat-Zinn J. Mindfulness in medicine. *JAMA*. 2008;300(11):1350-2.

180. Matchim Y, Armer JM, Stewart BR. Mindfulness-based stress reduction among breast cancer survivors: a literature review and discussion. *Oncol Nurs Forum*. 2011;38(2).

181. Raja-Khan N, Stener-Victorin E, Wu X, Legro RS. The physiological basis of complementary and alternative medicines for polycystic ovary syndrome. *Am J Physiol Endocrinol Metab*. 2011;301(1):E1-0.

182. Stefanaki C, Bacopoulou F, Livadas S, et al. Impact of a mindfulness stress management program on stress, anxiety, depression and quality of life in women with polycystic ovary syndrome: a randomized controlled trial. *Stress*. 2015;18:57-66.

183. Raja-Khan N, Agito K, Shah J, et al. Mindfulness-based stress reduction in women with overweight or obesity: A randomized clinical trial. *Obesity*. 2017;25(8):1349-59.

184. Sills ES, Perloe M, Tucker MJ, Kaplan CR, Genton MG, Schattman GL. Diagnostic and treatment characteristics of polycystic ovary syndrome: descriptive measurements of patient perception and awareness from 657 confidential self-reports. *BMC Womens Health*. 2001;1(1):3.

185. Arentz S, Smith CA, Abbott JA, Bensoussan A. A survey of the use of complementary medicine by a self-selected community group of Australian women with polycystic ovary syndrome. *BMC Complement Altern Med*. 2014;14:1.

186. Jedel E, Labrie F, Oden A, et al. Impact of electro-acupuncture and physical exercise on hyperandrogenism and oligo/amenorrhea in women with polycystic ovary syndrome: a randomized controlled trial. *Am J Physiol Endocrinol Metab.* 2011;300:E37-45.

187. Leonhardt H, Hellström M, Gull B, et al. Serum anti-Müllerian hormone and ovarian morphology assessed by magnetic resonance imaging in response to acupuncture and exercise in women with polycystic ovary syndrome: secondary analyses of a randomized controlled trial. *Acta Obstet Gynecol Scand*. 2015;94(3):279-87.

188. Johansson J, Redman L, Veldhuis PP, et al. Acupuncture for ovulation induction in polycystic ovary syndrome: a randomized controlled trial. *Am J Physiol Heart Circ Physiol*. 2013;304(9):E934-43..

189. Arentz S, Abbott JA, Smith CA, Bensoussan A. Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/amenorrhoea and hyperandrogenism; a review of the laboratory evidence for effects with corroborative clinical findings. *BMC Complement Altern Med*. 2014;14:511.

190. Wuttke W, Jarry H, Seidlová-Wuttke D. Cimicifuga racemosa extract for the treatment of climacteric complaints. *J Endocrinol Reprod*. 2006;10:106-110.

191. Shahin AY, Mohammed SA. Adding the phytoestrogen cimicifugae racemosae to clomiphene induction cycles with timed intercourse in polycystic ovary syndrome improves cycle outcomes and pregnancy rates–a randomized trial. *Gynecol Endocrinol*. 2014;30(7):505-10.

192. Kamel HH. Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2013;168:60-63.

193. Shahin AY, Ismail AM, Zahran KM, Makhlouf AM. Adding phytoestrogens to clomiphene induction in unexplained infertility patients–a randomized trial. *Reprod Biomed Online*. 2008;16:580-588.

194. Roussel A, Hininger I, Benaraba R, Ziegenfuss TN, Anderson RA. Antioxidant effects of a cinnamon extract in people with impaired fasting glucose that are overweight or obese. *J Am Coll Nutr*. 2009;28:16-21.

195. Wang JG, Anderson RA, Graham III GM, et al. The effect of cinnamon extract on insulin resistance parameters in polycystic ovary syndrome: a pilot study. *Fertil Steril*. 2007;88:240-243.

196. Heibashy M, Mazen G, Shahin M. Metabolic changes and hormonal disturbances in polycystic ovarian syndrome rats and the amelioration effects of metformin and/or cinnamon extraction. *J Am Sci*. 2013;9:54-p62.

197. Milewicz A, Gejdel E, Sworen H, et al. Vitex agnus castus extract in the treatment of luteal phase defects due to latent hyperprolactinemia. Results of a randomized placebo-controlled double-blind study. *Arzneimittelforschung*. 1993;64(7):752–756.

198. Gerhard I, Patek A, Monga B, Blank A, Gorkow C. Mastodynon® for female infertility. Randomized placebo controlled, clinical double-blind study. *Res Compl Med*. 1998;5(6):272–278.

199. Bergmann J, Luft B, Boehmann S, Runnebaum B, Gerhard I. The efficacy of the complex medication Phyto-Hypophyson L in female, hormone-related sterility. A randomized, placebo-controlled clinical double-blind study. *Forsch Komplementarmed Klass Naturheilkd.* 2000;7(4):190.

200. Kilicdag EB, Tarim E, Bagis T, et al. Fructus agni casti and bromocriptine for treatment of hyperprolactinemia and mastalgia. *Int J Gynecol Obstet*. 2004;85(3):292-3.

201. Armanini D, Castello R, Scaroni C, et al. Treatment of polycystic ovary syndrome with spironolactone plus licorice. *Eur J Obstet Gynecol Reprod Biol*. 2007;131(1):61-7.

202. Armanini D, Mattarello MJ, Fiore C, et al. Licorice reduces serum testosterone in healthy women. *Steroids*. 2004;69:763-766.

203. Grant P. Spearmint herbal tea has significant anti-androgen effects in polycystic ovarian syndrome. A randomized controlled trial. *Phytother Res*. 2010;24(2):186-8.

204. Ataabadi MS, Alaee S, Bagheri MJ, Bahmanpoor S. Role of essential oil of mentha spicata (spearmint) in addressing reverse hormonal and folliculogenesis disturbances in a polycystic ovarian syndrome in a rat model. *Adv Pharm Bull*. 2017;7:651.

205. Kalra B, Kalra S, Sharma JB. The inositols and polycystic ovary syndrome. *Indian J Endocrinol Metab*. 2016;20:720-724.

206. Unfer V, Facchinetti F, Orrù B, Giordani B, Nestler J. Myo-inositol effects in women with PCOS: a meta-analysis of randomized controlled trials. *Endocr Connect*. 2017;6(8):647-58.

