A MULTIDISCIPLINARY LIFESTYLE INTERVENTION FOR WOMEN WITH POLYCYSTIC OVARY SYNDROME: THE ROLE OF A PULSE-BASED DIET AND AEROBIC EXERCISE ON REPRODUCTIVE, CARDIO-METABOLIC, AND QUALITY OF LIFE OUTCOMES

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of PhD

in the College of Pharmacy and Nutrition

University of Saskatchewan

Saskatoon, Saskatchewan

Canada

By

Maryam Kazemi

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or a part, for scholarly purposes may be granted by the professor who supervised my thesis work:

Dr. Gordon Zello

College of Pharmacy and Nutrition

In his absence, permission may be granted by the Dean of the College of Pharmacy and Nutrition or College of Graduate and Postdoctoral Studies. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition should be given to the researcher and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis. Requests for permission to copy or to make other use of the material in this thesis in whole or part should be addressed to:

Dean	or	Dean
College of Pharmacy and Nutrition		College of Graduate and Postdoctoral Studies
University of Saskatchewan		University of Saskatchewan
104 Clinic Place 105		Administration Place,
Saskatoon, SK S7N 2Z4, Canada		Saskatoon, SK, S7N 5A2, Canada

ABSTRACT

Polycystic ovary syndrome (PCOS) is a complex endocrinopathy associated with adverse cardio-metabolic, reproductive, and quality of life outcomes. Controversy surrounds the optimal diet composition to mediate favourable health-related outcomes for women with PCOS. The main hypothesis of the present work was that a nutritionally balanced, low-glycemic index, pulse-based diet containing lentils, beans, split peas, and chickpeas would increase insulin sensitivity; thereby, improving multiple health-related outcomes of PCOS. The objectives of the present work were 1) to examine the prevalence and characteristics of metabolic syndrome (MetS), glucoregulation, type 2 diabetes, and risk factors for cardiovascular disease in women with PCOS, and 2) to compare the effect of a pulse-based diet to the National Cholesterol Education Program's Therapeutic Lifestyle Changes (TLC) diet on reproductive health measures, cardio-metabolic risk profile, health-related quality of life (HRQoL) indices, dietary intakes, and pulse consumption behaviours in women with PCOS.

To examine the study Objective 1, data were pooled from 2 prospective and cross-sectional studies on 237 women with PCOS and 42 (non-PCOS) controls (18-36y; Chapter 3). The prevalence of metabolic syndrome was 29.5% in the PCOS group, approximately 6-fold higher than age-matched controls ($P<0.001$), with worse glucose control, acanthosis nigricans, body mass index (BMI), systolic blood pressure (SBP), triglyceride (TG), high- (HDL-C) and low- (LDL-C) density lipoprotein cholesterol, total cholesterol to HDL-C (TC/HDL-C), and highly sensitive C-reactive protein levels ($P<0.001$ to $P=0.03$). Women with PCOS and MetS exhibited exacerbated levels of insulin and glucose responses to an oral glucose tolerance test (OGTT), TC, TC/HDL-C ratio, hirsutism, and acanthosis nigricans than BMI-matched counterparts without MetS ($P<0.001$ to $P=0.05$). Our observations support the opinion that MetS exacerbates hyperandrogenism, dyslipidemia, and glucose control in PCOS, possibly by aggravating inherent insulin resistance.

To address the main goal of the study, Objective 2, 95 women with PCOS (18-35y) were randomized to receive either the pulse-based diet or the TLC diet, without purposefully inducing calorie restriction, for 16 weeks. All women participated in an aerobic exercise program and received education and counselling about PCOS and lifestyle modification. Thirty women in the

pulse-based diet and 31 in TLC diet group completed the study. Chapter 4 represents the reproductive outcome results of Objective 2. Bilateral antral follicle count, ovarian volume, total testosterone (TT) levels, average and longest intervals between menses decreased over time in both groups ($P \leq 0.05$). Sex-hormone binding globulin levels increased ($P < 0.01$) in both groups with a tendency for a greater increase in the pulse-based diet group ($P = 0.07$). In Chapter 5, cardio-metabolic outcomes of Objective 2 are addressed. The pulse-based diet group had a greater reduction in total area under the curve (AUC) for insulin response to an OGTT than the TLC diet group ($P = 0.05$). Following the intervention, the pulse-based diet group exhibited lower diastolic BP, TG, LDL-C, and TC/HDL-C, as well as a greater increase in HDL-C when compared to the TLC diet group ($P \leq 0.05$). Body weight, waist circumference, percent body fat, SBP, homeostatic model assessment of insulin resistance, glucose AUC, and TC decreased in both groups ($P \leq 0.03$). Both groups maintained some of the improvements in cardio-metabolic and endocrine measures after 16 and 12 months post-intervention, including TT, HDL-C, and TC/HDL-C levels ($P \geq 0.05$); however, contrary to our hypothesis, groups had a tendency to revert to the baseline measures for certain cardio-metabolic and endocrine measures including fasting insulin and TC levels 6 and 12 months after the completion of the intervention ($P \leq 0.05$).

Following the 16-week intervention, the HRQoL scores of both groups increased in the domains of knowledge, concerns about PCOS, healthcare satisfaction, and lifestyle practices comprised of physical activity and healthy diets ($P < 0.05$; Chapter 6). The dietary component of Objective 2 is addressed in Chapter 7. Both intervention groups voluntarily reduced their average daily energy intake from baseline ($P < 0.001$). Dietary intakes increased for fiber, folate, magnesium, iron, and decreased for cholesterol in the pulse-based diet group compared to the TLC diet group ($P < 0.05$). Women in the pulse-based diet group exhibited higher scores in the domain of knowledge about the nutritional composition of pulses, recommended servings of legumes based on Canada's Food Guide, environmental, and economic benefits of pulse consumption when compared with the TLC diet group ($P < 0.05$). Both groups exhibited increased scores in the domain of attitudes about the palatability, accessibility, preparation, and affordability of pulse foods over the 16 weeks ($P < 0.01$).

Supported by the favourable health outcomes from the present intervention, an evidence-based pulse-based recipe resource guide has been developed for use by allied healthcare professionals and their clients (Appendix C).

In conclusion, both dietary interventions, without calorie restriction, in combination with aerobic exercise, education, and healthcare counselling yielded substantial improvements in multiple PCOS-specific health outcomes. The pulse-based diet was more effective than the TLC diet in decreasing insulin response to OGTT, improving many key risk factors for cardio-metabolic disease, and the overall dietary intakes in women with PCOS. In general, women did not maintain the reproductive and metabolic improvements. Further research is required to promote the consumption of nutrient-rich pulse foods and sustainable adherence to healthy lifestyle behaviours in women with PCOS in the long term.

ACKNOWLEDGEMENTS

I wish to thank Dr. Gordon Zello and Dr. Donna Chizen for their guidance, insight, and confidence that they bestow upon me. Equally, I wish to thank Drs. Philip Chilibeck and Roger Pierson and for their continued education and dedicated support during my research. I am truly honoured to have worked and being mentored by all of you as a member of our interdisciplinary research team.

I would like to express my gratitude to my committee chair Dr. Brian Bandy, and committee members Dr. Corey Tomczak and Dr. Wendy Dahl for their time and support. I wish to thank Drs. Jane Alcorn, Carol Henry, Susan Whiting, and Hassanali Vatanparast for their endless support, encouragement, and great guidance during my PhD journey. Also, I wish to acknowledge my colleagues and lab mates for their help and feedback.

To mother and best friend. Thank you for supporting my goals as I could not have done it without you. The same goes to my father, who not only introduced me to exercise physiology and science of nutrition but inspired me to pursue my interest in academic life; hence, I am a dietitian today.

The present thesis would not have been possible without financial support from the Agriculture and Agri-Food Canada, Saskatchewan Pulse Growers, the Canada Foundation for Innovation, and Saskatchewan Health Research Foundation. I also acknowledge the Colleges of Graduate and Postdoctoral Studies and Pharmacy and Nutrition at the University of Saskatchewan for granting me scholarships and awards to fulfill this accomplishment.

DEDICATION

To my mom and dad

To our interdisciplinary research team

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AE-PCOS	Androgen Excess and Polycystic Ovary Syndrome Society
AFC	Antral Follicle Count
AHA	American Heart Association
ANOVA	Analysis of Variance
ASRM	American Society for Reproductive Medicine
ATP III	Adult Treatment Panel III
AUC	Area Under the [the 2-Hour Variable versus Time] Curve
BMI	Body Mass Index
BP	Blood Pressure
CHC	Combined Hormonal Contraceptives
CI	Confidence Interval
CVD	Cardiovascular Disease
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic Blood Pressure
DM2	Type 2 Diabetes
DM2	Diabetes Mellitus Type 2
DXA	Dual Energy X-ray Absorptiometry
ESHRE	European Society for Human Reproduction and Embryology
EWCFG	Eating Well with Canada's Food Guide
FAO	Food and Agriculture Organization of the United Nations
FPG	Fasting Plasma Glucose
FSH	Follicle Stimulating Hormone
G:I ratio	Glucose to Insulin Ratio
GI	Glycemic Index
GnRH	Gonadotropin-Releasing Hormone
HbA1c	Hemoglobin A1c
HDL-C	High-Density Lipoprotein Cholesterol
HRQoL	Health-Related Quality of Life

HOMA-IR	Homeostatic Model Assessment
HPO axis	Hypothalamic-Pituitary-Ovarian Axis
hsCRP	Highly Sensitive C-reactive Protein
HTN	Hypertension
IDF	International Diabetes Federation
IGT	Impaired Glucose Tolerance
IR	Insulin Resistance
IYP	International Year of Pulses
LDL-C	Low-Density Lipoprotein Cholesterol
LH	Luteinizing Hormone
MetS	Metabolic Syndrome
NCEP	National Cholesterol Education Program
NHLBI	National Heart, Lung, And Blood Institute
NIH	National Institutes of Health
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
OV	Ovarian Volume
PCO	Polycystic Ovary
PCOS	Polycystic Ovary Syndrome
PCOM	Polycystic Ovarian Morphology
PTH	Parathyroid Hormone
RCT	Randomized Controlled Trials
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of the Mean
SCFA	Short Chain Fatty Acids
SFA	Saturated Fatty Acids
SHBG	Sex-Hormone Binding Globulin
SMD	Standardized Mean Differences
SPSS	Statistical Package for the Social Science

TC	Total Cholesterol
TC/HDL-C	Total Cholesterol/High-Density Lipoprotein Cholesterol
TG	Triglyceride
TLC	Therapeutic Lifestyle Changes
TT	Total Testosterone
Tx	Therapy
VLDL	Very Low-Density Lipoprotein Cholesterol
WC	Waist Circumference
WHO	World Health Organization

CHAPTER 1
INTRODUCTION

1.1. Background Information and Rationale

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy and a leading cause of anovulatory infertility, affecting up to one in five women of reproductive age worldwide in all ethnic groups (Carmina & Lobo, 1999; Knochenhauer, 1998; March et al., 2010; Teede et al., 2010). The PCOS is a complex multifactorial disorder, influenced by genetic and environmental factors contributing to its development.

The PCOS is classically characterized by hyperandrogenism, menstrual irregularity and polycystic appearing ovaries. The condition is associated with several reproductive features (i.e., hirsutism, acne, alopecia, ovulatory and menstrual dysfunction, infertility, pregnancy complications, miscarriage, endometrial hyperplasia, and endometrial adenoma) and substantial psychological distress (i.e., anxiety, depression, poor self-esteem, reduced quality of life, negative body image, psychosexual dysfunction, and eating disorders) (Azziz et al., 2006; "The revised Rotterdam ESHRE/ASRM criteria ", 2004).

Aside from the reproductive and psychologic burden, PCOS is associated with profound metabolic complications (Carmina & Lobo, 1999; A Dunaif, 1997). A majority (50-70%) of women with PCOS demonstrate significant peripheral insulin resistance (IR) and compensatory hyperinsulinemia, with higher rates compared to women without PCOS, matched by age, ethnicity and body mass index (BMI) (Diamanti-Kandarakis et al., 2008; Diamanti-Kandarakis & Papavassiliou, 2006). Women with PCOS exhibit other metabolic disturbances, including more dyslipidemia and impaired glucose metabolism compared with women without PCOS. A chronic inflammatory state and hypertension have been observed in PCOS, which can be attributed to high rates of obesity (Palomba et al., 2015). Thirty to 50% of women with PCOS are obese and overweight (A Dunaif, 1997; Gambineri et al., 2002; Norman et al., 2002). Obesity negatively affects metabolic abnormalities and complicates the management of the PCOS (Lau, 2007). Therapeutic modalities for PCOS have been shown to be less effective in women who are obese (Palomba et al., 2008). Impaired glucose metabolism, dyslipidemia, hypertension and abdominal obesity are key components of metabolic syndrome (MetS), a complex of five interrelated risk factors for cardiovascular disease (CVD) and type 2 diabetes

(DM2). The presence of MetS exacerbates the clinical and endocrine manifestations of PCOS (Apridonidze et al., 2005; Carmina et al., 2006; Glueck et al., 2003). The MetS is a major contributor to CVD and DM2 (Brien & Katzmarzyk, 2006; Cameron et al., 2008). With MetS, the risk of CVD doubles (Grundy, 2008). Women with PCOS have a 5-fold increased risk for DM2 (Alberti et al., 2006). Compared to other clinical presentations associated with PCOS, metabolic aberrations appear to be relatively more important contributors to increased morbidity and mortality rates in long term (Carmina & Lobo, 1999; Diamanti- Kandarakis & Dunaif, 2012; Wild, 2012; Wild et al., 2010).

Management of PCOS should focus on support and education, addressing psychological factors and strongly emphasizing healthy lifestyle with targeted medical therapy, as required (Teede et al., 2010). Lifestyle modification (comprising dietary, exercise and behavioural therapy), represents the first-line approach and the most effective strategy to manage PCOS. Lifestyle modification can positively impact the spectrum of morbidity associated with this disorder and reduce the risk of developing CVD and DM2 (Bruner et al., 2006; Marsh et al., 2010; Sweatt et al., 2015).

Adherence to lifestyle change is challenging and difficult for women with PCOS to maintain. While there is a general agreement on prioritizing body weight control, maintaining regular physical activity and acquiring cognitive behavioural skills for the management of syndrome, there is much debate about the optimal balance of dietary composition and exercise dose, type, intensity and frequency that would be uniformly beneficial for women with PCOS. There is limited evidence for the long-term effects of combined lifestyle interventions on PCOS health outcomes. Long-term therapeutic strategies are crucial to prevent or delay the occurrence of comorbidities and improve the overall prognosis associated with PCOS (Marsh & Brand-Miller, 2005).

Dietary pulses have received insufficient attention in the dietary management of PCOS. A pulse-rich diet has the potential to improve reproductive and metabolic aberrations associated with PCOS. Pulses, that is, split-peas, beans, lentils, and chickpeas, are high in fiber, contain complex carbohydrates with a low glycemic index (GI), are low in fat, contain high-quality protein, have low sodium content, and are a significant source of vitamins and minerals, such as iron, zinc, folate, calcium, and potassium (Mudryj et al., 2014). Chronic consumption of pulses in other populations has been associated with decreased postprandial

blood glucose and insulin concentrations, hypercholesterolemia, and obesity (Ha et al., 2014; McCrory et al., 2010; Sievenpiper et al., 2009).

1.2. Significance of the Study

Previous research has been limited in terms of reporting the degree of metabolic derangements in women with PCOS and the effects of MetS on PCOS prognosis. Previous studies suffered from several methodological limitations, including small sample size, marked heterogeneity in techniques employed, and the fact that older or less accurate criteria were used to diagnose PCOS, PCOM, and MetS. Unlike previous investigations, in the present study, we used a rigorous and comprehensive approach to diagnose PCOS and MetS to determine the prevalence of MetS in reproductive-age women with PCOS and the effect of MetS on many clinical and biochemical outcomes associated with PCOS.

Potential benefits of pulse consumption in women with PCOS has not been evaluated in available literature. The proposed study will examine a pulse-based diet as a nutrition intervention for a population of women exhibiting PCOS. The rationale for this intervention is supported by the association of improved glycemic control following consumption of pulse-rich diets by non-PCOS populations who similarly exhibit adverse cardio-metabolic DM2 risk profiles (Sievenpiper et al., 2009). It is proposed that a pulse-based diet can improve the metabolic and reproductive features of the syndrome, and the lifestyle intervention can enhance the overall quality of life and maintain lifestyle changes in women with PCOS. As complementary components of a comprehensive multidimensional lifestyle intervention program, the proposed intervention will profit from the additional benefits of exercise, education and healthcare counselling about PCOS and lifestyle modification. To isolate the effect of diet and healthcare counselling, exercise was prescribed as a portion of ethical Good Clinical Practice Guidelines. Extended follow-up times will be used in the study, in order to better explore the long-term effects of the nutritional intervention in women with PCOS. This research can provide insight on how a pulse-based diet may affect short-term and long-term outcomes for women with PCOS.

1.3. Main Research Hypotheses

A pulse-based diet is more effective than the National Cholesterol Education

Program (NCEP) Therapeutic Lifestyle Changes (TLC) diet to reduce cardio-metabolic risk profile, improve reproductive outcomes, the overall quality of life, dietary intakes, and pulse consumption behaviours in women with PCOS.

1.3.1. Primary Hypothesis

A 16-week pulse-based diet is more effective compared to the TLC diet to improve reproductive outcomes and reduce cardio-metabolic risk in women with PCOS, when the diet is combined with aerobic exercise and healthcare counselling.

1.3.2. Secondary Hypotheses

i. The prevalence of MetS is high in reproductive-age women with PCOS and MetS would exacerbate clinical and biochemical outcomes associated with PCOS.
ii. A 16-week pulse-based diet is more effective compared to the TLC diet to improve the quality of life of women with PCOS when combined with aerobic exercise and healthcare counselling.
iii. A 16-week pulse-based diet is more effective compared to the TLC diet to improve dietary intakes and pulse consumption behaviours in women with PCOS when combined with aerobic exercise and healthcare counselling.
iv. A 16-week pulse-based diet is more effective compared to the TLC diet after 6 and 12 months post-intervention to maintain improvements in cardio-metabolic and reproductive outcome measures.