207. Bizzarri M, Carlomagno G. Inositol: history of an effective therapy for polycystic ovary syndrome. *Eur Rev Med Pharmacol Sci*. 2014;18:1896-1903.

208. Ciotta L, Stracquadanio M, Pagano I, Carbonaro A, Palumbo M, Gulino F. Effects of myo-inositol supplementation on oocyte's quality in PCOS patients: a double blind trial. *Eur Rev Med Pharmacol Sci*. 2011;15:509-514.

209. Gerli S, Mignosa M, Di Renzo G. Effects of inositol on ovarian function and metabolic factors in women with PCOS: a randomized double blind placebo-controlled trial. *Eur Rev Med Pharmacol Sci*. 2003;7:151-160.

210. Gerli S, Papaleo E, Ferrari A, Di Renzo G. Randomized, double blind placebo-controlled trial: effects of myo-inositol on ovarian function and metabolic factors in women with PCOS. *Eur Rev Med Pharmacol Sci*. 2007;11:347-354.

211. Costantino D, Minozzi G, Minozzi E, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. *Eur Rev Med Pharmacol Sci*. 2009;13(2):105-10.

212. Papaleo E, Unfer V, Baillargeon JP, et al. Myo-inositol in patients with polycystic ovary syndrome: a novel method for ovulation induction. *Gynecol Endocrinol*. 2007;23(12):700-3.

213. Taylor MJ, Wilder H, Bhagwagar Z, Geddes J. Inositol for depressive disorders. *Cochrane Database Syst Rev*. 2004;(2).

214. Tang T, Lord JM, Norman RJ, Yasmin E, Balen AH. Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev*. 2012;(5).

215. Genazzani AD, Santagni S, Rattighieri E, et al. Modulatory role of D-chiro-inositol (DCI) on LH and insulin secretion in obese PCOS patients. *Gynecol Endocrinol*. 2014;30:438-443.

216. La Marca A, Grisendi V, Dondi G, Sighinolfi G, Cianci A. The menstrual cycle regularization following D-chiro-inositol treatment in PCOS women: a retrospective study. *Gynecol Endocrinol*. 2015;31:52-56.

217. Piomboni P, Focarelli R, Capaldo A, et al. Protein modification as oxidative stress marker in follicular fluid from women with polycystic ovary syndrome: the effect of inositol and metformin. *J Assist Reprod Genet*. 2014;31:1269-1276.

218. Nestler JE, Jakubowicz DJ, Reamer P, Gunn RD, Allan G. Ovulatory and metabolic effects of D-chiro-inositol in the polycystic ovary syndrome. *N Engl J Med*. 1999;340:1314-1320.

219. Colazingari S, Treglia M, Najjar R, Bevilacqua A. The combined therapy myo-inositol plus D-chiro-inositol, rather than D-chiro-inositol, is able to improve IVF outcomes: results from a randomized controlled trial. *Arch Gynecol Obstet*. 2013;288:1405-1411.

220. Ortega RM, Pérez-Rodrigo C, López-Sobaler AM. Dietary assessment methods: dietary records. *Nutr Hosp*. 2015;31(3):38-45.

221. Thompson FE, Subar AF. Dietary assessment methodology. *Nutrition in the Prevention and Treatment of Disease.* Elsevier; 2017:5-48.

222. Yang YJ, Kim MK, Hwang SH, Ahn Y, Shim JE, Kim DH. Relative validities of 3-day food records and the food frequency questionnaire. *Nutr Res Pract*. 2010;4:142-148.

223. Moshfegh AJ, Rhodes DG, Baer DJ, et al. The US Department of Agriculture Automated Multiple-Pass Method reduces bias in the collection of energy intakes. *Am J Clin Nutr*. 2008;88(2):324-332.

224. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr*. 1997;65:1220S-1228S; discussion 1229S-1231S.

225. Gemming L, Jiang Y, Swinburn B, Utter J, Mhurchu CN. Under-reporting remains a key limitation of self-reported dietary intake: an analysis of the 2008/09 New Zealand Adult Nutrition Survey. *Eur J Clin Nutr*. 2014;68:259-264.

226. Braam LA, Ocke MC, Bueno-de-Mesquita HB, Seidell JC. Determinants of obesity-related underreporting of energy intake. *Am J Epidemiol*. 1998;147(11):1081-1086.

227. Ballard-Barbash R, Graubard I, Krebs-Smith SM, Schatzkin A, Thompson FE. Contribution of dieting to the inverse association between energy intake and body mass index. *Eur J Clin Nutr*. 1996;50:98-106.

228. Lafay L, Basdevant A, Charles MA, et al. Determinants and nature of dietary underreporting in a free-living population: the Fleurbaix Laventie Ville Sante (FLVS) Study. *Int J Obes Relat Metab Disord*. 1997;21(7):567-73.

229. Goldberg GR, Black AE, Jebb SA, et al. Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr*. 1991;45(12):569-581.

230. Schofield WN. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr*. 1985;39 Suppl 1:5-41.

231. Douglas, CC, Norris, LE, Oster, RA, et al. Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. *Fertil Steril*. 2006;86:411-7.

232. Zhang J, Liu Y, Liu X, et al. High intake of energy and fat in Southwest Chinese women with PCOS: A population-based case-control study. *PloS one*. 2015;10:e0127094.

233. Georgopoulos NA, Saltamavros AD, Vervita V, et al. Basal metabolic rate is decreased in women with polycystic ovary syndrome and biochemical hyperandrogenemia and is associated with insulin resistance. *Fertil Steril*. 2009;92:250-255.

234. Robinson S, Chan S, Spacey S, Anyaoku V, Johnston DG, Franks S. Postprandial thermogenesis is reduced in polycystic ovary syndrome and is associated with increased insulin resistance. *Clin Endocrinol (Oxf)*. 1992;36:537-543.

235. Barr, S, Hart, K, Reeves, S, et al. Habitual dietary intake, eating pattern and physical activity of women with polycystic ovary syndrome. *Eur J Clin Nutr*. 2011;65:1126-32.

236. Wild, RA, Painter, P, Coulson, PB, et al. Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 1985;61:946-51.

237. Weickert, MO, Pfeiffer, AF. Metabolic effects of dietary fiber consumption and prevention of diabetes. *J Nutr*. 2008;138:439-42.

238. Jenkins DJ, Kendall CW, Augustin LS, et al. Glycemic index: overview of implications in health and disease. *Am J Clin Nutr*. 2002;76:266S-73S.