1.4. Research Objectives

i. To characterize the prevalence rate and individual components of MetS, impaired glucose control, DM2, and risk factors for CVD in women with PCOS.
ii. To determine whether the pulse-based diet is more effective compared to the TLC diet on the multiple reproductive health measures of PCOS, cardio-metabolic risk profile, dietary intakes, and quality of life after a 16-weeks intervention when the diets are combined with aerobic exercise and healthcare counselling.

The outcome variables are as follows:

- Measures of reproductive health related to PCOS, which include a) PCOM (i.e., antral follicle count [AFC], and ovarian volume [OV]); b) intervals between menstrual bleeds; and c) endocrine measures (i.e., ratio of luteinizing hormone [LH] to follicle stimulating hormone [FSH] and free androgen index [ratio of total testosterone {TT} to sex-hormone binding globulin {SHBG}]).
- Anthropometric measures, which include weight, BMI and waist circumference (WC).
- Measures of body composition, which include total body mass, fat mass, trunk fat mass, and muscle mass.
- Physiological measures, which include blood pressure (BP) and pulse rate.
- Measures of glucose control, which include fasting plasma glucose (FPG), 2-hour standard oral glucose tolerance test (OGTT), glycated hemoglobin (HbA1c), and insulin.
- Measures of blood lipids, which include total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, and TG.
- A measure of inflammation, which includes highly-sensitive C-reactive protein (hsCRP).
- Measures related to the quality of life, using a researcher-devised PCOS-specific health-related quality of life (HRQoL) survey (Appendix A).
- Dietary intake factors, using serial 24-hour dietary recalls and pulse consumption behavious using a researcher-devised Pulse Consumption Survey (Appendix B).

iii. To monitor women with PCOS 6 and 12 months after the completion of the intervention to determine long-term effects of the intervention on cardio-metabolic and endocrine outcome measures, as described in objective ii.

iv. To develop an evidence-based pulse recipe resource guide for use by clinicians, patients, and researchers.

1.5. Thesis Outline

In the next chapter, recent advances in understanding the PCOS paradigm and the syndrome-associated comorbidities were examined. Dietary and exercise interventions, which have been shown to possess important clinical implications on PCOS outcomes, were the focus of the review.

Findings of the research project have been presented in Chapters 3 to 7. Research Objective i has been addressed in Chapter 3. Various components of research Objective ii have been addressed in Chapter 4: the reproductive health outcomes; Chapter 5: cardio-metabolic risk, Chapter 6: the HRQoL indices, and Chapter 7: changes in dietary intakes and pulse consumption behaviours in women with PCOS following the 16-week lifestyle change intervention. Our observations about research Objective iii have been presented in relevant sections of Chapters 4 and 5. The last chapter, Chapter 8, discusses the findings of the research project while linking the findings to each other and to findings from previous studies. In Chapter 8, research gaps, limitations and future directions have been addressed. Overall conclusions have been established at the end of Chapter 8.

To address objective iv, a pulsed-based food recipe booklet has been developed. The booklet has been developed to promote pulse consumption and provide researchers and the general population with insight about preparation and consumption of pulse-based meals. The pulse-based diet recipe booklet is presented in Appendix C.

CHAPTER 2
LITERATURE REVIEW

2.1. Overview of Polycystic Ovary Syndrome

2.1.1. Definition and Epidemiology

"The PCOS is a syndrome of ovarian dysfunction along with the cardinal features of hyperandrogenism and polycystic ovary morphology in the absence of other explanatory endocrinopathies" (Fauser et al., 2012). Considered the most common endocrinopathy and the primary cause of anovulatory infertility amongst reproductive-age women worldwide, the prevalence of PCOS is up to 18% (Carmina & Lobo, 1999; Knochenhauer, 1998; March et al., 2010). The PCOS affects approximately 105 million women worldwide (Azziz et al., 2005), including an estimated 1.4 million women in Canada (Lujan et al., 2008). Determination of the exact prevalence of PCOS is challenging, owing to heterogeneity in employing the current diagnostic criteria, regional, lifestyle, and ethnic variations of women, as well as variability in clinical symptoms, laboratory values, and imaging studies across populations (Wild et al., 2005). A drastic increase in the prevalence of PCOS in recent years has accrued because of revisions in the PCOS diagnostic criteria, improved sonographic imaging and increased interest by researchers and clinicians. The global obesity epidemic, concomitant with a sedentary lifestyle has also contributed to an increased likelihood of recognizing PCOS. The PCOS represents an exasperating experience for affected women and has been a complicated scientific challenge for clinicians and researchers (Teede et al., 2010).

2.1.2. Etiology

The etiology of PCOS is multifactorial and still unclear. Compelling evidence exists to attribute genetic inheritance as a significant contributor to the origins of the syndrome (S. Franks et al., 2008; Legro et al., 1998). PCOS appears to be inherited as a common polygenic trait, with multiple susceptibility loci (Brower et al., 2015; Zhao et al., 2015); however, the available literature is not conclusive. More genome-wide association studies are required to identify the list of susceptibility genes contributing to PCOS risk, and potentially explain developmental origins of the syndrome.

While PCOS was initially considered a reproductive condition arising from a genetic predisposition, emerging evidence has highlighted the role of environmental determinants, including lifestyle factors (i.e., diet and physical activity), environmental toxins and socioeconomic status as contributing components to the development of the syndrome (E Diamanti-Kandarakis & A Dunaif, 2012; Dunaif & Fauser, 2013; Legro et al., 2013; Lim et al., 2013; Moran et al., 2011; Palomba et al., 2015). Obesity and poor dietary practices adversely affect symptoms and signs that lead to a diagnosis of PCOS, in part, by aggravating the syndrome associated metabolic abnormalities, including chronic low-grade inflammation, glucose intolerance, and IR. Obesity induces abnormal ovarian steroidogenesis, steatohepatitis, and neuroendocrine abnormalities, all of which can negatively influence women with PCOS (Dewailly et al., 2006; Kuchenbecker et al., 2011; Motta, 2012; Welt, 2006; Yildiz et al., 2008). Obesity increases the risk of developing DM2, CVD, dyslipidemia, and infertility in women with PCOS (Benson, 2008; Carmina, 2006; Diamanti-Kandarakis et al., 2006; Kiddy et al., 1990).

Environmental toxins are defined as "chemical pollutants in the environment, which negatively affect biological organisms" (Diamanti-Kandarakis et al., 2009). Examples of environmental pollutants include tobacco smoke, lead, pesticides, bisphenol A, and mercury. These pollutants have been hypothesized to act as endocrine-disrupting agents, with the potential to alter sex-steroids in women and sperm counts in men. Endocrine disruptors can alter sex hormone steroidogenesis to deteriorate human reproductive health in general, and enhance the development of PCOS symptoms in particular (*Environmental impacts on reproductive health and fertility 2010*, 2010; Kandaraki et al., 2010). There is limited research about how toxins may contribute to the development of PCOS, or possibly trigger or exacerbate symptoms of the syndrome.

There is limited research linking the relationship between socioeconomic status and PCOS. Low socioeconomic status and adverse health behaviours can influence an increase in the rate of obesity. An association between socioeconomic status and PCOS appears to be more pronounced among obese women; the relationship between socioeconomic status and obesity should be considered when examining the etiological origins and the threshold for the recognition of PCOS (Lim et al., 2013; Wang & Beydoun, 2007). Low socioeconomic status can indirectly and adversely influence hormonal reactions and unmask PCOS in predisposed women. Obesity can make it difficult to manage and treat PCOS, when there is a lack of healthcare access

and utilization for the onset of and treatment of obesity (Escobar-Morreale et al., 2005; Norman et al., 2007; Wang & Beydoun, 2007). Limited studies indicate some association between the exposure to low socioeconomic status, and the early development of PCOS in adolescents, as well as PCOS-related metabolic dysfunction and IR (E Diamanti-Kandarakis & A Dunaif, 2012; Lawlor et al., 2002).

Explaining the environmental factors related to PCOS allows us to understand the presentation, etiology and pathophysiology of the syndrome, and to improve the health status of the affected women.

2.1.3 Pathophysiology

A number of overlapping and interrelated processes underlie the pathophysiology of PCOS. IR and consequent hyperinsulinemia, androgen excess, and abnormal dynamics of FSH and LH secretion are key interdependent contributors to the pathophysiology of PCOS (Goodarzi et al., 2011). Due to the variability in degrees of insulin resistance, androgen excess and other complex, interrelated and interdependent contributors to the pathophysiology of PCOS, the clinical presentation of PCOS can be highly variable. These factors make it difficult to recognize and manage women with PCOS.

Elevated levels of androgens (mainly testosterone and androstenedione) and insulin in serum are the most consistently elevated biochemical abnormalities in PCOS (Franks, 1991). Overproduction of androgens occurs because of an intrinsic abnormality of ovarian theca cell function which is highly sensitive to insulin and leads to the pathology of PCOS (Figure 1). The hypothalamic-pituitary-ovarian (HPO) axis responds to changes in androgens and estrogens and contribute to the pathogenesis of ovulatory dysfunction in PCOS. Gonadotropin secretion changes, to include an elevated level of LH when compared to FSH and an elevated LH to FSH ratio during the follicular phase, which is longstanding when women do not grow follicles or ovulate. Hyperandrogenemia and hyperinsulinemia are important underpinnings to the ovulatory dysfunction in PCOS, acting to perpetuate a dysregulation of the HPO axis and stimulate further hyperandrogenism. Local production of androgens, in turn, contributes to ovarian follicular arrest, anovulation, and the morphologic hallmark of the syndrome–polycystic ovaries on ultrasound. Hyperandrogenism, in combination with an increase in the frequency and amplitude of gonadotropin-releasing hormone (GnRH) pulses from the hypothalamus, leads to hypersecretion of LH from pituitary gland. An increase in both LH pulse frequency and

amplitude, in turn, contributes to the mechanisms of anovulation (Brown, 1978; Pardridge, 1981). Additionally, PCOS is associated with lower follicular phase FSH levels when compared to the follicular phase levels of FSH in women with regular menstrual cycles (Chavez-Ross et al., 1997; Stephen Franks et al., 2008). The suboptimal level of FSH in women with PCOS contributes to impaired follicular development and anovulation (Baird et al., 1977; Rebar et al., 1976). Together, impaired follicle growth and anovulation result in infrequent and unpredictable menstrual bleeding, a heightened risk of infertility and an increase in the incidence of abnormal bleeding.

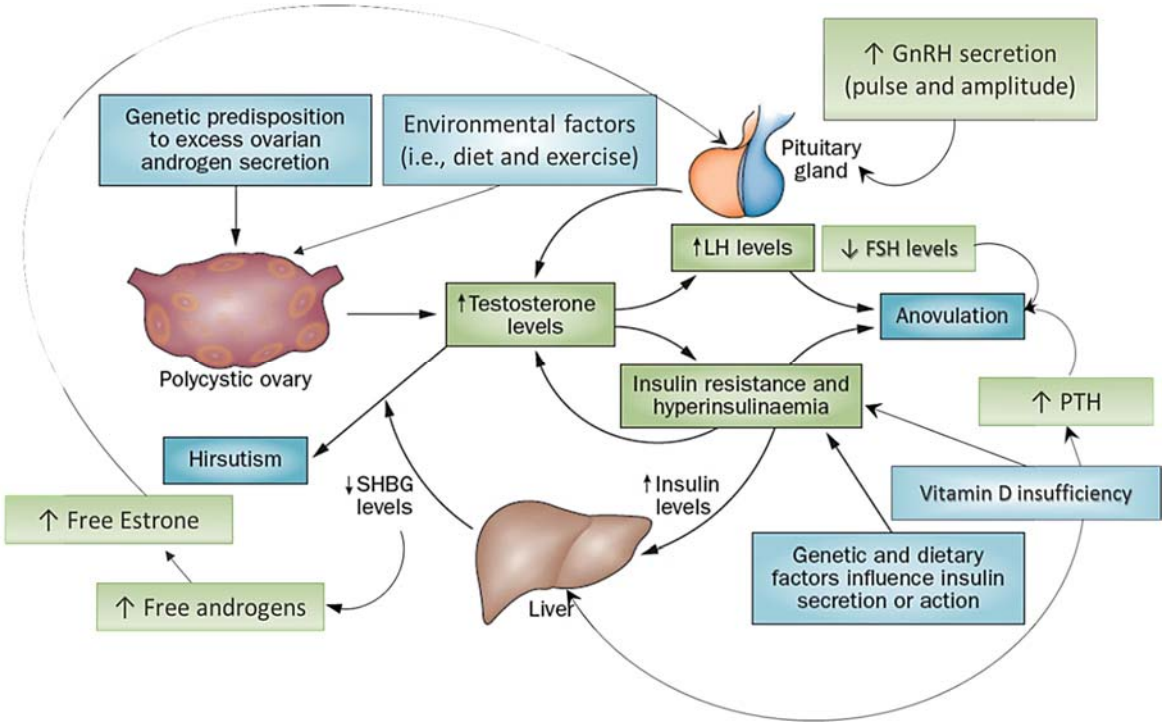
Insulin resistance and subsequent hyperinsulinemia are found in 50–70% of women with PCOS (Dunaif et al., 1989). Hyperinsulinemia stimulated ovarian-induced hyperandrogenemia via its own theca cells receptors interact with gonadotrophins to modulate steroidogenesis. In women with PCOS, the metabolic pathway of insulin activity is defective both at the cell receptors and at the post-receptor level in peripheral target tissues (myocytes and adipocytes), as well as in the ovarian tissue (Diamanti-Kandarakis et al., 2008). On the other hand, insulin sensitivity is negatively affected by central adiposity, and in part, by hyperandrogenism, both of which may exacerbate the mechanisms of anovulation (E Diamanti-Kandarakis & A Dunaif, 2012). Once established, both LH secretion and hyperinsulinemia exacerbate excess androgen production by ovarian theca cells (Franks, 2008; Franks et al., 2006). Hyperinsulinemia in women with PCOS suppresses the hepatic secretion of SHBG. Recognized as a modulator of circulating levels of free androgens, the hepatic SHBG is a glycoprotein recognized as the dominant carrier protein that binds circulating androgens, including testosterone, androstenedione, and dihydrotestosterone, with high affinity and minimizes the percentage of free androgen available to act at the target tissues. Sex-hormone binding globulin transports the sex-steroids within the bloodstream and to extravascular target tissues. Hepatic synthesis of SHBG is regulated by several metabolic and hormonal factors including insulin. The decline in SHBG levels, as seen in states of IR and hyperinsulinemia, is associated with an increase in circulating unbound sex-steroids. Down-regulation of SHBG levels, as seen in states of IR, is associated with an increase in the bioavailability of free androgen levels; hence, androgen-dependent hirsutism is mediated (E Diamanti-Kandarakis & A Dunaif, 2012; Plymate et al., 1988).

Vitamin D deficiency has been described in women with PCOS (He et al., 2015). A growing body of evidence indicates a relevance of vitamin D deficiency in the pathophysiology of PCOS, through its association with obesity, IR, hyperandrogenemia, dyslipidemia, ovulation dysfunction, and inflammation (Balen et al., 2005; A Dunaif, 1997; S Hahn et al., 2006; H. W. R. Li et al., 2011; Yasuda et al., 1975; Yildiz et al., 2008). Insulin secretion is a calcium-dependent process (Gedik & Zileli, 1977; Yasuda et al., 1975); IR and compensatory hyperinsulinemia are well described in the setting of PCOS. Vitamin D deficiency compromises the physiological mechanisms associated with insulin synthesis and release, downregulates insulin receptor expression, and upregulates the synthesis of proinflammatory cytokines that are recognized to play a role in the pathogenesis of IR (Chiu et al., 2004; Wang et al., 2008). Regulation of gene expression that is critical for glucose and lipid metabolism and is recognized to be downstream targets of vitamin D signalling, can be impaired by vitamin D deficiency (Chiu et al., 2004; Holick, 2007; Pittas et al., 2007). Vitamin D plays a vital role in oocyte activation, maturation, and eventually ovulation, through regulating calcium homeostasis (Kaufman & Homa, 1993; Steinhardt et al., 1974). Vitamin D deficiency has been hypothesized to increase free levels of androgens in the PCOS setting, with mechanisms associated with decreased SHBG, and increased parathyroid hormone (PTH) levels (Balen et al., 2005).

Despite great strides, our knowledge of the pathophysiology of PCOS is still evolving. The existing literature is limited predominantly to observational designs and small study samples. Sufficiently powered, well-designed studies can help to clarify the physiological mechanisms underlying the development of PCOS; therapeutic strategies can be developed once "cause and effect" mechanisms are clarified and linked with the many associated signs and symptoms of this syndrome

Figure 1. Proposed pathogenesis of PCOS (retrieved and modified (Jayasena & Franks, 2014)).

Starting from top left going in a clockwise direction: Genetic predisposition and environmental factors (i.e., diet and physical activity) stimulate ovarian theca cells steroidogenesis. An increase in GnRH secretion leads to abnormalities in the dynamics of FSH and LH, which can be aggravated by hyperandrogenism, mainly increased levels of total testosterone. Once established, hypersecretion of LH, sub-optimal secretion of FSH, and hyperinsulinemia further exacerbate ovarian theca cell androgen production. Central abnormalities in the pathogenesis of PCOS are IR, hyperinsulinemia and ovarian-induced hyperandrogenism, which may contribute to the mechanisms of anovulation. Vitamin D deficiency contributes to the pathogenesis of PCOS through mechanisms associated with IR, and SHBG and PTH secretion. IR and hyperinsulinemia suppress the hepatic synthesis of SHBG, resulting in increased mfree circulating bioactive testosterone and increased hyperandrogenemia, which can aggravate abnormal secretion of sex-hormones in PCOS. (Abbott et al., 2002; E Diamanti-Kandarakis & A Dunaif, 2012; Franks et al., 2006; S. Franks et al., 2008; Nelson et al., 1999; Pardridge, 1981). Abbreviations: PCOS, Polycystic ovary syndrome; LH, luteinizing hormone; FSH, follicle stimulating hormone; GnRH, gonadotropin-resealing hormone; PTH, parathyroid hormone; SHBG, sex hormone-binding globulin.



2.1.4. Diagnosis

PCOS was first established by Stein and Leventhal in 1935 to describe women who presented with obesity, hirsutism, and chronic anovulation, with enlarged cystic ovaries post-mortem (Stein & Leventhal, 1935). With the emergence of new evidence, the original characterization of the syndrome has been modified to include physical symptoms, biochemical profiles and ovarian ultrasonographic morphology. Subsequently, the PCOS diagnostic criteria have undergone multiple iterations in an attempt to refine and standardize the identification of this complex entity. Three iterations of the diagnostic criteria for PCOS have been established over time: the National Institutes of Health (NIH) criteria, the Rotterdam criteria, and the Androgen Excess and Polycystic Ovary Syndrome Society (AE-PCOS) criteria.

The NIH guidelines were outlined in 1990 in the first international expert workshop on PCOS and were based upon a consensus of the expert opinion of the attendees, without substantial research-based evidence. The criteria set forth included chronic anovulation with clinical and biochemical signs of hyperandrogenism (i.e., hirsutism and hyperandrogenemia). PCOS was classified as a disorder of exclusion; the diagnosis of PCOS was based on the exclusion of other endocrinopathies that mimicked the characteristics of PCOS, including adrenal dysfunction, Cushing's syndrome, congenital adrenal hyperplasia, androgen-secreting neoplasms, pituitary tumours, hyperprolactinemia, and thyroid dysfunction. The NIH criteria encompassed the strictest set of diagnostics to standardize research populations; however, because the clinical manifestations of PCOS appeared broader than that defined by the NIH criteria, newer criteria were adopted ("The revised Rotterdam ESHRE/ASRM criteria ", 2004; Zawadzki & Dunaif, 1992).

At a joint workshop meeting in the Netherlands between the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM), the NIH criteria were amended in favour of the Rotterdam consensus in 2003. The PCOS diagnostic consensus revised the criteria to include oligo-or anovulation, clinical and/or biochemical signs of hyperandrogenism, and polycystic appearing ovaries on imaging. The Rotterdam diagnosis of PCOS was defined by the presence of at least two of the three criteria; other conditions mimicking PCOS must be excluded similar to the NIH criteria ("The ACOG practice bulletin on PCOS," 2009). The Rotterdam criteria have been the most inclusive criteria. By widening the inclusion criteria for PCOS, women with potential disease risk factors

associated with PCOS would not be overlooked ("The revised Rotterdam ESHRE/ASRM criteria ", 2004).

In 2006, the AE-PCOS Society proposed a further modification of PCOS diagnostic criteria to emphasize that PCOS was an androgenic condition; The criteria included hyperandrogenism and ovarian dysfunction (where dysfunction was defined as oligo-anovulation and/or polycystic appearing ovaries). The AE-PCOS society also acknowledged that exclusion of other androgen excess or related disorders had been an essential element of the PCOS diagnosis (Azziz et al., 2006). Although the PCOS diagnostic criteria have not "officially" changed since 2006, in 2014, the AE-PCOS Society modified the diagnostic criteria for the polycystic ovary (PCO). The newest PCO criteria have refined the diagnosis of PCOS, by excluding women with a normal ovarian morphology, and has narrowed the population of women diagnosed with PCOS. The PCO criteria were defined as ≥ 25 antral follicles per ovary (Dewailly et al., 2014).

There continues to be controversy about which elements constitute the optimal criteria for the diagnosis of PCOS, partly because the various sets of criteria place different degrees of emphasis on specific phenotypes. Clinicians need discreet categorical criteria for ease of diagnosis. Because many features of PCOS overlap with multiple other endocrine disorders and clinical conditions, the diagnosis of PCOS is a "diagnosis of exclusion"; systemic evaluation is aimed at excluding conditions that could masquerade as PCOS (Dunaif & Fauser, 2013; Michelmore et al., 1999).

2.1.5. Clinical Manifestations and Associated Comorbidities

Clinically, PCOS is characterized by its heterogeneous presentation, including reproductive (e.g., hyperandrogenism, polycystic ovaries, and ovarian dysfunction), metabolic (e.g., impaired glucose tolerance [IGT], IR, dyslipidemia), and psychological (e.g., depression and anxiety) features (Barry et al., 2011; Deeks et al., 2011; Ehrmann, 2005; Ehrmann, 2006; Ehrmann et al., 1999; Kjerulff et al., 2011; Moran et al., 2010; Toulis et al., 2011; Weiner et al., 2004). Further, PCOS is associated with several medical comorbidities (i.e., type 2 diabetes and cardiovascular disease), that can negatively affect the quality of life and increase the risk of mortality in women with PCOS. ("The ACOG practice bulletin on PCOS," 2009; Azziz, 2004; Bethea & Nestler, 2008; Ehrmann, 2005; Elsenbruch et al., 2003; Hardiman et al., 2003a; Legro et al., 1999; Moran, 2010; Vassilatou et al., 2010; Weiner et al., 2004). It is crucial to identify

comorbidities that develop in women with PCOS, to improve the management and prognosis of women with the syndrome.

2.1.5.1. Reproductive Features

Reproductive presentations of PCOS are heterogeneous. Reproductive features of the syndrome include clinical and biochemical hyperandrogenism, polycystic appearing ovaries, and ovarian dysfunction. Associated clinical manifestations include abnormal uterine bleeding, amenorrhea, hirsutism, alopecia, and acne (Azziz et al., 2006; "The revised Rotterdam ESHRE/ASRM criteria ", 2004).

2.1.5.2. Hyperandrogenism

Hyperandrogenemia, defined as elevated blood levels of androgens (i.e., testosterone, androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone-sulfate), is observed in 60-80% of women with PCOS (Teede et al., 2010). Clinical presentations of hyperandrogenism are phenotypically identified as hirsutism, acne, and alopecia (Azziz et al., 2004).

Hirsutism is defined as “excessive terminal hair growth that follows a male pattern hair distribution” (Musrap & Diamandis, 2016). Development of hirsutism can be attributed to higher hair follicle sensitivity to androgenemia. Increased sensitivity to androgens leads to the overproduction of 5a-dihydrotestosterone in hair follicles, conversion of vellus hair to terminal hair, prolonged anagen phase of hair follicles sensitive to androgens and results in longer thicker terminal hairs (Archer & Chang, 2004). The most common, and recommended method used to score hirsutism is the Ferriman-Gallwey score. Twelve body areas (upper lip, chin, sideburns, neck, chest, upper back, lower back, upper and lower abdomen, upper arm, thigh, and perineum) are assigned a score from 0 to 4 depending on the position and density of hair growth. The clinical evaluation of hirsutism is subjective and prone to inter-observer variability. The requisite diagnostic threshold score for hirsutism has varied from 6 to 8 for Caucasian women (Ferriman & Gallwey, 1961). Is it important to note that the severity of hirsutism varies across ethnicities. There are racial variations in hair growth patterns with a lower prevalence of male pattern hair growth in women of East Asian descent, and a higher prevalence is seen in women of East Indian descent (Azziz et al., 2004). Assessment of hirsutism may be underestimated in women who have received therapies that suppress androgenemia when women receive contraceptive hormone therapies. Hirsutism may also be underestimated following electrolysis, depilatory therapies and laser hair removal.

Acne vulgaris is another cutaneous manifestation of hyperandrogenism that may be associated with PCOS (Panidis et al., 1995). Androgens form acne by increasing sebum production and follicular epithelial cell desquamation of sebaceous glands (Archer & Chang, 2004). Acne vulgaris affects approximately 12-14% of Caucasian women with PCOS. It is difficult to ascertain a causal relationship for acne in PCOS because acne vulgaris is also widely prevalent in the healthy non-PCOS population. There is also a paucity of evidence for women with PCOS who present with acne vulgaris but do not exhibit hirsutism (Azziz et al., 2009).

Alopecia is the loss of hair from the head or the body. Male pattern hair loss (androgenic alopecia) is another feature of hyperandrogenism and is a typical pattern of hair loss in women with PCOS. Androgenic alopecia involves thinning of hair at the crown with preservation of the anterior hairline (17). It is unclear whether the development of androgenic alopecia is correlated with hyperandrogenism or is independent of it. Androgenic alopecia typically has a genetic predisposition. (Azziz et al., 2009). As the relationship between PCOS and alopecia is unclear, studies are required to confirm the hypothesis that alopecia is a single dermatological sign of PCOS (O'Driscoll et al., 1994). While 75-80% of women with hirsutism have PCOS and 20-40% of women who exclusively present with acne vulgaris have PCOS, only 10% of women who present with alopecia will have a positive diagnosis of PCOS (Azziz et al., 2009).

Hyperandrogenism remains the main feature of PCOS because up to 80% of women diagnosed with the syndrome exhibit clinical manifestations of hyperandrogenism (Diamanti-Kandarakis et al., 2007). Ultrasonographic evidence of polycystic appearing ovaries is considered the third diagnostic criterion for PCOS (Lujan et al., 2013).

2.1.5.3. Polycystic Ovaries

The PCO is another reproductive marker for PCOS. The PCO can be determined by ultrasonographical examination of the ovaries. Non-PCO ovaries differ from the PCO by the distribution pattern and the number of ovarian follicles. The 2003 Rotterdam consensus defined the PCO by the appearance of a follicle count of ≥ 12 peripheral follicles measuring 2-9 mm in diameter in at least one ovary or an ovarian volume of at least 10 mL with follicles less than 10 mm diameter("The revised Rotterdam ESHRE/ASRM criteria ", 2004). Most recently, a higher threshold for follicle number (antral follicle count [AFC] ≥ 25) has been recommended to define PCO. The higher threshold for follicle number obviates the growing misconception that polycystic ovaries have been highly prevalent in the general population in women who do not

have PCOS (Dewailly et al., 2014). Decreased follicle atresia appears to underlie the increased follicle numbers in PCO. Morphological studies have demonstrated an increased density of follicles in the ovarian cortex predominantly at the primordial (resting) and primary stages (growing). It has been suggested that there may be an increased initiation of growth of follicles from the resting pool regardless of ovulatory status in women with PCOS compared with the non-PCO population. This observation has been suggested to occur via: an excessive transformation of the primordial germ cells in the fetal ovary and more mitotic divisions of the oogonia in the fetal ovary; enhanced assembly of somatic cells around the naked oocytes during follicle formation around the twelfth week of gestation; or a decreased rate of loss (atresia) of germ cells and the surrounding somatic cells in the PCO compared to non-PCO. Women with PCOS who are not ovulating have exhibited abnormalities in the later stages of antral follicle growth, including an arrest of follicular growth, typically at a diameter of approximately 5-8 mm. A trend toward a higher proportion of atretic follicles is seen in women with PCOS, compared with a non-PCOS group (Webber et al., 2003). Further, histologic features of atretic ovarian follicles have included a degenerated oocyte nucleus, uneven or folded nuclear membranes, vacuoles in the oocyte, and pyknotic nuclei in the granulosa cells (Hovatta et al., 1997; Webber et al., 2003). Women with PCOS have a lower proportion of healthy primordial cells, a higher proportion of early primary follicles, and a reciprocal decrease in primordial follicles when compared with non-PCOS counterparts (Webber et al., 2003).

2.1.5.4. Ovarian Dysfunction and Infertility

Women who ovulate regularly typically have regular menstrual cycles between 26-35 days (18). Ovarian dysfunction usually manifests as oligomenorrhoea/amenorrhoea resulting from chronic oligo-ovulation/anovulation, defined as ≤ 8 menstrual cycles per year or intervals of ≥ 35 days between menstrual cycles (Brassard et al., 2008). A study of menstrual cyclicity in 873 women aged 15-46 years revealed that 18% of the women had menstrual dysfunction and approximately 27% of women with menstruation dysfunction had PCOS. It has been suggested that approximately 25-33% of women who present with menstrual dysfunction have PCOS (Azziz et al., 2004). Of note, tracking reproductive cycles using the observation of bleeding patterns, which typically reflects a progression of follicle growth to ovulation to corpus luteum regression, is not necessarily a hallmark of regular ovulation. Women with PCOS who do not

ovulate have irregular, non-cyclic bleeding patterns. When estrogen-stimulated endometrial growth is not suppressed and controlled by progesterone, irregular bleeding occurs.

Androgens synthesized in excessive amounts in ovarian stromal tissue are aromatized to estrogen in peripheral tissues (adipose, muscle, and liver). Peripheral estrogen synthesis in the absence of ovulation is available to stimulate endometrial growth and enable irregular bleeding. Thus, women with PCOS who do not ovulate have variable endometrial bleeding episodes (Baird et al., 1977; DeVane et al., 1975; MacDonald et al., 1967).

In women, 7% of menstrual cycles of normal length are anovulatory (Harlow & Campbell, 2000). The length of each menstrual cycle is highly variable in ovulatory women without PCOS. Because of newer diagnostic criteria for PCOS, women with regular menstrual cycles may be diagnosed with PCOS (Azziz et al., 2006).

The most prevalent cause of anovulatory infertility is PCOS. Approximately 90-95% of women with anovulation attending infertility clinics had PCOS (Teede et al., 2010). At least 40% of women with PCOS are infertile, defined as the lack of ability to conceive within 12 months. Obesity is a strong risk factor that can exacerbate anovulation and cause infertility. Obesity is highly prevalent in women with PCOS, affecting over 90% of women with PCOS who have infertility (Brassard et al., 2008). Ideally, optimizing weight, and planning to conceive early in reproductive life are important factors not only in the treatment of infertility but also in the reduction of pregnancy complications and the risk of miscarriage in women with PCOS (Teede et al., 2010).

2.1.5.5. Endometrial Pathologies

Women with PCOS are predisposed to endometrial pathologies, including endometrial polyps, hyperplasia and endometrial adenocarcinoma (Azziz et al., 2006). Endometrial hyperplasia occurs in 35% of women with PCOS who do not have regular bleeding intervals. Endometrial adenocarcinoma represents 8% of all cancers occurring in women, and the highest risk populations are women who are obese, have hyperinsulinemia, DM2 and PCOS. Anovulation and abnormal uterine bleeding occur in women with PCOS, as ovarian androgens are aromatized to estradiol in peripheral tissues (i.e., adipose, muscle, and liver) (Hardiman et al., 2003b). Estradiol mediates endometrial growth, which is “unopposed” in the absence of ovulation. Irregular bleeding is common following anovulation because continuous estrogen-mediated endometrial growth lacks the stromal support normally produced by progesterone

following ovulation. Hyperinsulinemia plays a role in the etiology of endometrial pathology. With PCOS, hyperinsulinemia promotes androgen synthesis in ovarian theca cells and increases vascular unbound androgens by downregulating hepatic SHBG synthesis. Hyperinsulinemia accelerates cell proliferation, promotes tumour angiogenesis and has been implicated in endometrial proliferation, hyperplasia and carcinoma (González et al., 2006; Ortega et al., 2014; Piotrowski et al., 2005). Chronic systemic inflammation may increase the risk of endometrial hyperplasia and metabolic changes in women with PCOS (Hardiman et al., 2003b). Exposure to progesterone, either as replacement therapy or via spontaneous ovulation, more than 4 times per year may decrease neoplastic changes in endometrial tissue. Menstrual cyclicity following ovulation is associated with a lower risk of neoplastic change in endometrial tissue (Azziz et al., 2006).

2.1.5.6. Psychological Features

Most research has focused on the biological and physiological aspects of PCOS, and little attention has been focused on psychological features of the syndrome. Women with PCOS experience high rates of anxiety and depression. Physical challenges, such as acne vulgaris, alopecia, hirsutism, acanthosis nigricans (i.e., a striking cutaneous marker for tissue resistance to insulin (Goldenberg & Punthakee, 2013)), obesity, and alterations in voice, as well as long-term health-related comorbidities, such as diabetes, metabolic syndrome, heart disease, and infertility compromise quality of life and adversely impact mood and thr psychological well-being of affected women (Coffey et al., 2006; Deeks et al., 2010a; Dunaif et al., 1987). Women with PCOS are more prone to poor body image, psychosexual dysfunction, bipolar spectrum disorder, depression, anxiety, and eating disorders compared to counterparts without PCOS (Coffey & Mason, 2003; Deeks et al., 2010a).

No causal relationship between psychological, sexual, body image issues and PCOS have been established. However, psychological issues have been shown to adversely affect the levels of adherence of women with PCOS to therapeutic modalities. Negative impacts of mood disturbance, poor self-esteem, and reduced psychological well-being have been documented alongside the levels of motivation of women with PCOS to adopt healthy lifestyle options. Further, psychological issues associated with PCOS have been reported to reduce the ability of women with PCOS to implement and sustain successful long-term lifestyle change (Himelein & Thatcher, 2006; Moran et al., 2009). It is recommended that psychological issues that are linked

with PCOS be explored and addressed as part of routine PCOS assessment and management (Himelein & Thatcher, 2006).

2.1.5.7. Metabolic Features and Associated Long-Term Comorbidities

Polycystic ovary syndrome is associated with substantial metabolic derangements, including IR and compensatory hyperinsulinemia, glucose intolerance, and dyslipidemia. Women with PCOS are at increased risk for developing long-term comorbidities including DM2, and CVD, which can negatively impact the overall prognosis and increase all-cause mortality rates of women with PCOS (Moran et al., 2010; Teede et al., 2006).