239. Salmeron J, Manson JE, Stampfer MJ, Colditz GA, Wing AL, Willett WC. Dietary fiber, glycemic load, and risk of non—insulin-dependent diabetes mellitus in women. *JAMA*. 1997;277:472-477.

240. Afifi, L, Saeed, L, Pasch, LA, et al. Association of ethnicity, Fitzpatrick skin type, and hirsutism: a retrospective cross-sectional study of women with polycystic ovarian syndrome. *Int J Womens Dermatol*. 2017;3:37-43.

241. Karimah, P, Hestiantoro, A. The cut off of Ferriman Gallwey Score for PCOS in Asia and the degree of hyperandrogenism indicator. *KnE Med*. 2017;1:186-92.

242. Wijeyaratne, CN, Balen, AH, Barth, JH, et al. Clinical manifestations and insulin resistance (IR) in polycystic ovary syndrome (PCOS) among South Asians and Caucasians: Is there a difference? *Clin Endocrinol (Oxf*). 2002;57:343–50.

243. Dewailly, D, Lujan, M, Carmina, E, et al. Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update*. 2014;20:334-52.

244. Willett W. *Nutritional Epidemiology.* 3rd ed. Oxford University Press; 2012.

245. Stumbo, P. Considerations for selecting a dietary assessment system. *J Food Compost Anal*. 2008;21:S13-19.

246. Bonora E, Targher G, Alberiche M, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects

with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care*. 2000;23:57-63.

247. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. 1985;28:412-419.

248. Lansang MC, Williams GH, Carroll JS. Correlation between the glucose clamp technique and the homeostasis model assessment in hypertension. *Am J Hypertens*. 2001;14:51-53.

249. Kar, S. Anthropometric, clinical, and metabolic comparisons of the four Rotterdam PCOS phenotypes: a prospective study of PCOS women. *J Hum Reprod Sci*. 2013;6:194-200.

250. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake: basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes*. 2000;24:1119.

251. Toscani MK, Mario FM, Radavelli-Bagatini S, Spritzer PM. Insulin resistance is not strictly associated with energy intake or dietary macronutrient composition in women with polycystic ovary syndrome. *Nutr Res*. 2011;31:97-103.

252. Moran, LJ, Ranasinha, S, Zoungas, S, et al. The contribution of diet, physical activity and sedentary behaviour to body mass index in women with and without polycystic ovary syndrome. *Hum Reprod*. 2013;28:2276–83.

253. Threapleton, DE, Greenwood, DC, Evans, CE, et al. Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ*. 2013;347:f6879.

254. Yao, B, Fang, H, Xu, W, et al. Dietary fiber intake and risk of type 2 diabetes: a dose–response analysis of prospective studies. *Eur J Epidemiol*. 2014;29:79-88.

255. Liu, S, Willett, WC, Manson, JE, et al. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. *Am J Clin Nutr*. 2003;78:920-7.

256. Bo S, Durazzo M, Guidi S, et al. Dietary magnesium and fiber intakes and inflammatory and metabolic indicators in middle-aged subjects from a population-based cohort. *Am J Clin Nutr*. 2006;84:1062-1069.

257. Brown, L, Rosner, B, Willett, WW, et al. Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr*. 1999;69:30-42.

258. Shishehgar, F, Tehrani, FR, Mirmiran, P, et al. Comparison of dietary intake between polycystic ovary syndrome women and controls. *Glob J Health Sci*. 2016;8:302.

259. Jenkins DJ, Kendall CW, Axelsen M, Augustin LS, Vuksan V. Viscous and nonviscous fibres, nonabsorbable and low glycaemic index carbohydrates, blood lipids and coronary heart disease. *Curr Opin Lipidol*. 2000;11:49-56.

260. Goff, LM, Cowland, DE, Hooper, L, et al. Low glycaemic index diets and blood lipids: a systematic review and meta-analysis of randomised controlled trials. *Nutr Metab Cardiovasc Dis*. 2013;23:1-10.

261. Schwingshackl, L, Hoffmann, G. Long-term effects of low glycemic index/load vs. high glycemic index/load diets on parameters of obesity and obesity-associated risks: a systematic review and meta-analysis. *Nutr Metab Cardiovasc Dis*. 2013;23:699-706.

262. Brand-Miller, JC, Holt, SH, Pawlak, DB, et al. Glycemic index and obesity. *Am J Clin Nutr*. 2002;76:281S-5S.

263. Ou, S, Kwok, K, Li, Y, et al. In vitro study of possible role of dietary fiber in lowering postprandial serum glucose. *J Agric Food Chem*. 2001;49:1026-29.

264. Livadas, S, Chaskou, S, Kandaraki AA, et al. Anxiety is associated with hormonal and metabolic profile in women with polycystic ovarian syndrome. *Clin Endocrinol (Oxf)*. 2011;75:698-703.

265. Bazarganipour F, Ziaei S, Montazeri A, Foroozanfard F, Kazemnejad A, Faghihzadeh S. Health-related quality of life in patients with polycystic ovary syndrome (PCOS): A model-based study of predictive factors. *J Sex Med*. 2014;11:1023-1032.

266. Barbieri RL, Ryan KJ. Hyperandrogenism, insulin resistance, and acanthosis nigricans syndrome: a common endocrinopathy with distinct pathophysiologic features. *Am J Obstet Gynecol*. 1983;147(1):90-101.

267. Katcher HI, Kunselman AR, Dmitrovic R, et al. Comparison of hormonal and metabolic markers after a high-fat, Western meal versus a low-fat, high-fiber meal in women with polycystic ovary syndrome. *Fertil Steril*. 2009;91:1175-1182.

268. O'Reilly, M, Kempegowda, P, Jenkinson, C, et al. 11-oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2017;102(3):840-8.

269. Fox C, Ramsoomair D, Carter C. Magnesium: its proven and potential clinical significance. *South Med J*. 2001;94:1195-1202.

270. Rumawas ME, McKeown NM, Rogers G, Meigs JB, Wilson PW, Jacques PF. Magnesium intake is related to improved insulin homeostasis in the framingham offspring cohort. *J Am Coll Nutr*. 2006;25:486-492.

271. Sharifi F, Mazloomi S, Hajihosseini R, Mazloomzadeh S. Serum magnesium concentrations in polycystic ovary syndrome and its association with insulin resistance. *Gynecol Endocrinol*. 2012;28:7-11.