2.1.5.8. Insulin Resistance and Abnormal Glucose Metabolism

Insulin stimulates glucose uptake in tissues such as skeletal myocytes, adipocytes, and cardiac myocytes, and suppresses hepatic gluconeogenesis, glycogenolysis, proteolysis, and lipolysis (DeFronzo, 1988; Saltiel & Kahn, 2001). Insulin resistance is defined by a decreased ability of a certain amount of insulin to efficiently mediate glucose uptake, glucose production, and lipolysis; IR is characterized by an increased amount of circulating insulin (Bergman et al., 1985). A majority (50-70%) of women with PCOS demonstrate significant peripheral hyperinsulinemia and IR, with significantly higher prevalence rates compared to women without PCOS, matched by age, ethnicity, and BMI (A Dunaif, 1997; Gambineri et al., 2002).

Mechanisms involved in the development of IR are complex, with genetic and environmental contributors. Specific abnormalities of insulin metabolism identified in PCOS include reduced pancreatic secretion, reduced hepatic extraction, impaired hepatic gluconeogenesis suppression, defects in peripheral insulin receptor signalling, and impaired insulin-mediated glucose transport pathways (A Dunaif, 1997; Dunaif & Finegood, 1996; Dunaif et al., 1989; O'Meara et al., 1993). Further, obesity can contribute to the development of PCOS, via endocrine pathways of IR that are mechanistically distinct from the IR present in lean women with PCOS (Corbould et al., 2005; Corbould et al., 2006; E Diamanti-Kandarakis & A Dunaif, 2012; Andrea Dunaif, 1997; Skov et al., 2007). Thirty-five to 50% of women with PCOS have abdominal obesity and an increased waist circumference (WC) (Norman et al., 2002). Up to 95% of obese (BMI $\geq$ 30 kg/m^2) women with PCOS have some degree of IR (DeUgarte et al., 2005; Legro et al., 1999) compared with 20–25% of lean counterparts (BMI < 25 kg/m^2) (Ovesen et al., 1993). In the state of obesity, adipose tissue releases excessive amounts of proinflammatory cytokines (mainly tumour necrosis factor-α and interleukin-6), non-esterified fatty acids,

glycerol, leptin, resistin, and other factors (e.g., retinol-binding protein-4) that are involved in the development of IR. Further, in obesity, A reduction of adiponectin secretion by adipose tissue has been linked to the development of IR and atherosclerosis (Kern et al., 2003). The distribution of body fat is a critical determinant of insulin sensitivity. Individuals with a more peripheral accumulation of fat are generally more insulin sensitive when compared to individuals with central fat accumulation in abdominal and chest areas (Kahn et al., 2006).

Insulin resistance and consequent hyperinsulinemia can aggravate PCOS symptoms and unmask PCOS in predisposed women, by inducing hyperandrogenemia, oligo-anovulation, and infertility (E Diamanti-Kandarakis & A Dunaif, 2012). Impaired insulin function can dysregulate lipid and protein metabolism and contribute to the development of the long-term comorbidities associated with PCOS, including atherosclerotic CVD, DM2, and MetS. Hyperinsulinemia and IR act in concert to cause dysfunction and apoptosis of insulin-releasing pancreatic islet β-cells; impaired glycemic control, glucose intolerance, and ultimately DM2 subsequently develop in women with PCOS. Women with PCOS are at an increased risk of developing IGT and DM2 with prevalence rates of 31.3% and 7.5% respectively, compared to 14% for IGT and 0% for DM2 in age-matched and weight-matched women without PCOS (Legro et al., 1999). Abnormal glucose metabolism develops in women with PCOS at a younger age compared with non-PCOS counterparts. Further, women with PCOS and impaired glucose tolerance have a tendency toward an increased rate of developing DM2 when compared with non-PCOS counterparts (Ehrmann et al., 1999). Women with PCOS are at higher risk of developing gestational diabetes when compared with women without PCOS. The development of gestational diabetes is independent of obesity, but can be exacerbated by obesity (Boudreaux et al., 2006; Legro et al., 1999). Acanthosis nigricans is an important cutaneous manifestation of IR, which is frequently observed in women with PCOS. The altered insulin signalling in PCOS accelerates the growth of keratinocytes and dermal fibroblasts and leads to the development of acanthosis nigricans (Panidis et al., 1995).

Few adequately powered studies have examined the evolution of IGT, DM2, and CVD in PCOS. Nevertheless, the International Diabetes Federation has identified PCOS as a significant and modifiable risk factor associated with DM2 (Alberti et al., 2007). Given the high prevalence rates and the clinical significance of the metabolic complications associated with IR and IGT in PCOS, the American College of Obstetricians and Gynecologists and the Endocrine Society

recommended routine clinical screening for IGT and DM2 for all women with PCOS, using a standard 2-hour 75-g OGTT. The OGTT is a sensitive and relatively specific test, recommended as the gold standard screening tool for the diagnosis of IGT and DM2 in women with PCOS. The OGTT cannot be replaced by simpler alternatives, such as fasting plasma insulin levels, HbA1C, fasting plasma glucose (FPG) levels, homeostatic model assessment score, fasting glucose to insulin ratio, and quantitative insulin index test. For example, the FPG test fails to diagnose women with IGT in PCOS population, as a majority of women with IGT have normal FPG (Legro et al., 1999). The OGTT can provide information about peripheral insulin action and pancreatic β-cell function and can be performed in office laboratory settings. The reproducibility of the test can be enhanced by the standardizing of the carbohydrate content of the prior days' meals and ensuring the blood samples will be collected within a 2-hour limit (Legro et al., 2004; Salley et al., 2007).

2.1.5.9. Dyslipidemia

Dyslipidemia is a frequent metabolic abnormality in women with PCOS. Dyslipidemia is characterised by elevations of TG, very low-density lipoprotein cholesterol (VLDL-C), and total cholesterol, as well as reduced HDL-C levels. Approximately 70% of women with PCOS exhibit abnormal serum lipid levels ("The NCEP ATP III final report," 2002). Depressed HDL-C and elevated TG are the most frequent lipid abnormalities observed in women with PCOS (Legro et al., 1999; Talbott et al., 1998; Wild et al., 1985). Dyslipidemia is one of the most perplexing metabolic complications associated with PCOS which can increase the risk of DM2 and CVD in the affected women, in a complex interplay with IR.

Hyperinsulinemia can exacerbate the lipidemic aberrations of PCOS, in part, by stimulating lipogenesis and altering the expression of lipase enzymes which regulate lipid metabolism (E Diamanti-Kandarakis & A Dunaif, 2012; Victor et al., 2009; Wild et al., 1985). Hyperinsulinemia induces the hepatic overproduction of apo-B-containing VLDL-C and upregulates the expression of proteins involved in the VLDL-C production, thereby, significantly increasing VLDL-C levels (Au et al., 2003; Taghibiglou et al., 2000). Hyperinsulinemia has been reported to suppress the clearance of TG-rich proteins during the postprandial period, which may be another possible mechanism of dyslipidemia in PCOS (Harbis et al., 2001).

Dyslipidemia in PCOS has frequently been reported independent of BMI (Wild & Bartholomew, 1988; Wild et al., 1985). However, increased BMI has synergistic deleterious

effects on the development of dyslipidemia in PCOS (Wild et al., 1985). Accumulation of intra-abdominal visceral fat alters lipid metabolism and accelerates lipolysis, mainly because of the upregulation of hormone-sensitive lipases. Insulin resistance and consequent hyperinsulinemia are the primary pathological contributors to the development of dyslipidemia in PCOS.

Hyperandrogenemia may contribute to the development of dyslipidemia (von Eckardstein, 1998; Whitsel et al., 2001). Hyperandrogenemia, specifically increased testosterone, induces androgen receptor-mediated IR, a mechanism for further potentiation of IR. Interactions between androgens and androgen receptors decrease the catabolic clearance and increase the circulating levels of LDL-C (Croston et al., 1997). Elevated levels of sex-steroids alter lipoprotein lipase activity through regulations that occur at the transcriptional and post-transcriptional levels and lead to the development of dyslipidemia (Enerbäck & Gimble, 1993). Androgens have been shown to impair HDL-C metabolism and lead to depressed levels of HDL-C, by altering enzymes involved in HDL-C remodeling, lipid transfer, and cell surface receptors. Additionally, hyperandrogenemia may induce the expression of genes involved in the catabolism of HDL-C (Arai et al., 1999; Kozarsky et al., 2000). To better understand the mechanistic interplay between metabolic and hormonal abnormalities in PCOS and dyslipidemia, more research is required.

2.1.5.10. Cardiovascular Disease

Women with PCOS are at high risk of developing CVD. In addition to the classical risk determinants for CVD such as obesity, dyslipidemia, MetS, IGT, DM2, and IR, (Wild et al., 2010), women with PCOS are predisposed to novel CVD risk factors, including inflammation, oxidative stress, and impaired fibrinolysis and coagulation (Mannerås-Holm et al., 2011; Moran & Teede, 2009). Results of a systematic review and meta-analysis of studies evaluating circulating markers of oxidative stress in women with PCOS showed a: 23% increase in homocysteine concentration with a 0.6 standardized mean difference (SMD; a measure of effect size) (95% CI, 0.4-0.8); 47% increase in malondialdehyde levels, 1.9 SMD (95% CI 1.2-2.6); 36% increase in asymmetric dimethylarginine levels, 1.1 SMD (95% CI 0.6-1.6); 34% increase in superoxide dismutase activity, 1.0 SMD (95% CI 0.5-1.4); 50% decrease in glutathione concentration, −3.7 SMD (95% CI −6.2 to −1.2); and 32% decrease in paraoxonase-1 activity −0.9 SMD (95% CI −1.3 to −0.4) when compared with control subjects without PCOS (Murri et al., 2013). The PCOS group exhibited higher levels of plasminogen activator inhibitor-1 activity

and fibrinogen when compared with controls. Differences between groups remained significant after adjustments for age and BMI. An impaired prothrombotic state also contributes to the increased risk of CVD in women with PCOS (Mannerås-Holm et al., 2011). An elevated CRP level has frequently been observed in PCOS. Elevation of CRP has been an indicator of low-grade chronic inflammation. Increased CRP level has also been a reliable and independent predictor of CVD (Duleba & Dokras, 2012).

Women with PCOS are at high risk for IGT and DM2 (Moran et al., 2010), which can increase the risk of developing premature CVD. The risk of CVD mortality was estimated to be up to 60% among PCOS population with impaired glucose metabolism (Barr et al., 2007).

Obesity is a common comorbidity present in PCOS (A Dunaif, 1997; Gambineri et al., 2002). Obesity can induce chronic systemic inflammation. Obesity can exacerbate the development of early clinical indicators of atherosclerosis in PCOS, including endothelial dysfunction, pulse wave velocity, carotid intima-media wall thickness, presence of carotid plaques, and coronary artery calcification in women with PCOS (Legro et al., 1999; Legro et al., 2001; C Meyer et al., 2005; C. Meyer et al., 2005).

While evidence from retrospective studies is limited, it has been suggested that there is an increased risk of CVD with PCOS (Shaw et al., 2008). Evidence from well-conducted longitudinal studies to address CVD in the PCOS population from different age and race groups is not available. There is still a paucity of appropriate methods to evaluate or predict the risk of CVD in women with PCOS, particularly in reproductive-age women, and further research is required.

2.1.5.11. Metabolic Syndrome

The MetS is a complex clustering of five interrelated risk factors for CVD and DM2, including elevated FPG, BP and TG levels, central adiposity, and low HDL-C levels (Alberti et al., 2009). The prevalence of MetS is high in women with PCOS and varies depending upon the chosen diagnostic criteria, and regional, lifestyle, and ethnic variations (Apridonidze et al., 2005; Carmina et al., 2006; Glueck et al., 2003). Metabolic syndrome affects 47-53% of women with PCOS (Apridonidze et al., 2005; Ehrmann et al., 1999). The MetS is a significant contributor to CVD and DM2 (Brien & Katzmarzyk, 2006; Cameron et al., 2008). With MetS, the risk of CVD doubles and the risk of DM2 increases fivefold (Alberti et al., 2006; Grundy, 2008).

There are several definitions for MetS based on central features, including IR, visceral adiposity, atherogenic dyslipidemia, and endothelial dysfunction. The 1998 World Health Organization (WHO) criteria, the 1999 European Group for the Study of Insulin Resistance (EGIR) criteria, the 2005 (revised) NCEP Adult Treatment Panel (ATP) III criteria, and the 2005 International Diabetes Federation and American Heart Association/National Heart, Lung, and Blood Institute (IDF and AHA/NHLBI) criteria are amongst the most widely recognized criteria for diagnosing MetS (Alberti et al., 2006; Alberti & Zimmet, 1998; Huang, 2009; "The NCEP ATP III final report," 2002; Takamiya et al., 2004). The revised NCEP ATP III, the IDF and the AHA/NHLBI criteria are the most widely used to characterize MetS in PCOS research (Apridonidze et al., 2005; Carmina et al., 2006; Glueck et al., 2003; Teede et al., 2006). According to the modified NCEP ATP III criteria, MetS is diagnosed in women with PCOS if $\geq$ 3 of the following is satisfied: FPG $\geq$ 100 mg/dl ($\geq$ 5.6 mmol/L) or a positive diagnosis of DM2, WC $\geq$ 88 cm, BP $\geq$ 130/85 mmHg or the use of medication for the control of hypertension, TG $\geq$ 150 mg/dl ($\geq$ 1.7 mmol/L) or the use of medication for elevated TG levels, and reduced HDL-C $\leq$ 50 mg/dl ($\leq$ 1.3 mmol/L) or the use of medication for reduced HDL-C levels. The IDF 2005 criteria use similar cut-points for FPG, TG, HDL-C, and BP. However, the IDF and AHA/NHLBI recommend using race-specific WC cut-points to determine abdominal obesity. The present recommendation accounts for the fact that different populations, ethnicities, and nationalities have different distributions of normative values for body weight and WC. The 2005 IDF criteria reflect that there is a relationship between the proposed WC cut-points and the risk for DM2 or CVD which varies in different populations (Alberti et al., 2006). For example, South Asian populations have an increased risk for DM2 and CVD at a WC that is smaller than the criteria for MetS in Western populations. The variability in MetS criteria may explain the observed disparities in the prevalence rates of MetS among different populations. Table 1 shows the 2005 IDF and AHA/NHLBI thresholds for abdominal obesity in females, which are currently recommended in several different populations and ethnic groups.

Table 1. Recommended population and country-specific waist circumference thresholds for abdominal obesity (K. G. M. M. Alberti et al., 2006)

Population	Waist circumference thresholds
Canada, United States, and Europe	≥88 cm
Middle East, Mediterranean, Sub-Saharan African, and West Asian	≥80 cm
Asian, Latin American, Ethnic Central and South American	≥80 cm
Chinese	≥80 cm

The pathophysiology of MetS is complex and is not fully understood. However, the pathophysiology of MetS appears to run in parallel to the pathophysiology of PCOS in many ways. Two features appear to stand out as potential shared contributing factors: IR and abnormal fat distribution, predominantly central obesity. Other factors have also been implicated in the development of MetS, including genetic profile, physical inactivity, ageing, a chronic proinflammatory state, and hormonal aberrations. The role and influence of the proposed factors may vary depending on ethnic variations (Anderson et al., 2001; Saad et al., 1991).

Many (42-74%) women with PCOS are obese or overweight, and lead a sedentary lifestyle, both of which contribute to the development of MetS (Corbould et al., 2005; Corbould et al., 2006; E Diamanti-Kandarakis & A Dunaif, 2012; Andrea Dunaif, 1997; Skov et al., 2007). Accumulation of excess adipose tissue that culminates in obesity, especially visceral fat interferes with normal adipose tissue function as a metabolically active endocrine and immunological organ and impairs adipose tissue metabolism (Legro et al., 1999). Obesity can impair immunity, induce inflammation, and lead to metabolic derangements and endocrine imbalance. Obesity is a proinflammatory state that induces oxidative stress (Vendrell et al., 2004; Vincent & Taylor, 2005). Obesity-induced inflammation and oxidative stress are pronounced more in the setting of MetS, rather than in PCOS per se, and may stand out as a distinguishing factor in the pathophysiology of MetS (Boulman et al., 2004; Tarkun et al., 2004). Inflammation can trigger a cascade of molecular events that lead to metabolic aberrations in PCOS. Also, inflammation can directly induce excess ovarian hyperandrogenism in PCOS (Ortega et al., 2014; Piotrowski et al., 2005). Diet-induced inflammation and oxidative stress have been proposed to aggravate proinflammatory signalling in PCOS, independent of obesity. Dietary factors can induce molecular alterations, which may mediate the development of IR,

hyperandrogenism, atherogenesis, and ovarian dysfunction in PCOS (Abdelhadi et al., 2013; González, 2015; González et al., 2013; González et al., 2012).

The PCOS and MetS share overlapping pathophysiological mechanisms. Aside from the pathophysiological pathways, many of the anthropometric and metabolic features of PCOS overlap with the characteristics of MetS, including increased WC, hypertriglyceridemia, low levels of HDL-C, hypertension, and elevated FPG levels. Many women with PCOS have MetS, and almost all women with PCOS present with least one adverse CVD risk factor, suggesting an increased risk of developing CVD and DM2 with PCOS. In a retrospective chart review of women with PCOS, Apridonidze et al. reported a 43% prevalence rate of MetS in women with PCOS, using the revised NCEP ATP III criteria, compared with 24% in age-adjusted controls obtained from the Third National Health and Nutrition Examination Survey (Apridonidze et al., 2005). The differences remained significant after adjustments for age and BMI differences between groups. The researchers concluded that the prevalence rate of MetS and its risk factors, particularly abdominal obesity and reduced HDL-C levels, were high in women with PCOS and may increase the risk of premature CVD and DM2. Increased age and obesity can aggravate the development of CVD and DM2 in post-menopausal age in women with PCOS, mainly due to a progressive shift toward androgen dominance in the hormonal profile and estrogen deficiency (Burger et al., 2000; Carr, 2003; Lasley et al., 2002).