272. Cahill F, Shahidi M, Shea J, et al. High dietary magnesium intake is associated with low insulin resistance in the Newfoundland population. *PLoS One*. 2013;8:e58278.

273. Olatunji LA, Soladoye AO. Effect of increased magnesium intake on plasma cholesterol, triglyceride and oxidative stress in alloxan-diabetic rats. *Afr J Med Med Sci*. 2007;36:155-161.

274. Simental-Mendía LE, Simental-Mendía M, Sahebkar A, Rodríguez-Morán M, Guerrero-Romero F. Effect of magnesium supplementation on lipid profile: a systematic review and meta-analysis of randomized controlled trials. *Eur J Clin Pharmacol*. 2017;73:525-536.

275. Dibaba DT, Xun P, He K. Dietary magnesium intake is inversely associated with serum C-reactive protein levels: meta-analysis and systematic review. *Eur J Clin Nutr*. 2014;68(4):510.

276. Ebrahimi FA, Foroozanfard F, Aghadavod E, Bahmani F, Asemi Z. The effects of magnesium and zinc co-supplementation on biomarkers of inflammation and oxidative stress, and gene expression related to inflammation in polycystic ovary syndrome: a randomized controlled clinical trial. *Biol Trace Elem Res*. 2018;184:300-307.

277. Matthews, KA, Rhoten, WB, Driscoll, HK, et al. Vitamin A deficiency impairs fetal islet development and causes subsequent glucose intolerance in adult rats. *J Nutri*. 2004;134:1958-63.

278. Amisten S, Al-Amily IM, Soni A, et al. Anti-diabetic action of all-trans retinoic acid and the orphan G protein coupled receptor GPRC5C in pancreatic β-cells. *Endocr J*. 2017:EJ16-0338.

279. Rajeswari G, Gopal PS, Veerabhadrudu B, Suresh E. Study of magnesium levels in polycystic ovarian syndrome. *IJAR*. 2016;2:610-613.

280. Boivin J, Takefman J, Braverman A. Development and preliminary validation of the fertility quality of life (FertiQoL) tool. *Hum Reprod*. 2011;26(8):2084–91.

281. Lovibond SH, Lovibond PF. Manual for the Depression Anxiety Stress Scales. 2nd ed. Sydney: Psychology Foundation; 1995.

282. Hahn S, Janssen OE, Tan S, et al. Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. *Eur J Endocrinol*. 2005;153:853-860.

283. Månsson M, Holte J, Landin-Wilhelmsen K, Dahlgren E, Johansson A, Landén M. Women with polycystic ovary syndrome are often depressed or anxious—a case control study. *Psychoneuroendocrinology*. 2008;33(8):1132-8.

284. Tiggemann M, Kenyon SJ. The hairlessness norm: The removal of body hair in women. *Sex Roles*. 1998;39(11-12):873–885.

285. Basow, SA, Braman, AC. Women and body hair: Social perceptions and attitudes. *Psychol Women Q*. 1998;22(4):637–645.

286. Wharton W, E Gleason C, Sandra O, M Carlsson C, Asthana S. Neurobiological underpinnings of the estrogen-mood relationship. *Curr Psychiatry Rev*. 2012;8:247-256.

287. Murakami K, Sasaki S. Dietary intake and depressive symptoms: a systematic review of observational studies. *Mol Nutr Food Res*. 2010;54:471-488.

288. Akbaraly TN, Brunner EJ, Ferrie JE, Marmot MG, Kivimaki M, Singh-Manoux A. Dietary pattern and depressive symptoms in middle age. *Br J Psychiatry*. 2009;195(5):408-413.

289. Lai JS, Hiles S, Bisquera A, Hure AJ, McEvoy M, Attia J. A systematic review and meta-analysis of dietary patterns and depression in community-dwelling adults. *Am J Clin Nutr*. 2013;99:181-197.

290. Jacka FN, O'Neil A, Opie R, et al. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES'trial). *BMC Medicine*. 2017;15:23.

291. Boyle N, Lawton C, Dye L. The effects of magnesium supplementation on subjective anxiety and stress—a systematic review. *Nutrients*. 2017;9:429.

292. McKercher CM, Schmidt MD, Sanderson KA, Patton GC, Dwyer T, Venn AJ. Physical activity and depression in young adults. *Am J Prev Med*. 2009;36:161-164.

293. Yuenyongchaiwat K. Effects of 10,000 steps a day on physical and mental health in overweight participants in a community setting: a preliminary study. *Braz J Phys Ther*. 2016;20(4):367-73.

294. Penckofer S, Kouba J, Byrn M, Estwing Ferrans C. Vitamin D and depression: where is all the sunshine? *Issues Ment Health Nurs*. 2010;31(6):385-93.

295. Hoogendijk WJ, Lips P, Dik MG, Deeg DJ, Beekman AT, Penninx BW. Depression is associated with decreased 25-hydroxyvitamin D and increased parathyroid hormone levels in older adults. *Arch Gen Psychiatry*. 2008;65:508-512.

296. Lin MW, Wu MH. The role of vitamin D in polycystic ovary syndrome. *Indian J Med Res*. 2015;142:238-240.

297. Łagowska K, Bajerska J, Jamka M. The role of vitamin d oral supplementation in insulin resistance in women with polycystic ovary syndrome: A systematic review and meta-analysis of randomized controlled trials. *Nutrients*. 2018;10(11):1637.

298. Tolkien K, Bradburn S, Murgatroyd C. An anti-inflammatory diet as a potential intervention for depressive disorders: A systematic review and meta-analysis. *Clin Nutr*. 2018;S0261-5614(18)32540-8.

299. Sánchez-Villegas A, Martínez-González MA, Estruch R, et al. Mediterranean dietary pattern and depression: the PREDIMED randomized trial. *BMC Medicine*. 2013;11:208.

300. Esposito K, Marfella R, Ciotola M, et al. Effect of a Mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. *JAMA*. 2004;292:1440-1446.

301. Sozou PD, Hartshorne GM. Time to pregnancy: a computational method for using the duration of non-conception for predicting conception. *PLoS One*. 2012;7(10):e46544.

302. Legro RS, Dodson WC, Kris-Etherton PM, et al. Randomized controlled trial of preconception interventions in infertile women with polycystic ovary syndrome. *J Clin Endocrinol Metab*. 2015;100(11):4048–58.

303. Domar AD, Seibel MM, Benson H. The mind/body program for infertility: a new behavioral treatment approach for women with infertility. *Fertil Steril*. 1990;53(2):246–9.