Given the high prevalence and staggering morbidity burden of the above metabolic abnormalities, it is no longer feasible to consider PCOS a purely gynaecological disorder. Instead, PCOS is a systemic metabolic endocrinopathy. Early recognition of PCOS metabolic sequelae, including IR, IGT, DM2, dyslipidemia, and CVD seems to be crucial in the management of women with PCOS. Identification of metabolic abnormalities associated with PCOS may assist healthcare professionals and the affected women to adopt available therapeutic modalities for the management of PCOS and prevent or improve the risk of CVD and DM2 in the long-term.

2.1.6. Management

2.1.6.1. Pharmaceutical, Surgical, and Cosmetic Management

The complex pathophysiological mechanisms that underlie PCOS and extensive clinical manifestations of the syndrome require multidisciplinary medical management. Aside from lifestyle therapies, clinical management of PCOS includes pharmaceutical, surgical, and

cosmetic therapeutic approaches, tailored to the individual needs of each patient. In the present section, current medical, surgical, and cosmetic approaches for the management of PCOS were examined.

In general, most PCOS pharmacological therapeutic modalities have targeted excess ovarian androgen production, androgen action at the receptor level, insulin sensitization and menstrual control (Badawy & Elnashar, 2011; Ganie et al., 2015). Combined hormonal contraceptives (CHC), containing ethinyl estradiol and progestin, indirectly inhibit intra-ovarian androgen production by suppressing GnRH release. The CHC is a conventional method for the management of menstrual abnormalities, hirsutism, and acne in women with PCOS (Legro et al., 2013). The CHC increase circulating levels of SHBG. As indicated previously, SHBG plays a vital role in regulating bioavailable sex-steroid concentrations and decreases free sex steroids levels in women with PCOS. Decreased level of SHBG has been associated with androgenization in women with PCOS. The CHC might also inhibit binding of dihydrotestosterone to the androgen receptors (Eli & Edelson, 1984; Givens et al., 1974; Wild et al., 1982).

Antiandrogens (e.g., cyproterone acetate, flutamide, and spironolactone), and 5-α reductase inhibitors (e.g., finasteride) act as competitive antagonists, by competing for binding sites at the receptor level on the normally androgen-sensitive tissues in the body. Thus, antiandrogens inhibit androgen activity. Antiandrogens have been combined with ethinyl estradiol to manage hirsutism and menstrual irregularity (Azziz et al., 2009).

IR is a significant abnormality in PCOS, leading to metabolic and reproductive complications for women with PCOS. Efforts have been made to use medications that improve insulin sensitivity and modulate IR (E Diamanti-Kandarakis & A Dunaif, 2012). There are two main groups of insulin-sensitising agents: biguanides, with metformin as the principal agent, and thiazolidinediones (TZDs; also known as glitazones) (Baillargeon et al., 2003; Legro et al., 2013). Metformin is the most widely used insulin reducing agent, which improves metabolic and glycemic abnormalities and decreases menstrual irregularities in women with PCOS. Metformin exerts its beneficial effects through decreasing intestinal glucose uptake, reducing IR by inhibition of hepatic gluconeogenesis, and improving peripheral insulin sensitivity (Diamanti-Kandarakis et al., 2010; Palomba et al., 2009). TZD improve insulin signalling, enhance insulin-mediated glucose uptake and utilization in adipose tissue and muscle, and confer multiple effects on lipid metabolism and inflammatory pathways. While TZDs are most effective at increasing

peripheral glucose uptake, metformin was found to be more efficient in decreasing hepatic glucose production (Dunaif, 2008). However, the use of TZDs have been discouraged in women with PCOS, because of the safety concerns associated with it, such as increased risk of CVD, hepatotoxicity, bladder cancer, and drug-induced weight gain (Baillargeon et al., 2003; Dunaif, 2008; Lago et al., 2007; Lewis et al., 2011).

Many medications are available to induce ovulation for anovulatory women who desire pregnancy. Clomiphene citrate is an estrogen receptor antagonist; the negative feedback exerted by estradiol on gonadotropin secretion is inhibited by clomiphene. Thus, FSH secretion increases, which in turn, increases follicle growth and improves the chance of ovulation. The medication was recommended as the first-line drug therapy for infertility treatment for women with PCOS ("The ESHRE/ASRM PCOS Consensus on infertility," 2008). Recent off-label use of aromatase inhibitors, such as letrozole, has been recommended to increase FSH secretion, follicle growth and ovulation. Letrozole suppresses the activity of aromatase enzyme and prevents the aromatase from converting androgens to estrogens by competitive, reversible binding to the heme of its cytochrome P450 unit (Franik et al., 2015; Palomba, 2015). Roque et al. performed a meta-analysis on seven RCTs (n=1833) comparing the effects of letrozole with clomiphene citrate on ovulation induction in women with PCOS. The letrozole treatment appeared to be superior to the clomiphene citrate treatment in increasing live birth and pregnancy rates in women with PCOS (relative risk = 1.55, 95% CI: 1.26–1.90, and 1.38, 1.05–1.83, respectively). Groups were not different in their rates of multiple pregnancy, miscarriage and ovulation (Roque et al., 2015).

Orlistat (tetrahydrolipstatin), a semisynthetic derivative of lipstatin, inhibits the absorption of fat from the gut by binding irreversibly to gastric and pancreatic lipases. Orlistat is considered a safe alternative in the management of obesity for women with PCOS. Orlistat is used alone, or in combination with appetite regulator agents, such as sibutramine.

Bariatric surgical procedures are effective at reducing weight in overweight or obese women with PCOS and are used as adjuvant therapies within a weight management program (Panidis et al., 2014; Rubio et al., 2007). Many women are able to discontinue medications for the control of DM2 and HTN following bariatric surgical weight loss (Ikramuddin et al., 2013; Sjöström et al., 1999). Significant weight regains, and micronutrient (iron, vitamin B12, folate, calcium, and vitamin D) deficiency have been reported over the long-term follow up after

bariatric surgery. Therefore regular monitoring, follow up visits, and interventions are recommended to maintain weight loss (Rashti et al., 2015; Shah et al., 2006).

Numerous therapies for hirsutism exist, but most therapies are temporary. Cosmetic treatments for hirsutism include temporary hair removal methods such as waxing, shaving, threading, and bleaching. Eflornithine and spironolactone are antiandrogens that are used as pharmacological agents to slow male-pattern hair growth for the treatment of hirsutism. Long-term hair removal procedures include laser therapy and electrolysis. Each therapy is effective for variable duration and serves to reduce the discomfort, improve hair removal, increase the self-image of PCOS women with hirsutism (Balfour & McClellan, 2001; Legro et al., 2013; Trueb, 2002). Hair regrowth recurs most quickly when other therapies aimed at decreasing androgen production are not used on a long-term basis (O'Driscoll et al., 1994).

Surgical procedures have been developed to induce ovulation and achieve pregnancy in infertile women with PCOS. Two primary surgical methods have been used for those who desire pregnancy: ovarian wedge resection and ovarian drilling (ovarian diathermy) completed at laparotomy or laparoscopy) (Adashi et al., 1981; Buttram & Vaquero, 1975). Ovarian wedge resection at laparotomy, performed by taking multiple biopsies or a single larger ovarian biopsy, became obsolete after the introduction of the minimally invasive surgical techniques, because of the frequent rates of complications such as, premature ovarian failure and periovarian adhesions (Adashi et al., 1981; Buttram & Vaquero, 1975). Ovarian drilling involves destroying multiple small areas of ovarian tissue by creating a variable number of holes (drilling) in the ovarian capsule and subcapsular stroma in anovulatory women with PCOS. The drilling procedure is also associated with periovarian adhesion and may provide only temporary induction of ovulation. The destruction of the ovarian stroma is believed to decrease androgen synthesis and therefore decrease peripheral conversion of androgen to estrogen, thereby allowing a decline in estrogen and spontaneous GnRH release. Surgical procedures are considered as the second-line alternative after the failure of or lack of supply of conventional medical treatment, such as treatment with clomiphene citrate and gonadotropins (Vause et al., 2010). In vitro fertilization and intracytoplasmic sperm injection cycles have been proposed as the third line therapy for infertile women suffering from PCOS ("The ESHRE/ASRM PCOS Consensus on infertility," 2008). In vitro fertilization has been adopted when women with PCOS are at greater risk of multiple ovulation, multifetal conception and risk for ovarian hyperstimulation syndrome. Single embryo

transfer, cryopreservation, and delayed embryo transfer have decreased the risk of multiple gestations and ovarian hyperstimulation syndrome in vitro fertilization (Smith et al., 2015; Tiitinen et al., 2001). However, some women with PCOS who complete IVF have poor rates of conception due to unexplained poor-quality oocyte fertilization and embryonic development.

The medical management of women with PCOS remains a very complicated issue. No single therapy has been established to address all aspects of the syndrome simultaneously. However, any clinical intervention in the management of PCOS should be an adjuvant treatment to lifestyle modification.

2.1.6.2. Lifestyle Interventions

Lifestyle modification, comprising dietary, exercise, and behavioural therapies, is, without question, the most important and first-line therapy in the management of PCOS. Diet and exercise interventions have been demonstrated to improve insulin sensitivity, reduce insulin levels, modify glucose metabolism, improve lipid profiles, and improve the overall physical and mental health of women with PCOS (Domecq et al., 2013; Harris-Glocker et al., 2010; Moran et al., 2011; Norman et al., 2002). Adopting healthy lifestyle behaviours have been recommended to improve the prognosis for women affected by PCOS.

2.1.6.2.1. Dietary Interventions

Dietary modification is an integral part of therapeutic strategies for the management of PCOS. However, the optimal dietary composition that will mediate reproductive, metabolic, and psychological improvements in women with PCOS has yet to be determined (Moran, Ko, et al., 2013). This section will focus on the available literature regarding the dietary interventions to improve PCOS outcomes.

There is much debate concerning the overall diet quality and dietary intake of women with PCOS compared to counterparts without PCOS, mainly due to regional, ethnic, life stage, and lifestyle variations in PCOS populations (S Barr et al., 2011; Crystal C. Douglas et al., 2006; Graff et al., 2013; Moran, Ko, et al., 2013; Tsai et al., 2013; Wright et al., 2004). However, there is a general agreement that women with PCOS have higher intakes of energy (mainly from foods with high GI) and saturated fat (SFA), as well as insufficient fiber intake, specifically from pulses and vegetables, when compared to women without PCOS (Crystal C Douglas et al., 2006; Eslamian et al., 2016; Lin & Lujan, 2014; Shishehgar et al., 2016b). Positive energy balance is a strong determinant of excess body weight, which can exacerbate PCOS outcomes and confer the

risk of chronic disease in women with PCOS. Targeting weight loss in overweight and obese PCOS populations and maintaining long-term healthy weight should be a priority in the management of the syndrome. A modest weight loss (5-10%) has been shown to improve CVD risk factors, including abdominal obesity and lipid profile, modulate blood glucose, increase insulin sensitivity, attenuate hyperandrogenemia, regulate menstrual cyclicity, restore ovulation, enhance fertility, and improve overall psychological well-being and quality of life of women with PCOS, irrespective of dietary composition (Badawy & Elnashar, 2011; Crosignani et al., 2003; Elsenbruch et al., 2003; Gronbaek et al., 2012; M.-M. Huber-Buchholz et al., 1999; Moran, Ko, et al., 2013; Sweatt et al., 2015). Moran et al. (2013) conducted a systematic review to assess the favourable dietary composition that can uniformly improve the features of PCOS. Dietary interventions showed subtle differences in multiple outcomes. There was a lack of conclusive evidence for the optimal dietary composition that may confer benefit over caloric restriction in PCOS. Instead, the positive effects of weight loss per se were highlighted in overweight and obese women through short-term calorie restriction. The proposed hypocaloric dietary interventions have had a minimal emphasis on optimizing dietary composition for women with PCOS. A low GI and low carbohydrate diet was reported to result in higher improvements in IR, fibrinogen, total cholesterol, HDL-C, and quality of life; a high protein diet was shown to improve depression and self-esteem; a monounsaturated fat-enriched diet was reported to enhanced weight loss, and a high-carbohydrate diet was shown to increase free androgen index (Moran, Ko, et al., 2013). Asemi and colleagues (2014) examined the effects of a calorie-restricted Dietary Approaches to Stop Hypertension (DASH) diet on lipid profile and the biomarkers of oxidative stress in overweight and obese women with PCOS (Asemi et al., 2014). The DASH diet is a diet rich in fruits, vegetables, whole grains, and low-fat dairy products and low in SFA, total cholesterol, and refined grains (Sacks et al., 2001a). The DASH diet combined with calorie restriction for 8 weeks was reported to improve insulin, TG and VLDL-C levels, and increased plasma total antioxidant capacity and total glutathione levels (Asemi et al., 2014). However, the dietary intervention was on a background of a calorie restriction; thus, the isolated benefits of the DASH eating plan on PCOS metabolic and oxidative stress outcomes are difficult to extrapolate.

Studies comparing the benefits of one diet over another are scarce. Conventional healthy diets, containing low carbohydrate, GI, SFA, and sodium contents, which are rich in fiber

specifically from whole grains, fruits, and vegetables, are most commonly recommended for women with PCOS. It has been proposed that insulin sensitivity, glucose tolerance, lipid profile, body fat distribution, and visceral fat content improve following the adherence to conventional healthy diets (Barr et al., 2013; Crystal C. Douglas et al., 2006; Goss et al., 2014; Gower et al., 2013; Marsh & Brand-Miller, 2005; Marsh et al., 2010; Stamets et al., 2004; Sweatt et al., 2015; Turner-McGrievy et al., 2014a).

Emerging evidence supports the use of dietary supplements, to modulate hyperglycemia and dyslipidemia, and to alleviate the manifestations of hyperandrogenemia in PCOS. Omega-3 fatty acids have been shown to improve glucose metabolism. Results of three 8-week RCTs, which were conducted separately, have shown improvements in fasting glucose and insulin levels in women with PCOS following dietary supplementation therapy. A dose of 1200 mg/d omega-3 long-chain polyunsaturated fatty acids (Rafraf et al., 2012) and selenium supplementation with a dose of 200 μg/d (Jamilian et al., 2015) have been reported to decrease insulin, TG, and VLDL-C levels. Zinc supplementation at a dose of 220 mg/d has resulted in modified insulin, glucose, and TG levels in women with PCOS (Foroozanfard et al., 2015).

Inositol supplementation has been proposed as a safe therapy to improve insulin sensitivity in PCOS (Dinicola et al., 2014; Unfer et al., 2016). Inositols are 6-carbon compounds found in many foods, including fruits, beans, cereals, and nuts (Dinicola et al., 2014). Inositol isomers, myo-inositol and D-chiro-inositol, were shown to improve IR, glucose uptake, hyperandrogenemia, FSH signalling, and dyslipidemia in PCOS. A 40:1 ratio of myo-inositol to D-chiro-inositol has been proposed to be optimal to both reproduce the physiological plasma ratio, and to improve insulin sensitivity in PCOS (Dinicola et al., 2014; Unfer et al., 2016). Results of a systematic review and meta-analysis of 12 RCTs indicated supplementation with myo-inositol, alone, or in combination with D-chiro-inositol, can restore spontaneous ovulation, improve fertility, and modify hormonal and reproductive alterations in women with PCOS (Unfer et al., 2016).

The efficacy of vitamin D3 supplementation on various features of PCOS is still controversial. Vitamin D3 deficiency appears to exacerbate IR and increase FPG in women with PCOS (He et al., 2015). Supplementation with 20,000-50,000 IU vitamin D3 weekly for 8 weeks has been reported to improve FPG, TG, and menstrual cyclicity in 30-50% of vitamin D-deficient women with PCOS who presented with oligomenorrhea (Wehr E et al., 2011).

Additionally, vitamin D3 supplementation with a dose of 50,000 IU weekly for 8 weeks appears to decrease the intervals between menstrual bleedings, hirsutism, acne, and TG levels in women with PCOS with vitamin D3 deficiency. Vitamin D3 replenishment has been proposed to regulate the bioavailability of transforming growth factor (TGF)-β1, a cytokine with essential roles in immunity and modulation of many cellular functions, including the control of cell growth, cell proliferation, cell differentiation and apoptosis (Irani et al., 2015).

The available literature on the effects of various nutritional supplementation on PCOS is limited by sample size, supplementation duration, supplementation dosage, and study design. Further adequately powered, well-designed RCTs are needed to confirm the safety and effectiveness of dietary supplementation on multiple PCOS outcomes.

2.1.6.2.2. Proposed Dietary Interventions with Potential to Improve PCOS Outcomes

2.1.6.2.2.1. The Therapeutic Lifestyle Changes Diet

The TLC diet is an integral component of nonpharmacologic healthy lifestyle habits program, recommended by the NCEP ATP III, to lower LDL-C and reduce CVD risk. In the TLC program, ATP III endorses a multifactorial lifestyle approach: Reducing high-saturated fat atherogenic diet; optimizing body weight; increasing physical activity; and smoking cessation ("The NCEP ATP III final report," 2002). Many women with PCOS present with obesity, dyslipidemia, and adverse lifestyle habits. The TLC diet appears to be appropriate for women with PCOS. The TLC diet encourages a nutritionally balanced diet focusing on improving CVD risk and reducing LDL-C levels by diminishing fat intake and increasing fiber consumption. Hence, the TLC diet, as a component of the TLC program that promotes healthy changes in lifestyle, can be considered a standard conventional healthy diet, with the potential to improve PCOS outcomes.

The TLC program has been designed according to a number of factors: 1) the benefits of keeping cholesterol and trans fatty acid intake low and the addition of LDL-C lowering dietary options (i.e., viscous, soluble fiber and plant stanol/sterol esters); 2) the encouragement of health-promoting aspects of the diet that include, among other things, fish and omega-3 fatty acids; and 3) the endorsement of regular physical activity, weight loss, and smoking cessation as critical first steps in reversing the unwanted metabolic effects of the MetS (Stone & Van Horn, 2002).