304. Li J, Long L, Liu Y, He W, Li M. Effects of a mindfulness-based intervention on fertility quality of life and pregnancy rates among women subjected to first in vitro fertilization treatment. *Behav Res Ther*. 2016;77:96–104.

305. Bergh CM, Moore M, Gundell C. Evidence-based management of infertility in women with polycystic ovary syndrome. *J Obstet Gynecol Neonatal Nurs*. 2016;45(1):111–22.

306. Berni T, Morgan C, Berni E, Rees A. Polycystic ovary syndrome is associated with adverse mental health and neurodevelopmental outcomes: a retrospective, observational study. *J Clin Endocrinol Metab*. 2018;103(6)2116-2125.

307. Regidor P, Schindler AE. Myo-inositol as a safe and alternative approach in the treatment of infertile PCOS women: a German observational study. *Int J Endocrinol*. 2016;2016:1–5.

308. Unfer V, Carlomagno G, Papaleo E, Vailati S, Candiani M, Baillargeon J. Hyperinsulinemia alters myo-inositol to d-chiroinositol ratio in the follicular fluid of patients with PCOS. *Reprod Sci*. 2014;21(7):854–8.

309. Milewska EM, Czyzyk A, Meczekalski B, Genazzani AD. Inositol and human reproduction. From cellular metabolism to clinical use. *Gynecol Endocrinol*. 2016;32(9):690–5.

310. Chiu TT, Rogers MS, Law EL, Briton-Jones CM, Cheung LP, Haines CJ. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF: Relationship with oocyte quality. *Hum Reprod*. 2002;17(6):1591–6.

311. Pizzo A, Laganã AS, Barbaro L. Comparison between effects of myo-inositol and D-chiro-inositol on ovarian function and metabolic factors in women with PCOS. *Gynecol Endocrinol*. 2014;30(3):205.

312. Palatnik A, Frolov K, Fux M, Benjamin J. Double-blind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder. *J Clin Psychopharmacol*. 2001;1;21(3):335-9.

313. McAlister FA, Straus SE, Sackett DL, Altman DG. Analysis and reporting of factorial trials: a systematic review. *JAMA*. 2003;289(19):2545–53.

314. Jaki T, Vasileiou D. Factorial versus multi-arm multi-stage designs for clinical trials with multiple treatments. *Stat Med*. 2017;36:563–80.

315. Raffone E, Rizzo P, Benedetto V. Insulin sensitizer agents alone and in co-treatment with r-FSH for ovulation induction in PCOS women. *Gynecol Endocrinol*. 2010;26(4):275–80.

316. Yanovski SZ. Dieting and the development of eating disorders in overweight and obese adults. *Arch Intern Med*. 2000;160(17):2581–9.

317. Himelein MJ, Thatcher SS. Polycystic ovary syndrome and mental health: a review. *Obstet Gynecol Surv*. 2006;61(11):723–32.

318. Palomba S, Falbo A, Giallauria F, et al. Six weeks of structured exercise training and hypocaloric diet increases the probability of ovulation after clomiphene citrate in overweight and obese patients with polycystic ovary syndrome: a randomized controlled trial. *Hum Reprod*. 2010;25:2783–91.

319. Zeinalzadeh M, Basirat Z, Esmailpour M. Efficacy of letrozole in ovulation induction compared to that of clomiphene citrate in patients with polycystic ovarian syndrome. *J Reprod Med*. 2010;55(1–2):36–40.

320. Karimzadeh MA, Javedani M. An assessment of lifestyle modification versus medical treatment with clomiphene citrate, metformin, and clomiphene citrate-metformin in patients with polycystic ovary syndrome. *Fertil Steril*. 2010;94(1):216–20.

321. Cutler DA, Pride SM, Cheung AP. Low intakes of dietary fiber and magnesium are associated with insulin resistance and hyperandrogenism in polycystic ovary syndrome: A cohort study. *Food Sci Nutr*. 2019;00:1–12.

322. Cutler DA, Shaw AK, Pride SM, Bedaiwy MA, Cheung AP. A randomized controlled trial comparing lifestyle intervention to letrozole for ovulation in women with polycystic ovary syndrome: a study protocol. *Trials*. 2018;19:632.

323. Subar AF, Freedman LS, Tooze JA, et al. Addressing current criticism regarding the value of self-report dietary data. *J Nutr*. 2015;145:2639-2645.

324. Karayiannis D, Kontogianni MD, Mendorou C, Mastrominas M, Yiannakouris N. Adherence to the Mediterranean diet and IVF success rate among non-obese women attempting fertility. *Hum Reprod*. 2018;33:494-502.

325. Barton J, Hine R, Pretty J. The health benefits of walking in greenspaces of high natural and heritage value. *J Integr Environ Sci*. 2009;6:261-278.

326. Van Cauter E. Sleep disturbances and insulin resistance. *Diabetic Med*. 2011;28:1455-1462.

327. Ehrmann DA. Metabolic dysfunction in PCOS: Relationship to obstructive sleep apnea. *Steroids*. 2012;77:290-294.

328. Spinedi E, Cardinali DP. The polycystic ovary syndrome and the metabolic syndrome: A possible chronobiotic-cytoprotective adjuvant therapy. *Int J Endocrinol*. 2018;2018:1349868.

329. Brotto LA, Clark C, Geoffrion R, et al. Knowledge translation in obstetrics and gynaecology: A pilot study of physical and online media. *Obstet Gynecol Int J*. 2016;5(2):00154.

Appendices

Appendix A: Consent Forms

Title: **Diet, Activity and Stress Levels in Women with Polycystic Ovary Syndrome and/or Women Undergoing Fertility Treatment**

Principal Investigator: A. P. Cheung, MB BS, MPH, MBA, FRACOG, FRCSC
Assistant Professor, Division of Reproductive Endocrinology & Infertility
Department of Obstetrics and Gynaecology, Faculty of Medicine, UBC
Medical Director, Grace Fertility Centre, #210 - 604 West Broadway

Co-Investigator: D. Cutler, PhD Candidate, BSc
Department of Obstetrics and Gynaecology, Faculty of Medicine, UBC
Grace Fertility Centre, #210 - 604 West Broadway

A. Shaw, ND, BSc
Naturopathic Physician, Grace Fertility Centre, #210 - 604 West Broadway `
Clinic Supervisor, Boucher Institute of Naturopathic Medicine

S. Pride, MD, FRCSC
Clinical Professor, Division of Reproductive Endocrinology and Infertility
Department of Obstetrics and Gynecology, Faculty of Medicine, UBC
D6-4500 Oak Street (604) 875-2000 ext: 5685

Invitation:
You are invited to take part in this study because you are or will be undergoing fertility treatments. Some of you will have been diagnosed with polycystic ovary syndrome. This study is entirely *voluntary*; you may withdraw from it at any time and your ongoing care will not be affected. Please review the consent document and decide whether or not you wish to be a participant.