The general approach to a TLC diet involves reduced intake of saturated fats and cholesterol and incorporating other therapeutic dietary options, such as increased consumption of viscous fiber to reduce LDL-C. The major LDL-C-raising dietary constituents are saturated fat and cholesterol. There is a dose-response relationship between saturated fatty acids and LDL-C levels ("The NCEP ATP III final report," 2002). For every 1% increase in calories from saturated fatty acids, as a percent of total energy, the serum LDL-C rises about 2% (Ginsberg et al., 1998). The primary sources of saturated fatty acids in the diet are high-fat dairy products (e.g., whole milk, cheese, butter, and cream), high-fat meats, and tropical oils (e.g., palm oil, coconut oil, and baked products containing dairy fats, tropical oils, and shortening oils). Trans fatty acids (fatty acids in which double-bonds are in the trans configuration) have a dose-response effect on lowering HDL-C, and thus, are associated with increased risk of CVD. Significant sources of trans fatty acids in the diet include products made from partially hydrogenated oils, such as baked products, including cookies and doughnuts, French fries, or chicken fried in partially hydrogenated shortening. Animal sources of trans fatty acids include dairy products, which contain a relatively smaller amount of trans fatty acids. Other major nutrients, such as unsaturated fats, proteins, and carbohydrates, do not raise cholesterol levels significantly compared to saturated fats and trans fatty acids. The TLC diet recommends to minimize the intake of trans fatty acids, and reduce the intake of saturated fatty acids to less than 7% of total calorie intake. Moreover, intake of cholesterol is recommended to be confined to less than 200 mg per day, to maximize the amount of LDL-C lowering that can be achieved through a reduction in dietary cholesterol. Substitution of mono- and polyunsaturated fats for saturated fats, and limiting total caloric fat intake up to 10% and 20% respectively can cause a reduction in LDL-C levels ("The NCEP ATP III final report," 2002).

Based on the TLC diet recommendations, carbohydrate intake should be limited to 60% of total calories, with focus on the dietary sources from grain products, especially whole grains, vegetables, fruits, and fat-free and low-fat dairy products ("The NCEP ATP III final report," 2002). Consumption of complex carbohydrates along with high fiber diets modifies the rise in TG and fall in the HDL-C concentration (Jenkins et al., 1993; Turley et al., 1998; Vuksan et al., 2000), which were reported in diets when carbohydrates are substituted for high saturated fatty acids diets (Garg, 1998; Mensink & Katan, 1992; Turley et al., 1998).

The TLC diet recommends receiving 15% of daily calories from proteins. Dietary protein, in general, has little effect on serum LDL-C level or other lipoprotein functions. However, substituting soy protein for animal protein has been reported to reduce LDL-C concentrations ("The NCEP ATP III final report," 2002). Plant sources of protein include soybeans, pulses, nuts, grain products and vegetables, which tend to be low in saturated fats and cholesterol. Animal sources of protein should be consumed in moderation; the recommended animal protein sources which contain lower amounts of saturated fat and cholesterol include low-fat dairy products, egg whites, fish, skinless poultry, and lean meats. Choosing lean forms of animal protein and plants sources of protein are encouraged in the TLC diet guideline ("The NCEP ATP III final report," 2002).

On average, the dietary increase of 5-10 g viscous soluble fiber per day is accompanied by an approximately 5% reduction in LDL-C. Accordingly, the ATP III panel recommended the enrichment of the Therapeutic Diet by foods that provide a total of at least 5-10 g of viscous soluble fiber daily. Even higher intakes of 10-25 g per day can be beneficial. Soluble fiber can be increased by emphasizing intake of certain foods, including cereal grains, fruits, vegetables, dried beans, peas, and lentils ("The NCEP ATP III final report," 2002).

Plant sterols and stanols are phytosterols and are essential components of plant membranes that “resemble the chemical structure of animal cholesterol and carry out similar cellular functions in plants” ("Functional foods fact sheet: Plant stanols and sterols," 2003). Plant sterols and stanols are effective and safe cholesterol-lowering functional food ingredients, which are naturally present in small quantities in many fruits, vegetables, nuts, seeds, cereals, legumes, vegetable. Intestinal cholesterol absorption is reduced by the consumption of plant sterols and stanols. Subsequently, LDL-C is reduced when plant sterols & stanols mimick LDL-C and compete with cholesterol for intestinal absorption, influence cellular LDL-C metabolism within intestinal enterocytes, and enhance, receptor-mediated lipoprotein cholesterol uptake (Plat & Mensink, 2005). Plant-derived stanols/sterol esters at dosages of a 2-3 g per day lower LDL-C levels by 6-15%, with no or little change in HDL-C or TG levels (Brown et al., 1999). However, dietary intake of the recommended dosage of stanols/sterols to improve LDL-C may be challenging in conventional dietary regimens. Most diets provide a few plant sterols and stanols. Vegetarian diets may contain more plant sterols and stanols; however, the amount of diet-derived sterol and stanols may not be sufficient to have LDL-C lowering effects. The intake of dietary

supplements or foods fortified with sterols and stanols may be an option for patients with hypercholesterolemia. Further research is required to clarify the applicability, efficacy, safety, and generalizability of fortification and supplementation with plant-derived sterols and sterols to lower LDL-C cholesterol.

After TLC dietary therapy achieved a maximum reduction of LDL-C, emphasis on ways to reduce other lipid risk factors associated with MetS, such as elevated TG and low HDL-C were addressed within the TLC program. Weight reduction therapy in combination with increased regular physical activity is recommended by the TLC program, to obtain further CVD risk reduction beyond LDL-C lowering achieved through dietary changes. At all stages of diet therapy, healthcare professionals are encouraged to refer clients to registered dietitians for dietary counselling and nutritional education. The ATP III essential components of the TLC program and recommendations for ranges of macronutrients are described in Tables 2 and 3 ("The NCEP ATP III final report," 2002). Please see Tables 2 and 3, below.

Table 2. Essential components of the Therapeutic Lifestyle Changes program ("The NCEP ATP III final report," 2002)

Component of the TLC program	Recommendations
LDL-C-raising nutrients • Saturated fats and trans fatty acids • Dietary cholesterol	 Less than 7% of total calories Less than 200 mg/d
Therapeutic options for LDL-C lowering • Plant stanols/sterols • Increased viscous (soluble) fiber	 2 g per day 10-25 g per day
Total calories (energy)	Adjust total caloric intake to maintain desirable body weight/prevent weight gain
Physical activity	Include moderate exercise to expend at least 200 kcal/d

Abbreviations: LDL-C, low-density lipoprotein cholesterol.

Table 3. Macronutrient recommendations for the Therapeutic Lifestyle Changes diet ("The NCEP ATP III final report," 2002)

Nutrient	Recommended intake
Polyunsaturated fat	Up to 10% of total calories
Monounsaturated fat	Up to 20% of total calories
Total fat	25-35% of total calories*
Carbohydrate†	50-60% of total calories*
Dietary fiber	20-30 g/d
Protein	Approximately 15% of total calories

*ATP III recommends an increase of total fat to 35% of total calories and a reduction in carbohydrate to 50% for individuals with MetS. Any increase in fat intake should be in the form of either polyunsaturated or monounsaturated fat. †Carbohydrates should be derived predominantly from foods rich in complex carbohydrates including grains (especially whole grains), fruits, and vegetables.

2.1.6.2.2.2. Dietary Pulses and their Health Benefits

Pulse consumption has received insufficient attention in PCOS research. Pulses have a favourable nutritional composition, which includes an exceptionally low-glycemic index (GI; i.e., “a ranking of carbohydrates in foods, based on their effects on blood glucose levels”) (Jenkins et al., 1981). Further, pulses have high fibre, are low in fat, and are an excellent source of starch and vegetable protein (Hanefeld et al., 2004). Chronic consumption of pulses has been associated with several favourable effects, including reduced postprandial blood glucose, lower insulin levels, and enhanced lipid profile (Abeysekara et al., 2012). Consuming pulses can be beneficial in the dietary management of PCOS. The health benefits of pulse consumption are addressed in the present section.

According to the Food and Agriculture Organization of the United Nations (FAO), pulses are a type of annual leguminous crop yielding from one to 12 grains or seeds of variable size, shape and colour within a pod. The term pulse is limited to leguminous crops that are exclusively harvested for the dry grain, including dry beans, dry broad beans, dry peas, chickpeas, cow peas, pigeon peas, lentils, Bambara beans, vetches and lupins. Therefore, the term “pulse” excludes oil-seed legumes (e.g. soybean and groundnuts), which are harvested for their oil, crops

harvested green for food (green peas and green beans), and leguminous crops used for forage (e.g. seeds of clover and alfalfa) (FAO, 2014). Pulses are also referred to as pulse grains or grain legumes. Legumes are the pods or fruits of plants in the botanical family Fabaceae or Leguminosae. Legumes include dry beans, chickpeas, lentils, alfalfa, clover, lupin, green beans, and peas, peanuts, and soybean (McCrory et al., 2010).

Canada has emerged as the world's largest producer and exporter of dry peas and lentils ("Saskatchewan Pulse Growers," 2016a). Saskatchewan is "at the heart of the Canadian pulse industry". Saskatchewan grows 99% of Canada's chickpeas, 96% of lentils, and 60% of dry peas (Farm and Food Care, 2016), while most beans are grown in Alberta, Manitoba, Ontario, and Quebec. Over 80% of pulse crops are sold to international markets ("Saskatchewan Pulse Growers," 2016b). Peas are mainly exported to India, Spain, and China; a large percentage of beans are exported to the US and UK; and chickpeas are exported to Pakistan, India, Jordan, and other countries ("Pulse facts," 2016). Pulses are Canada's fifth largest crop, after wheat, canola, corn, and barley ("Saskatchewan Pulse Growers," 2016a).

Consumption of pulses is low in Canada, and the Canadian consumption reports indicate that domestic consumers do not eat pulse foods on a regular basis ("Pulse facts," 2016). For example, Canada's pea consumption is estimated at 1% of its total production ("Pulse facts," 2016). The median intake level of pulses in the US is 0.2 servings daily, and only 7.9% of American adults consume pulses on a given day (Guenther et al., 2006; Mitchell et al., 2009). In Canada only 13% consume dietary pulses on a given day, with a median intake of only about 0.5 serving daily (Ha et al., 2014). A recent analysis of data from the Food Habits of Canadians study demonstrated that energy in the diet of Canadian adults was derived mainly from bread, pasta, rice, grains, and fluid milk. Protein intake was primarily derived from meat and dairy products, while legumes, nuts, seeds, and eggs were not significant sources of protein (Johnson-Down et al., 2006).

Pulse producers seek potential domestic markets. An opportunity exists for Canadians to consume more pulses, given the availability of the food supply and that Canadians currently have low consumption rates. Pulse producers invest in research to examine opportunities to increase pulse consumption locally, including the adaptation of pulses into Canadian daily eating patterns and advertising the nutritional benefits of pulses ("Saskatchewan Pulse Growers," 2016c). The FAO 68th UN General Assembly declared 2016 to be the International Year of Pulses (IYP). The

IYP 2016 was recommended to increase public awareness of the nutritional benefits of pulses, as part of sustainable food production to improve global food security and nutrition. A second goal of the nomination was to help the UN implement its 2030 Agenda for Sustainable Development, which aims to eliminate global poverty and hunger (FAO, 2016).

A growing body of evidence supports the positive effects of pulses on diet quality. Pulses are the leading source of dry vegetable protein, an excellent source of fiber, and a significant source of vitamins and minerals, such as iron, zinc, folate, calcium, and magnesium. Pulses contain approximately double the protein content of some grains, and double the amount found in whole grain cereals, such as wheat, and three times the protein content of rice. Pulses are a good source of potassium and have a low sodium content. Pulses are gluten-free, cholesterol-free, low in fat, have a low GI, and high protein quality, which can considerably fulfil the essential amino acid requirement of human diet (Iqbal et al., 2006; Mudryj et al., 2014). Consuming half a cup of pulses has been shown to enhance the overall diet quality (Mudryj et al., 2014). Consumption of pulses has been shown to increase the intake of fiber, protein, carbohydrate, folate, magnesium, iron and zinc (Mitchell et al., 2009). Pulses can mediate satiety and weight management, due to high fiber content, moderate energy density, and complex and slowly digestible carbohydrates (McCrory et al., 2010). Pulses have the potential to improve diet quality as indicated by the Healthy Eating Index, a numerical scale to assess diet quality of specific populations on a scale of 1 to 100) (Garriguet, 2009). In Health Canada's resource: Eating Well with Canada's Food Guide (EWCFG), pulses are considered nutritious foods and are recommended as a part of a healthy diet. The EWCFG classified pulses as a meat alternative (Health Canada, 2007). Interestingly, the United States Department of Agriculture's MyPyramid food guidance system includes pulses in both the Meat and Beans group as well as the Vegetable group (USDA Center for Nutrition and Policy Promotion, 2016). Contrary to the United States Department of Agriculture 2005 Dietary Guidelines, which recommends consumption of 3 cups of legumes per week for most adults (USDA Center for Nutrition and Policy Promotion, 2016), Health Canada does not have a recommended amount of legume or pulse consumption for adults. Rather, Health Canada recommends "having meat alternatives such as beans, lentils…often". The EWCFG defines one serving of pulses as 175 ml (3/4 cup) (Health Canada, 2007). See Table 4 for some nutrient values of common pulses (*Health Canada. Nutrient Value of Some Common Foods*, 2008).

Table 4. Nutrients per 175ml (3/4 cup) of pulses (Health Canada. Nutrient Value of Some Common Foods, 2008)

Measure	White beans (Canned)	Chickpeas (Canned)	Lentils (Boiled)	Split peas (Boiled)
Weight (g)	194	178	146	145
Energy (kcal)	227	211	170	171
Protein (g)	14	9	13	12
Carbohydrate (g)	43	40	29	31
Dietary fiber (g)	9.3	7.8	6.2	4.2
Total fat (g)	1	2	1	1
Iron (mg)	5.8	2.4	4.9	1.9

The favourable nutrient profile of pulse foods has led to research findings related to their potential health benefits in the dietary management of chronic diseases. Specifically, pulses have demonstrated health benefits in CVD, DM2, weight maintenance, and cancer prevention (Abeysekara et al., 2012; Ha et al., 2014; McCrory et al., 2010; Mudryj et al., 2014). Pulses have received particular attention for their ability to reduce the risk of CVD (Ha et al., 2014). Pulse consumption improves serum lipid profiles and positively affects some CVD risk factors, such as BP, LDL-C, platelet activity, and inflammation (Ha et al., 2014; Jenkins et al., 2003). These observations may be, in part, due to the remarkable antioxidant capacity of pulses, attributed to tannins, flavonoids, polyphenols, phytates, and saponins. Pulses may inhibit the oxidation of LDL-C and modulate total cholesterol levels, and thus, improve arterial health and reduce cardiometabolic risk factors. Approximately 60% of the fiber in pulses is insoluble, and 31% is soluble, both of which can bind to, and prevent the reabsorption of bile acids in the intestine (Abeysekara et al., 2012). Also, fermentation of fiber in the colon can produce short-chain fatty acids and improve in the gut microbiota composition, which contributes to the decreased hepatic synthesis of cholesterol and glucose (Chibbar et al., 2010; Kishimoto et al., 1995; van Bennekum et al., 2005). Results of a meta-analysis of eleven clinical trials evaluating effects of non-soy legume interventions with serum lipids revealed that regular pulse consumption lowered LDL-C, with no significant effects on HDL-C levels (Anderson & Major, 2002). Researchers attributed the hypocholesterolemic effects of pulses to be multi-factorial but ranked their soluble fiber content, vegetable protein and oligosaccharides as the top three important traits. The positive effects of pulse consumption on total cholesterol and LDL-C in individuals at risk for MetS are further supported by data originating from our research group. Two months of a pulse-based diet

(i.e., 2 servings per day of pulses, or 150 g/d dry weight) reduced total cholesterol and LDL-C-cholesterol by 8%, and improved body composition (i.e., reduced body fat%) in men and women at risk for MetS of 50 years of age and older, which is an age-group with an increased risk of CVD (Abeysekara et al., 2012). Results of a recent systematic review and meta-analysis on 26 RCTs showed a modest reduction, −0.17 mmol/L (95% CI, −0.25 to −0.09) of LDL-C concentration following the consumption of a median dose (130 g/d dry weight) of pulses (Ha et al., 2014). Similarly, results of a meta-analysis of 10 RCTs showed −0.2 mmol/L (95% CI, −0.3 to −1.2) decrease in LDL-C levels following the consumption of legume-rich diets (Bazzano et al., 2011). However, the assessed RCTs were limited by methodological and intervention duration heterogeneity. Large-scale, long-term RCTs are required to confirm LDL-C lowering findings. In addition to improving blood lipid concentrations, pulses have been shown to improve BP. Jayalath et al. performed a meta-analysis on 8 isocaloric trials (n=554 participants) and showed pulse consumption (~162 gram/day) over 10 weeks reduced mean arterial BP by -0.75 mmHg in middle-aged subjects with or without hypertension (Jayalath et al., 2014). Pulses can confer BP-lowering effects by increasing dietary intakes of low-GI foods, dietary fiber, plant protein, potassium, and magnesium, as well as decreasing sodium intake through pre-established mechanisms (Aburto et al., 2013; Altorf–van der Kuil et al., 2010; Jee et al., 2002; Mudryj et al., 2014; Tielemans et al., 2013).