Background information*:*
Studies suggest that diet, activity and stress levels influence ovulation and affect the response of the ovaries to fertility medications or pregnancy success to fertility treatments. Polycystic ovary syndrome (PCOS) is a hormonal condition that affects approximately 6-18% of women during their reproductive years. There are a wide range of symptoms and biochemical changes with which PCOS patients may present such as absent or infrequent menstrual cycles, cosmetic changes (acne, increased facial and body hair), and weight gain. Pre-diabetic and elevated cholesterol states can be present in some women. Many women with PCOS have difficulty conceiving due to infrequent ovulation.

What is the purpose of the study?
This study will examine the diet, activity and stress levels of women undergoing infertility treatments to determine differences among various groups of women, particularly women with PCOS and those without. This study will provide further insight into whether these factors impact PCOS symptoms, ovulation and fertility. It will also provide an opportunity to assess whether these factors can predict ovarian response to fertility medications in women with or without PCOS.

Who can participate in this study?

- all women with PCOS
- all women trying to conceive

What does the study involve?

You will be asked to fill in two questionnaires, which will take no longer than 30 minutes (the Depression Anxiety Stress Scale (DASS) and the Fertility Quality of Life questionnaire (FertiQoL)). You will then keep a detailed food and exercise diary for three days (two weekdays and one weekend day). Additionally, you will wear a small pedometer on your belt or pants (provided by us) which will measure your physical activity.

What information will be collected?

Asides from the stress questionnaires, food diary and exercise diary, your medical records at Grace Centre will be accessed to correlate study results with relevant clinical data for meaningful interpretation. This set of data, with your identification removed, may form a registry to follow-up long-term health effects related to PCOS; however, participation is voluntary and you will be contacted at a later date to ask for your permission and consent.

What are the risks of being a participant?

There is a small possibility that you may feel uncomfortable answering some of the questions. However, the study results will be more meaningful if you can answer all questions.

What are the benefits of being a participant?

Possible benefits of being a participant could include gaining a higher level of awareness and knowledge of your nutritional habits, physical activity, and level of stress. Nutritional software will identify and calculate your nutrient intake, such as important vitamins and minerals, and we will be able to provide you with a written report of this information. However, there is the possibility you will not receive any benefit from participating. We anticipate that the information gained from this study will 1) help women with PCOS, and clinicians to better understand this complex syndrome, and 2) help us identify if there are any correlation of these factors and response of the ovaries to fertility medications.

Will participants receive remuneration or reimbursement?

There will be no cost incurred to you; nor will you receive any payment as a participant. We will appreciate if you return all materials provided, such as journals and pedometers, to the Grace Centre so that other participants can use them.

What happens if I decide to withdraw my consent to participate?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained, without revealing your identity, for analysis.

How will you ensure that my medical information is kept confidential?

All information is strictly confidential. Your confidentiality will be respected. However, research records and health records identifying you may be inspected by the REB in the presence of the Investigator, or his designate, for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number,

SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to ensure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

You do not waive any legal rights by signing this consent form.

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complain Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

CONSENT FORM CHECKLIST

Project: **Characterizing Diet, Physical Activity and Stress in Women with PCOS**

Principal Investigator (P.I.): Dr. A. P. Cheung
***Co-investigators*:** Dylan Cutler
Dr. Alana Shaw
Dr. Sheila Pride

	YES	NO
Do you understand that you have been asked to be a participant in this characterization study analyzing the diet, activity, and stress of women?	☐	☐
I have been told that I will receive a dated and signed copy of this form.	☐	☐
Do you understand the purpose of this characterization study, and the benefits or risks?	☐	☐
Have you had an opportunity to ask questions about this characterization study?	☐	☐
Do you understand that you are free to withdraw from this study at any time, without having to provide a reason and without affecting your medical care?	☐	☐
Has the issue of confidentiality been explained to you, and do you understand who will have access to your medical information?	☐	☐
Would you like to be notified on any future studies that you might be interested in (e.g., the voluntary PCOS registry, or fertility treatments)?	☐	☐

I, (print your name) _____
Last Name *First Name*
agree to become a participant in this characterization study.
Signature of Registrant **_____Date:_____**

***Signature of P.I. or his designate* _____Date:_____**

Appendix B: 3-Day Food and Activity Diary Template

Name:

Instructions

Use this booklet to record your daily food and drink intake for three days. Please record two days during the week (Mon-Fri) and one day on the weekend (Sat or Sun).

Food & Drink Items Consumed: Please be as specific as possible. This means including information such as the brand/producer, type, flavor, or restaurant eaten at. For food items such as a 'McDonald's hamburger' include everything in it (ex. 1 tomato slice, 1 slice of cheddar cheese, etc.) For mixed dishes such as salads, include each individual ingredient and its quantity (ex. 1 cup of organic spinach, 1 tablespoon of sliced blanched almonds, etc.) List one food item on each row.

Amount: Use measurements whenever possible such as cups, tablespoons, teaspoons, grams, milliliters, etc. If these measurements are not feasible to obtain (such as when eating at a restaurant) then use comparisons such as a golf ball size, or tennis ball size. Please refer to charts in the handout to ensure accurate recording.

If you happen to have a smartphone, please take a picture of your meals which may make it easier to identify specific food or drink items.

If you have any questions please contact Dylan Cutler at

research@fertilitywithgrace.com

Day 1 **Date:**

Time	Food & Drink Items Consumed	Amount
8:00 am	ex. Quaker Steel Cut Oats (hot cereal)	ex. 1 cup oats
	ex. 2% milk	ex. ½ cup

Day 2 **Date:**

Time	Food & Drink Items Consumed	Amount

Day 3 **Date:**

Time	Food & Drink Items Consumed	Amount

Appendix C: Depression Anxiety and Stress Scales (English & Chinese)

DASS *Name:* *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found myself getting upset by quite trivial things	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I just couldn't seem to get going	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I had a feeling of shakiness (eg, legs going to give way)	0	1	2	3
8	I found it difficult to relax	0	1	2	3
9	I found myself in situations that made me so anxious I was most relieved when they ended	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting upset rather easily	0	1	2	3
12	I felt that I was using a lot of nervous energy	0	1	2	3
13	I felt sad and depressed	0	1	2	3
14	I found myself getting impatient when I was delayed in any way (eg, elevators, traffic lights, being kept waiting)	0	1	2	3
15	I had a feeling of faintness	0	1	2	3
16	I felt that I had lost interest in just about everything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I perspired noticeably (eg, hands sweaty) in the absence of high temperatures or physical exertion	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life wasn't worthwhile	0	1	2	3

Reminder of rating scale:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

22	I found it hard to wind down	0	1	2	3
23	I had difficulty in swallowing	0	1	2	3
24	I couldn't seem to get any enjoyment out of the things I did	0	1	2	3
25	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
26	I felt down-hearted and blue	0	1	2	3
27	I found that I was very irritable	0	1	2	3
28	I felt I was close to panic	0	1	2	3
29	I found it hard to calm down after something upset me	0	1	2	3
30	I feared that I would be "thrown" by some trivial but unfamiliar task	0	1	2	3
31	I was unable to become enthusiastic about anything	0	1	2	3
32	I found it difficult to tolerate interruptions to what I was doing	0	1	2	3
33	I was in a state of nervous tension	0	1	2	3
34	I felt I was pretty worthless	0	1	2	3
35	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
36	I felt terrified	0	1	2	3
37	I could see nothing in the future to be hopeful about	0	1	2	3
38	I felt that life was meaningless	0	1	2	3
39	I found myself getting agitated	0	1	2	3
40	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
41	I experienced trembling (eg, in the hands)	0	1	2	3
42	I found it difficult to work up the initiative to do things	0	1	2	3

情緒自評量表

填表說明：

請小心閱讀以下每一句子，並在其右方圈上一數字，表示在「過往一星期」如何適用於你。請不要花太　多時間在某一句子上。

評估量表：

0 = 不適用
1 = 頗適用，或間中適用
2 = 很適用，或經常適用
3 = 最適用，或常常適用

1.	我發覺自己為很細微的事而煩惱	0	1	2	3
2.	我感到口乾	0	1	2	3
3.	我好像不能再有愉快、舒暢的感覺	0	1	2	3
4.	我感到呼吸有困難 （例如呼吸過促，氣喘）	0	1	2	3
5.	我真的好像提不起勁	0	1	2	3
6.	我對事情往往作出過敏反應	0	1	2	3
7.	我感到身體打震（如有腳軟的感覺）	0	1	2	3
8.	我感到很難放鬆自己	0	1	2	3
9.	我發覺自己在某些場合非常緊張，極渴望立刻離開，鬆一口氣	0	1	2	3
10.	我覺得自己沒有甚麼可盼望將來	0	1	2	3
11.	我發覺自己很容易感到不快	0	1	2	3
12.	我覺得自己消耗很多精神	0	1	2	3
13.	我感到憂愁悲哀	0	1	2	3
14.	若受到阻延（例如交通擠塞），我會感到很不耐煩	0	1	2	3
15.	我有暈眩的感覺	0	1	2	3
16.	我感到對所有事情都失去興趣	0	1	2	3
17.	我覺得自己不怎麼配做人	0	1	2	3
18.	我發覺自己很容易被觸怒	0	1	2	3

評估量表：
0 = 不適用
1 = 頗適用，或間中適用
2 = 很適用，或經常適用
3 = 最適用，或常常適用

19.	我無故流汗（例如手腳冒汗)	0	1	2	3
20.	我無緣無故地感到害怕	0	1	2	3
21.	我感到生命沒有價值	0	1	2	3
22.	我覺得很難讓自己安靜下來	0	1	2	3
23.	我感到吞嚥困難	0	1	2	3
24.	我覺得不能從所作的事取得樂趣	0	1	2	3
25.	我平時也感覺到心跳或心律不正常	0	1	2	3
26.	我感到憂鬱沮喪	0	1	2	3
27.	我感到自己很容易煩躁	0	1	2	3
28.	我感到快要恐慌了	0	1	2	3
29.	受了刺激後，我感到很難去平伏自己	0	1	2	3
30.	我害怕被一些瑣碎而不熟識的事情難倒	0	1	2	3
31.	我對任何事也不能熱衷	0	1	2	3
32.	我很難忍受工作時的障礙	0	1	2	3
33.	我神經緊張	0	1	2	3
34.	我覺得自己很無價值	0	1	2	3
35.	我無法容忍那阻礙我繼續工作的事情	0	1	2	3
36.	我感到驚惶	0	1	2	3
37.	我對未來完全失去希望	0	1	2	3
38.	我感到生命毫無意義	0	1	2	3
39.	我感到忐忑不安	0	1	2	3
40.	我憂慮一些令自己恐慌或出醜的場合	0	1	2	3
41.	我感到顫抖（例如手震）	0	1	2	3
42.	我感到很難去開始工作	0	1	2	3

Appendix D: Fertility Quality of Life Questionnaire (English & Chinese)

FertiQoL International

Fertility Quality of Life Questionnaire (2008)

For each question, kindly check (tick the box) for the response that most closely reflects how you think and feel. Relate your answers to your current thoughts and feelings. Some questions may relate to your private life, but they are necessary to adequately measure all aspects of your life.

Please complete the items marked with an asterisk (*) only if you have a partner.