Pulse consumption has positive effects on the control of IGT and DM2. Most pulses have an exceptionally low GI, which is approximately half of that reported for commonly eaten carbohydrate-rich foods. For example, using a reference value of GI = 100 for 50 g glucose, the GIs of 150 g green lentils, chickpeas, and kidney beans are 30±4, 28±6, and 28±4 respectively, compared to weight-matched long-grain white rice with a GI of 56±2 (*Health Canada. Nutrient Value of Some Common Foods*, 2008). Chronic consumption of pulses has been shown to modulate glucose and insulin responses of patients with DM2 and improve weight control, by reducing appetite and energy intake (McCrory et al., 2010). However, the isolated effect of pulses on glycemic response in diabetes is less clear. Many trials have classified pulses as a whole grain, and thus, interpretation of the isolated positive effects of pulses is difficult to ascertain (Jacobs et al., 1998; Jang et al., 2001). Further, the reported low intake and the duration of pulse consumption were likely too small to claim pulse consumption reflected a genuine effect on diabetes control. Nevertheless, “there is strong evidence to suggest eating a variety of whole

grains and legumes is beneficial in the treatment and management of diabetes" (Venn & Mann, 2004). Theoretically, some unprocessed whole grains share the nutritional profile of pulses including a high fiber content and lower GI. However, it is important to investigate the isolated effects of pulses on diabetes management. Results of a systematic review and meta-analysis of 41 RCTs on the effects of pulse interventions on glycemic control in adults with and without DM2, were in favour of pulse consumption (Sievenpiper et al., 2009). Results of the meta-analysis were stratified according to the effects of pulses alone, pulses as a component of low-GI diets, or as a component of high-fibre diets on glycemic control measures. Eleven trials from the meta-analysis revealed that pulses alone lowered the FPG −0.82 SMD (95%CI −1.36 to −0.27) and fasting insulin −0.49 SMD (95% CI −0.93 to −0.04). Nineteen trials showed that pulses as a component of low-GI diets lowered blood glycosylated proteins, measured as HbA1c or fructosamine −0.28 SMD (95% CI −0.42 to −0.14). The remaining eleven trials examined pulses as a component of high-fibre diets and showed lowered FPG −0.32 SMD (95% CI −0.49 to −0.15) and glycosylated proteins −0.27 SMD (95%CI −0.45 to −0.09). However, there was high heterogeneity in results, and researchers were unable to conclude the optimal type and dose of pulses that would improve DM2 outcomes (Sievenpiper et al., 2009).

Pulses are rich sources of dietary fiber. Fermentation of non-digestible dietary fiber in cecum and colon leads to the exogenous synthesis of short-chain fatty acids (SCFA) and branch-chain amino acids (valine, isoleucine, leucine) derivatives of gut microbiota. The microbial conversion of fiber into functionally relevant metabolites has been proposed to improve host gut microbiota composition, immunity, and metabolism, protect against metabolic diseases and improve IR. SCFAs, in particular, acetate, propionate, and butyrate, are critical bacterial metabolites which can promote host-microbial symbiosis as effective prebiotics. SCFAs are minor energy and essential signalling molecules, which are implicated in improving insulin sensitivity, glucose tolerance, and lipid metabolism; regulation of blood-brain barrier permeability; regulation of satiety; regulation of hepatic and intestinal gluconeogenesis; regulation of cytokines expression; regulation of vaginal physiological environment; and potential improvement of mood, depression, and anxiety (Koh et al., 2016).

Consumption of pulses, as nutritionally balanced foods with moderate energy density, has been recommended to reduce energy intake, increase satiety, and control obesity (Li et al., 2014; Marinangeli & Jones, 2012; McCrory et al., 2010; Mollard et al., 2012). Pulses can induce

satiety via their favourable macronutrient profile. Pulses are low in fat, high in protein and fiber, contain a high level of complex carbohydrate that is slowly digested, amylase inhibitors and phytochemicals(McCrory et al., 2010). Aside from weight loss, coupling pulse consumption with energy restriction has been shown to lower BP and dyslipidemia and decrease inflammation (Hermsdorff et al., 2011).

Gastrointestinal symptoms and poor dietary compliance have been reported with the diets comprised of high quantities of pulses. Upset stomach, flatulence, bloating, diarrhoea, and increased stool frequency are among the reported adverse effects. Of note, gas production is a normal physiological process that, to some extent, aids in digestion by softening and helping to move stool through the colon (Hellendoorn, 1979). There is evidence that compliance to dietary pulses may be improved with regular consumption of pulse foods (Fleming et al., 1985; O'Donnell & Fleming, 1984). Chronic consumption of dietary pulses can improve gut microflora composition, with mechanisms associated with the intestinal fermentation of dietary fiber in cecum and colon, which could, in turn, influence gastrointestinal symptoms and improve the overall tolerance to pulse consumption (Roberfroid & Slavin, 2000; Swennen et al., 2006). Also, there are practices to remove galacto-oligosaccharide sugars, the primary flatulence-producing compounds in legumes), including overnight soaking, followed by pressure cooking, discarding both soaking and cooking liquids, irradiation, germination, and enzyme treatments (Minorsky, 2003). Paradoxically, pulse foods are consumed in many cultures without reports of adverse effects that would limit consumption (McCrory et al., 2010).

Pulses contain nutritive and non-nutritive components and have potential anti-carcinogenic effects (Mathers, 2002; Wiseman, 2008). The theories surrounding the mechanism for cancer protection from pulse-containing-diets are multi-dimensional (Aune et al., 2009; Mathers, 2002; Wiseman, 2008). Pulses contain a rich variety of components, which may help to reduce cancer risk if consumed in sufficient quantities (Mathers, 2002). Nutrients such as resistant starch, non-starch polysaccharides, oligosaccharides, folate, selenium, zinc and bioactive macroconstituents such as protease inhibitors, phytosterols, lectins, phytochemicals, saponins, tannins, and phytates are purported to have anti-carcinogenic effects. The anti-carcinogenic effects of pulses have been attributed to mechanisms associated with digestion, fermentation by gut microbiota, repair of DNA damage, and apoptosis of damaged cells (Mathers, 2002). Of note, the proposed nutritive components may only have a beneficial effect in

the case of deficiency. Therefore, a food-first approach is warranted (Duffield-Lillico et al., 2003; Wiseman, 2008).

The Second World Cancer Research Fund/American Institute for Cancer Research Expert Report recommends the consumption of "relatively unprocessed cereals (grains) and/or pulses, and other foods that are natural sources of dietary fiber, to contribute to a population average daily consumption of 25 grams of non-starch polysaccharides". The inclusion of mostly plant-based foods in the diet is recommended. Red meat consumption is recommended to be limited to less than 11 ounces weekly with little, if any, meat processing. A study assessing cancer risk in Uruguay conducted a multi-site case-control on 3539 cancer cases and 2032 controls to explore the association between legume intake and cancer risk (Aune et al., 2009). Legume intake was tiered into low, medium and high for each test group. Higher intake of legumes was associated with a decreased risk of several cancers including those of the upper digestive tract (odds ratio [OR] = 0.50, 95% CI: 0.40–0.63), stomach (OR = 0.69, 95% CI: 0.49–0.97), colorectum (OR = 0.43, 95% CI: 0.32–0.59), and kidney (OR = 0.41, 95% CI: 0.24–0.71; all $P<0.05$). Comparisons between low and high intakes of legumes indicated that higher bean consumption was associated with lower risk of oral cavity/pharynx (OR = 0.54, 95% CI: 0.37–0.79), oesophagal (OR = 0.59, 95% CI: 0.40–0.86), larynx (OR = 0.50, 95% CI: 0.34–0.73), upper digestive (OR = 0.53, 95% CI: 0.41–0.68), stomach (OR = 0.54, 95% CI: 0.37–0.80), colorectum (OR = 0.44, 95% CI: 0.31– 0.61), and bladder cancers (all $P<0.006$). Lentil intake had similar results. However, unlike bean intake, no significant associations were observed for bladder (OR = 1.06, 95% CI: 0.71–1.57; P= 0.81) or stomach cancers (OR = 0.84, 95% CI: 0.57–1.23; P=0.33), but, a reduced odds ratio was reported for kidney cancer (OR = 0.46, 95% CI: 0.25– 0.82; P=0.01). No significant associations with cancers of the lung, breast or prostate were observed for any of the legume-based diets. Hartman et al. detected an improvement in biomarkers of inflammation when comparing usual diets to both a high legume-containing diet and a healthy American diet. No difference was found between the legume-diet and the healthy American diet for inflammatory biomarkers; however, results were possibly confounded given the heterogeneity in the degree of weight maintenance among subjects (Hartman et al., 2010).

Pulses in the diet can improve the overall diet quality and reduce the risk of several chronic diseases. Recent research supports the notion that pulses may have further positive effects on health (Mudryj et al., 2014). The most optimal dietary composition that will mediate

the reproductive, metabolic, and psychological improvements in women with PCOS is unclear. No published research addresses the potential benefits of pulse consumption in the PCOS population. The optimal dietary composition needs to be further explored to evaluate the potential to enhance outcomes for women with PCOS. It has been proposed that a pulse-based diet can improve the metabolic and reproductive outcomes and the overall quality of life and lifestyle of women with PCOS.

2.1.6.3. Physical Activity

Physical exercise has been accepted as an integral component of lifestyle therapy in the management of PCOS. Physical activity can improve the clinical manifestations, metabolic profile, and quality of life of women with PCOS (Harrison et al., 2011). Regular physical activity has been shown to decrease the risk of chronic diseases including CVD and DM2, and the overall prognosis of PCOS (Brown et al., 2009; J. A. Hawley, 2004; Vigorito et al., 2007). Exercise positively affects mood, anxiety, body image, depression, and overall psychological well-being of women with PCOS (Liao et al., 2008; Ramos et al., 2016). Exercise is a significant predictor of successful and sustainable weight maintenance in women with PCOS (Harrison et al., 2011; J. A. Hawley, 2004; Vigorito et al., 2007).

Currently, there is no specific guideline for the amount or type of exercise that is recommended for women with PCOS. Exercise recommendations for women with PCOS are generally based on the Canadian and American Physical Activity Guidelines (Tremblay et al., 2011; "The Department of Health and Human Services guidelines for exercise ", 2008). These guidelines have been established for all North American adults, based on evidence regarding the minimum amount of aerobic activity that is necessary to produce substantial health benefits. The guidelines support the recommendation that physical activity should not be less than 150 minutes total per week of moderate-intensity exercise, or 75 minutes of vigorous-intensity activity per week, or an equivalent combination of moderate- and vigorous-intensity physical activity. Activities such as brisk walking, water aerobics, ballroom and line dancing, tennis (doubles), general gardening, or sports in which one catches and throws (e.g., volleyball, baseball, and softball) are all considered to reflect moderate-intensity physical activity. Vigorous intensity activities include racewalking, aerobic dance, biking faster than 10 miles per hour, hiking uphill, heavy gardening, jumping rope, martial arts (e.g., Karate), jogging and running, swimming fast laps, tennis (singles), and any sports that involve a significant amount of running (e.g.,

basketball, field hockey, and soccer). Activities that increase muscle mass, improve balance and preserve bone, such as weight training using resistance bands, weight-bearing aerobics, and heavy gardening, are recommended to be included 2 or more days per week (Tremblay et al., 2011; "The Department of Health and Human Services guidelines for exercise ", 2008).

The benefits of exercise training and its recommendation as a cornerstone of PCOS management are well-documented. However, few well-controlled studies have evaluated the benefits of exercise training on PCOS (Bruner et al., 2006; Hoeger et al., 2004; Norman et al., 2002). Vigorito et al. evaluated the effects of a three-month structured exercise training program on the cardiopulmonary functional capacity of young women with PCOS. Subjects were randomly assigned to either the trained group, who underwent a three-month structured aerobic exercise training program for 3 times per week, or the untrained group, who did not exercise. Exercise regimen comprised of a 5-minutes warm-up, followed by a 30-minutes biking with the target of 60–70% of the maximal oxygen consumption (VO_{2max}), and a 5-min cool-down. After 3 months, trained women showed significant improvement in peak oxygen consumption, maximal workload, insulin sensitivity, BP, CRP, and BMI levels compared with an untrained group (Vigorito et al., 2007). Structured exercise training for 6-months has been shown to be superior to CHC therapy, comprised of 3 mg drospirenone plus 30 μg ethinyl estradiol, in improving the CVD risk factors of endothelial dysfunction, including intima-media thickness and flow-mediated dilation. The observation might be attributed to the positive effects of physical activity on cardiopulmonary health and function (Orio et al., 2016). Exercise has been shown to improve the lipid profile in women with PCOS. Results of a 8–12-week ramp-up followed by a 12-week moderate-intensity exercise program (16–24 weeks total, ~228 minutes per week at 40–60% of the $VO_{2max)}$ on sedentary women with PCOS demonstrated the exercise, without significant weight loss, improved HDL-C, VLDL-C/chylomicrons, and TG levels in women who exercised compared with non-exercisers (Brown et al., 2009). Results of a prospective cohort of obese women with PCOS and anovulatory infertility who underwent supervised treadmill sessions for 24 weeks showed greater increase in insulin sensitivity compared with women in a hypocaloric high-protein diet group. Although diet induced greater weight loss, exercise resulted in greater improvement in insulin sensitivity (Palomba et al., 2008). Researchers concluded that exercise might improve IR in PCOS by a mechanism other than weight loss.

Exercise training can improve insulin sensitivity by two major mechanisms. First, exercise can improve insulin sensitivity by modulating visceral adipose tissue, which is a metabolically active endocrine organ with a well-established contribution to the development of IR (Després et al., 2001; Lord et al., 2006). Second, physical activity can improve insulin sensitivity by the enhancement of muscle metabolism at the cellular level. Skeletal muscle is the main site of glucose deposition implicated in IR. Physical exercise mediates the expression and the activity of proteins involved in insulin signal transduction in skeletal muscles (John A Hawley, 2004). Exercise may enhance insulin sensitivity by affecting the oxidative capacity of skeletal muscle (Kirk et al., 2003). A few other mechanisms have been proposed by which exercise may improve PCOS outcomes. Physical activity may modulate exaggerated sympathetic nerve activity in women with PCOS (Lindmark et al., 2003; Stener-Victorin et al., 2009). Physical exercise can mediate an improvement of the psychological well-being of women with PCOS by improving body image, self-esteem, depression, and anxiety (Brown et al., 2009; Hoeger, 2008; Thomson et al., 2009).

Physical activity has been attributed to positive changes in the mental health of women with PCOS (Conte et al., 2015). However, there is little evidence to support the isolated effects of exercise on psychological well-being in PCOS. A small nonrandomized study of a 6-month self-directed brisk walking program for overweight and obese women with PCOS demonstrated decreased body image distress scores (Liao et al., 2008). Improved scores on quality of life and depression have been reported following a 20-week clinical trial of energy-restricted diet combined with exercise (i.e., aerobic only or combined aerobic-resistance) in overweight women with PCOS (Thomson et al., 2010). Most recently, results of a 16-week structured resistance exercise training program showed modest improvements in the functional capacity, vitality, social aspects, and mental health of women with PCOS (Ramos et al., 2016). Resistance training was reported to improve depression and anxiety, as well as to enhance the indices of female sexual function including sexual desire, sexual excitement, and lubrication in women with PCOS (Lara et al., 2015). Banting et al. showed that active women with PCOS had fewer depressive symptoms compared with inactive counterparts. However, active women with PCOS still reported more depressive symptoms than active women without PCOS (Banting et al., 2014). Further high-quality research is required to clarify the relationship between physical activity and mental health outcomes in PCOS.

Few studies have examined the isolated effects of exercise training on reproductive outcomes in PCOS. Results of a 14-week medically supervised fitness program showed a 46% increase in the conception rate of women with PCOS who desired pregnancy, independent of changes in body composition or eating behaviour before pregnancy occurrence (Aubuchon et al., 2009). A 3-month aerobic training program was reported to restore regular menstrual cyclicity in 60% of women with PCOS who were previously diagnosed with anovulatory infertility (Palomba et al., 2008; Vigorito et al., 2007). Similarly, a 24-week program of dieting or aerobic exercise improved menstrual cyclicity and ovulation in overweight women with PCOS, with no differences in the quantity or lengths of menstrual cycles between treatments. However, the frequency of menses and rates of ovulation were higher in the exercising group. The exercising group exhibited a trend toward higher pregnancy rates and greater improvements in hormonal profile compared with the dieting group (Palomba et al., 2008). Results of a small nonrandomized study of progressive resistance training program for 16 weeks showed improvements in total testosterone, SHBG, and menstrual cyclicity in women with PCOS (Kogure et al., 2016).

Physical activity appears to improve cardiopulmonary function, body composition, IR, hyperandrogenism, menstrual cyclicity, ovulation, quality of life, and psychological well-being of women with PCOS. No guideline has been established for the amount and type of physical activity that can improve the outcomes of PCOS. Additional research is needed to determine the optimal dose, type, intensity, and frequency of exercise that would improve multiple PCOS outcomes.

2.1.6.4. Combined Dietary and Exercise Lifestyle Interventions

There are surprisingly limited and mixed results on the short- and long-term impacts of combined dietary, exercise, and behavioural interventions on PCOS outcomes. Moran et al. conducted a systematic review on 21 RCTs to examine the effect of lifestyle interventions comprising dietary, exercise, behavioural management (separately/or combination of all three) compared with minimal treatment for women with PCOS (Moran et al., 2011). The outcomes measures assessed were reproductive (i.e., pregnancy, menstrual cyclicity, ovulation, total testosterone levels, SHBG levels, and clinical hyperandrogenism), metabolic (i.e., OGTT, fasting glucose and insulin levels, and lipid profile), and anthropometric (i.e., BMI, WC, android body fat distribution, and waist to hip ratio). Out of 21 articles retrieved, 13 were excluded due to lack

of a control group, not being an RCT or no access to a full-text article. Six studies were included in the review (Badawy & Elnashar, 2011; Guzick et al., 1994; Hoeger et al., 2008; Stener-Victorin et al., 2009; Vigorito et al., 2007). The studies were relatively small, with samples sizes of 11-90 participants, and relatively high drop-out rates in relation to the intervention period: 0% drop out at 12 weeks (Hoeger et al., 2008; Vigorito et al., 2007), 18% at 24 weeks (Stener-Victorin et al., 2009), 25% at 24 weeks, 35% at 48 weeks (Hoeger, 2008), and 43%-46% at 16 weeks (Badawy & Elnashar, 2011; Guzick et al., 1994). Researchers concluded an overall positive effect of lifestyle intervention on anthropometric measurements, including a 7% weight loss, with no significant improvements on reproductive or metabolic measures in women with PCOS undergoing lifestyle interventions, compared with minimal treatment. However, it is likely that the comparisons between studies were confounded by several factors, including variable clinical phenotypes and life stages of subjects, high drop-out rates of participants, low compliance to intervention protocols, lack of statistical power to detect a true effect if it existed, and small sample size.