	For each question, check the response that is closest to your current thoughts and feelings	Very Poor	Poor	Neither Good nor Poor	Good	Very Good
A	How would you rate your health?	☐	☐	☐	☐	☐
	For each question, check the response that is closest to your current thoughts and feelings	Very Dissatisfied	Dissatisfied	Neither Satisfied Nor Dissatisfied	Satisfied	Very Satisfied
B	Are you satisfied with your quality of life?	☐	☐	☐	☐	☐
	For each question, check the response that is closest to your current thoughts and feelings	Completely	A Great Deal	Moderately	Not Much	Not At All
Q1	Are your attention and concentration impaired by thoughts of infertility?	☐	☐	☐	☐	☐
Q2	Do you think you cannot move ahead with other life goals and plans because of fertility problems?	☐	☐	☐	☐	☐
Q3	Do you feel drained or worn out because of fertility problems?	☐	☐	☐	☐	☐
Q4	Do you feel able to cope with your fertility problems?	☐	☐	☐	☐	☐
	For each question, check the response that is closest to your current thoughts and feelings	Very Dissatisfied	Dissatisfied	Neither Satisfied Nor Dissatisfied	Satisfied	Very Satisfied
Q5	Are you satisfied with the support you receive from friends with regard to your fertility problems?	☐	☐	☐	☐	☐
*Q6	Are you satisfied with your sexual relationship even though you have fertility problems?	☐	☐	☐	☐	☐
	For each question, check the response that is closest to your current thoughts and feelings	Always	Very Often	Quite Often	Seldom	Never
Q7	Do your fertility problems cause feelings of jealousy and resentment?	☐	☐	☐	☐	☐
Q8	Do you experience grief and/or feelings of loss about not being able to have a child (or more children)?	☐	☐	☐	☐	☐
Q9	Do you fluctuate between hope and despair because of fertility problems?	☐	☐	☐	☐	☐
Q10	Are you socially isolated because of fertility problems?	☐	☐	☐	☐	☐
*Q11	Are you and your partner affectionate with each other even though you have fertility problems?	☐	☐	☐	☐	☐
Q12	Do your fertility problems interfere with your day-to-day work or obligations?	☐	☐	☐	☐	☐
Q13	Do you feel uncomfortable attending social situations like holidays and celebrations because of your fertility problems?	☐	☐	☐	☐	☐
Q14	Do you feel your family can understand what you are going through?	☐	☐	☐	☐	☐

FertiQoL International

Fertility Quality of Life Questionnaire (2008)

For each question, kindly check (tick the box) for the response that most closely reflects how you think and feel. Relate your answers to your current thoughts and feelings. Some questions may relate to your private life, but they are necessary to adequately measure all aspects of your life.

Please complete the items marked with an asterisk (*) only if you have a partner.

	For each question, check the response that is closest to your current thoughts and feelings	An Extreme Amount	Very Much	A Moderate Amount	A Little	Not At All
*Q15	Have fertility problems strengthened your commitment to your partner?	☐	☐	☐	☐	☐
Q16	Do you feel sad and depressed about your fertility problems?	☐	☐	☐	☐	☐
Q17	Do your fertility problems make you inferior to people with children?	☐	☐	☐	☐	☐
Q18	Are you bothered by fatigue because of fertility problems?	☐	☐	☐	☐	☐
*Q19	Have fertility problems had a negative impact on your relationship with your partner?	☐	☐	☐	☐	☐
*Q20	Do you find it difficult to talk to your partner about your feelings related to infertility?	☐	☐	☐	☐	☐
*Q21	Are you content with your relationship even though you have fertility problems?	☐	☐	☐	☐	☐
Q22	Do you feel social pressure on you to have (or have more) children?	☐	☐	☐	☐	☐
Q23	Do your fertility problems make you angry?	☐	☐	☐	☐	☐
Q24	Do you feel pain and physical discomfort because of your fertility problems?	☐	☐	☐	☐	☐

FertiQoL International

Simplified CH.

2008 生育生活质量问卷调查

请针对每个问题选择最能贴切反映您所想所感的回答（在回答处方框中打勾）。将您的回答与您目前的想法和感受联系起来。部分问题可能与您的隐私生活有关，但这是充分衡量您生活各方面的必要问题。

带星号（*）的项目，只有在您有伴侣的情况下才需要填写。

	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	非常差	差	普通	好	非常好
A	您如何评价您的健康状况？	□	□	□	□	□
	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	非常不满意	不满意	既非满意也非不满意	满意	非常满意
B	您对您的生活质量是否满意？	□	□	□	□	□
	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	完全	很大程度	中度	轻度	完全不
Q1	您的注意力和专注力是否受到不孕不育的想法的影响？	□	□	□	□	□
Q2	您是否认为由于生育问题的困扰，您不能在生活上继续前行和实现其它生活目标及计划？	□	□	□	□	□
Q3	您是否因为生育问题的困扰而感到筋疲力尽或疲惫不堪？	□	□	□	□	□
Q4	您是否认为您有能力应对您的生育问题？	□	□	□	□	□
	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	非常不满意	不满意	既非满意也非不满意	满意	非常满意
Q5	您是否对朋友就您的生育问题所提供的支持感到满意？	□	□	□	□	□
*Q6	尽管您有生育问题的困扰，您是否对您的性关系感到满意？	□	□	□	□	□
	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	总是	很经常	比较经常	很少	从未
Q7	您的生育问题是否为您带来妒忌或不满的情绪？	□	□	□	□	□
Q8	您是否就您不能生育孩子或者更多的孩子而存在悲伤及/或失落感？	□	□	□	□	□
Q9	您是否由于生育问题而在希望和失望的情绪中徘徊？	□	□	□	□	□
Q10	您是否因为生育问题而在社交关系中处于孤立状态？	□	□	□	□	□
*Q11	尽管您有生育问题，您和您的伴侣间是否仍充满感情？	□	□	□	□	□
Q12	您的生育是否干扰到您的日常工作或职责？	□	□	□	□	□
Q13	您是否因为您的生育问题而在出席度假或庆祝活动等社交场合感到不适？	□	□	□	□	□
Q14	您是否认为您的家庭能够理解您正经历的一切？	□	□	□	□	□

FertiQoL International

Simplified CH.

	请针对每个问题选择与您目前的想法和感受最为贴切的回答。	极度	非常	适度	轻度	完全不
*Q15	生育问题是否增进了您对您的伴侣的承诺？	□	□	□	□	□
Q16	您是否就您的生育问题而感到悲伤和抑郁？	□	□	□	□	□
Q17	您的生育问题是否让您觉得自己不及有孩子的人士？	□	□	□	□	□
Q18	您是否因为生育问题而受到疲劳的困扰？	□	□	□	□	□
*Q19	生育问题是否对您与您的伴侣的关系带来了负面影响？	□	□	□	□	□
*Q20	您是否认为很难与您的伴侣就您关于不孕不育一事的感受进行交流？	□	□	□	□	□
*Q21	尽管您有生育问题，您是否对您与伴侣的关系感到满足？	□	□	□	□	□
Q22	您是否感到就您生育（或者生育更多）孩子一事有来自社会的压力？	□	□	□	□	□
Q23	您的生育问题是否让您感到生气？	□	□	□	□	□
Q24	您是否因为您的生育问题而感到痛苦和身体不适？	□	□	□	□	□