Long-term adherence to lifestyle interventions is difficult. Results of a systematic review and meta-analysis of RCTs compared the effects of a combined diet and exercise intervention versus diet-only intervention on both long-term and short-term weight loss success in women with PCOS. The combination of diet and exercise showed greater weight loss −0.25 SMD (95% CI −0.36 to −0.14). However, in studies lasting 2 years or longer, both diet plus exercise and diet-only programs were associated with partial long-term weight regain (Wu et al., 2009). Carefully monitored long-term PCOS studies with control subjects and adequate power to observe an effect are recommended to discern the impacts of combined lifestyle intervention on PCOS outcomes in short- and long-term evaluations/observations. Future research should address the compliance, attrition rate, and barriers to lifestyle modifications in PCOS. Attempts should be made to develop effective strategies for more successful engagement of women with PCOS with lifestyle modification that is sustainable.

2.2. Significance of the Review and Relevance of the Study Objectives

The PCOS is a complex endocrine condition in women of reproductive age, associated with metabolic and reproductive compilations and compromised HRQoL. Lifestyle modifications are recommended as the first-line strategy in the management of PCOS. The

optimal diet composition that would uniformly be beneficial for women with PCOS is not known.

None of the reviewed studies focused on the implications of a pulse-rich dietary intervention on PCOS health outcomes. Instead, lifestyle modifications focused on the effects that weight loss have through short-term calorie restricted dietary interventions in PCOS, with minimal emphasis on a PCOS-optimized dietary composition. It is proposed that a pulse-based diet (i.e. a low GI diet including dry peas, beans, lentils, and chickpeas) can positively affect the metabolic, reproductive, and HRQoL outcome associated with PCOS. Pulses have a favourable nutritional composition, are high in fiber, have a low GI, are low in fat, and contain high-quality protein. Chronic consumption of pulses in non-PCOS populations has been associated with positive metabolic effects, including reduced postprandial blood glucose and insulin concentrations, and a more favourable lipid profile. Therefore, the influence of a pulse-based diet needs to be studied on multiple physiologic, metabolic, reproductive, and HRQoL indices in women with PCOS. As complementary components of a comprehensive multidimensional lifestyle intervention program, the proposed intervention will profit from the additional benefits of exercise, education and healthcare counselling about PCOS and lifestyle modification. To isolate the effect of diet and healthcare counselling, exercise was prescribed as a portion of ethical Good Clinical Practice Guidelines. Extended follow-up times will be used, to better understand the long-term effects of the pulse-based diet and aerobic exercise intervention on PCOS outcomes. Prior to presenting the results of the proposed lifestyle change program, the prevalence rate and individual components of MetS, impaired glucose control, DM2, and risk factors for CVD were evaluated in women with PCOS (Objective i). The results have been presented in Chapter 3.

CHAPTER 3

CHARACTERISING THE RISK OF CARDIOVASCULAR DISEASE AND TYPE 2 DIABETES IN WOMEN WITH PCOS

Kazemi M, Pierson RA, Lujan ME, Chilibeck PD, McBreairty LE, Gordon JJ, Serrao SB, Zello GA, Chizen DR.

A comprehensive evaluation of type 2 diabetes and cardiovascular disease risk factors in polycystic ovary syndrome*

**format and structure of the original manuscript have been revised to provide a better flow within the thesis and be consistent with the format of previous chapters*

In this chapter, the prevalence and characteristics of MetS, glucoregulation, DM2, and risk factors for CVD in Canadian women with PCOS is examined.

Abstract

Background and objectives: PCOS is associated with profound metabolic disruptions. Limited research has comprehensively examined the contribution of PCOS-related alterations to MetS using the latest recommendations to diagnose PCOS and polycystic ovaries. The objective of the present work was to examine the prevalence and characteristics of MetS, glucoregulation, DM2, and risk factors for CVD in Canadian women with PCOS.

Methods: Data were pooled from 2 prospective and cross-sectional studies on 237 women with PCOS (AE-PCOS Society criteria) and 42 (non-PCOS) controls (18-36y). Clinical and biochemical measures of MetS (International Diabetes Federation consensus definition) and DM2 (Canadian Diabetic Association criteria), and CVD risk factors (AE-PCOS Society statement) were evaluated.

Results: The prevalence of MetS was 29.5% in the PCOS group, approximately 6-fold higher than age-matched controls ($P<0.001$), with worse glucose control, acanthosis nigricans, BMI, systolic blood pressure (SBP), TG, HDL-C, LDL-C, TC/HDL-C, and hsCRP levels ($P<0.03$). Women with PCOS and MetS exhibited exacerbated levels of insulin and glucose

responses to an oral glucose tolerance test, TC, TC/HDL-C ratio, hirsutism, and acanthosis nigricans than BMI-matched counterparts without MetS (P≤0.05).

Conclusion: Reproductive-age women with PCOS have a high prevalence of MetS and exhibit adverse cardiometabolic and DM2 risk profiles. MetS exacerbates hyperandrogenicity, dyslipidemia, and glucose control in PCOS, possibly by aggravating inherent IR, with concomitant obesity having synergistic effects.

Keywords: Polycystic ovary syndrome, obesity, insulin, cardiovascular disease, glucose intolerance

3.1. Introduction

PCOS is the most common hyperandrogenic disorder and cause of anovulatory infertility among reproductive-age women worldwide, with a prevalence of up to 18% (Carmina & Lobo, 1999; March et al., 2010). PCOS is primarily characterized by hyperandrogenism, ovulatory dysfunction, and polycystic appearing ovaries on ultrasonographic examination and associated clinical manifestations, including oligo-amenorrhea, hirsutism, and infertility (Azziz et al., 2006). Aside from reproductive complications, PCOS is associated with profound metabolic disruptions which represent important contributors to increased rates of long-term morbidity and mortality (Carmina & Lobo, 1999; E Diamanti-Kandarakis & A Dunaif, 2012; Wild et al., 2010). Women with PCOS exhibit IR and compensatory hyperinsulinemia, with associated metabolic disruptions, including impaired glucose metabolism, dyslipidemia, hypertension, and abdominal obesity. These symptoms are key components of MetS, which is defined as a complex of five interrelated risk factors for CVD and DM2. The prevalence rate of MetS in women with PCOS is estimated at 8.2-43% and varies depending upon the chosen diagnostic criteria, as well as regional, lifestyle, and ethnic variations (Apridonidze et al., 2005; Carmina et al., 2006; Glueck et al., 2003). Previous research has used older criteria for diagnosing polycystic ovarian morphology (PCOM), using a threshold of ≥12 follicles per ovary measuring 2-9 mm in diameter which appear to be obsolete after the introduction of advanced imaging technology that affords maximal resolution of ovarian follicles. No study has previously evaluated the metabolic derangements of PCOS using the recently introduced thresholds (≥25 AFC)) for PCOM. Further, the extended variability in the prevalence rates of MetS may in the previous reports be due, in part, to observations of small sample sizes, marked heterogeneity in diagnostic techniques

employed, and the fact that older or less accurate criteria were used to diagnose PCOS and MetS, and variations in ages at diagnosis. The adverse effects of obesity on MetS have been exhaustively documented (Alberti et al., 2009; Grundy, 2008; Kahn et al., 2006; Vendrell et al., 2004). Over the past three decades the prevalence of obesity has increased steadily in Canada, and is predicted to continue to increase (Twells et al., 2014). To our knowledge, there has been no coordinated effort to address the contribution of MetS and obesity to PCOS in Canada. Therefore, in the present study, we used a rigorous and comprehensive approach to diagnose PCOS using multiple clinical, biochemical, and ultrasonographic tests. Further, a precise methodology was implemented to determine the presence of MetS in PCOS population. The severity of metabolic derangements was evaluated in the affected women using multiple outcome measures across age and BMI categories.

With MetS the risk of CVD doubles (Grundy, 2008) and the risk of DM increases 5-fold (Alberti et al., 2006). A high prevalence of CVD and DM2 risk factors in PCOS is associated with an increased risk of early-onset CVD and DM2. Early recognition and prevention of PCOS-related metabolic sequelae can be attained through screening, diagnosis, and interventions ("American College of Obstetricians and Gynecologists. Polycystic ovary syndrome. ACOG Practice Bulletin no. 108," 2009; Legro et al., 2013). Identification of concomitant metabolic abnormalities may assist healthcare professionals and patients to adopt feasible therapeutic modalities for the management and prognosis of PCOS. In the present study, we re-assessed the prevalence of MetS and risk factors for DM2 and CVD in a relatively large group of women in reproductive ages, using a carefully-defined PCOS population, advanced ultrasound technology, and rigorous outcome measures. We hypothesized that in women with PCOS 1) the prevalence of MetS, which can contribute to increased risk of CVD and DM2, is high during their reproductive-years; and 2) that MetS would exacerbate clinical and biochemical outcomes associated with PCOS. Our primary objective was to examine the prevalence rate and individual components of MetS, impaired glucose control, DM2, and risk factors for CVD in young women with PCOS. Secondly, we investigated the relevance of MetS to various clinical and biochemical characteristics in women who were diagnosed with PCOS.

3.2. Materials and Methods

3.2.1. Participants and Setting

Data were pooled from two separate prospective and cross-sectional studies on 279 participants. Women were recruited by local newspaper advertisements, online bulletin posts, flyers available in physician offices, and placement of posters at the University of Saskatchewan and Royal University Hospital in Saskatoon, Canada between 2006 and 2016 for studies designed to evaluate ovarian morphology and the effects of lifestyle interventions in women with PCOS. The present study evaluated the baseline characteristics of women recruited for the purposed of the two abovementioned studies. Women aged 18-36 years that had any or a combination of irregular periods, unwanted facial and body hair growth, and/or a family history of DM2 were included and assessed to confirm the diagnosis of PCOS. Women with the following conditions were excluded: used hormonal and/or fertility medications within the last 3 months of recruitment; women who took anti-seizure or anti-psychotic medications which are known to induce IR and PCO; women diagnosed with untreated hyperprolactinemia or thyroid disease; or, excessive adrenal androgen production confirmed by a diagnosis of congenital adrenal hyperplasia, Cushing's syndrome, or an adrenal tumor. Age-matched healthy women were recruited for the control group and were assessed to confirm the absence of PCOS. A subset of participants evaluated in the present study was used to explore the prevalence of PCOS using updated sonographic thresholds for polycystic ovaries (Clark et al., 2014). PCOS was diagnosed according to the AE-PCOS Society criteria (Azziz et al., 2006), which also complied with the newest AE-PCOS Society recommendations for polycystic ovarian morphology as described elsewhere (Dewailly et al., 2014). The 2005 International Diabetes Federation consensus statement in collaboration with American Heart Association/National Heart, Lung, and Blood Institute was applied for the diagnosis of MetS. The presence of at least three of the following five metabolic risk factors constituted a diagnosis of MetS: FPG levels ≥5.6 mmol/L; TG levels ≥1.7 mmol/L; HDL-C levels ≤1.3 mmol/L; elevated BP ≥130/85 mmHg; and, abdominal obesity (using ethno-specific cut-points) (Alberti et al., 2009). Study protocols were approved by the University of Saskatchewan Biomedical Research Ethics Board. Participants provided informed written consent to be enrolled in the studies. All procedures were conducted in compliance with the Declaration of Helsinki principles and the Guidelines for Good Clinical Practice in research.

3.2.2. Study Procedures

A standardized medical and physical examination was performed to obtain demographic, anthropometric, physiologic, and gynecologic measures, familial history of diseases, and

menstruation patterns as described previously (Clark et al., 2014; McBreairty et al., 2017). The presence and degree of acne vulgaris were recorded. Acanthosis nigricans was observed as velvety, dark-pigmented, hyperkeratotic areas affecting areas of flexural skin, including neck, axillae, under the breast, abdomen, and groin. Ovarian ultrasound scans were performed as described previously (Clark et al., 2014; McBreairty et al., 2017).

Women were screened for glucose intolerance and IR using a standard 75-g OGTT, as described elsewhere (Goldenberg & Punthakee, 2013; McBreairty et al., 2017). Fasting blood samples were collected between 8:00 and 9:00 am, following a 10-12 hour overnight fast.

3.2.3. Hormonal and Biochemical Analyses

Hormonal and biochemical metabolites evaluated were testosterone, FSH, LH, SHBG, total cholesterol, LDL-C, HDL-C, TC/HDL-C ratio, TG, hsCRP, and HbA1c. Estradiol, progesterone, prolactin, 17-hydroxyprogesterone, dehydroepiandrosterone sulfate, and thyroid hormones were evaluated to exclude the endocrinopathies that mimicked the clinical symptoms and signs of PCOS. Fasting blood samples were taken when dominant follicles and corpora lutea were sonographically absent on days 1-5 of regular, predictable cycle lengths, or on random days for women with irregular/absent periods. All samples were analyzed immediately, or during the first week of the collection after freezing at -80 °C.

Concentrations of plasma insulin concentrations and serum total testosterone (by solid-phase, enzyme-labeled, competitive chemiluminescent immunoassay) were measured on Immulite 2000 Systems Analyzers (Tarrytown, USA) with Roche kits (Basel, Switzerland). The remaining metabolites were analyzed using Roche Cobas Modular Analyzers and Roche kits, with HbA1c analyzed using the turbidimetric inhibition immunoassay, plasma glucose using the hexokinase method, serum SHBG using the solid-phase, enzyme-labeled competitive chemiluminescent immunoassay, hsCRP using a particle-enhanced immunoturbidimetric assay, serum TG and cholesterol using the colorimetric assay, and serum FSH, and LH using the electrochemiluminescence immunoassay. Inulin resistance was estimated by the homeostasis model assessment of IR (HOMA-IR) method using the formula fasting plasma insulin (μIU/mL) × fasting plasma glucose (mg/dL)/405. The intra- and inter-assay coefficients of variation were <6.8% consistent with good assay performance in both PCOS and control populations.

3.2.4. Statistical Analysis

Statistical analyses were performed using the SPSS version 22.0 (Chicago, USA). Continuous and categorical variables were presented as mean±SD and percentages, respectively. For between-group comparisons, Student's t-tests were used for continuous variables, with chi-squared analyses with the Pearson and Fisher's Exact tests for categorical variables. Analysis of covariance was used to adjust for body mass index (BMI) differences between groups (control vs PCOS and PCOS with MetS [PCOS-MetS] versus PCOS without MetS [PCOS-noMetS]) for continuous variables, with logistic regression, when comparing the categorical outcome measures. Results were considered significant at $P \leq 0.05$.

3.3. Results

3.3.1. Demographic, Anthropometric, and Clinical Characteristics

Of the 279 women examined, two-hundred and thirty-seven women were diagnosed with PCOS and 42 were designated as controls. Women with PCOS were subcategorized according to the presence or absence of MetS: PCOS-MetS (n=70) or PCOS-noMetS (n=167). Participant characteristics are presented in Table 5. Age and ethnicity were not different between groups. BMI was higher in the PCOS group versus controls ($P<0.001$) and in the PCOS-MetS group versus the PCOS-noMetS group ($P<0.001$).

Women with PCOS had a higher maximum duration between bleeding intervals ($P=0.005$) and a tendency ($P=0.06$) for shortest intervals between bleedings to be increased compared to controls. The PCOS-MetS group had earlier menarche than the PCOS-noMetS group ($P=0.03$), with differences remaining significant after adjustment for BMI ($P=0.02$, Table 5).

Women with PCOS had a higher prevalence of hirsutism and acanthosis nigricans than controls ($P<0.001$). Hirsutism and acanthosis nigricans were more frequent in PCOS-MetS group than in the PCOS-noMetS group ($P<0.001$). The observed hirsutism ($P=0.04$) and acanthosis nigricans ($P <0.001$) trends remained significant after adjustment for BMI (i.e., PCOS versus controls and PCOS-MetS versus PCOS-noMetS groups). The PCOS-MetS group had increased severity of acne vulgaris than the PCOS-noMetS group ($P=0.05$). Groups did not differ in the prevalence of DM2, CVD and hypertension, among first- and second-degree relatives (Table 5).

3.3.2. Prevalence and Characteristics of Metabolic Syndrome and Cardiovascular Disease Risk Factors

The overall prevalence of MetS was 29.5% (70/237) in the PCOS group versus 4.8% (2/42) in controls. FPG, systolic BP, WC, and TG levels were higher, and HDL-C lower in the PCOS group than in controls ($P \leq 0.05$, Table 5). The most prevalent components of the MetS in women with PCOS were increased WC (73.8% [175/237]) and reduced HDL-C levels (53.2% [126/237]), followed by hypertriglyceridemia (21.1% [50/237]) and hypertension (19.0% [45/237]). Elevated FPG (8.4% [20/237]) was the least common component of MetS. The PCOS-MetS group had higher levels of all MetS components when compared with PCOS-noMetS group; trends remained significant after BMI adjustments ($P \leq 0.001$). The prevalence of MetS across BMI and age groups among women with PCOS is shown (Figure 2). The prevalence of MetS increased with BMI and age in women with PCOS ($P<0.001$). We observed no differences in the overall prevalence rate and the prevalence of individual components of MetS by ethnic background among groups.

Women with PCOS had increased levels of WC, LDL-C, TC/HDL-C ratio, and hsCRP ($P \leq 0.03$) compared with controls. The PCOS-MetS group exhibited higher TC, LDL-C, TC/HDL-C ratio, and hsCRP ($P \leq 0.006$) levels than the PCOS-noMetS group. The TC ($P=0.01$) and TC/HDL-C ($P<0.001$) levels remained higher in PCOS-MetS group compared with the PCOS-noMetS group after adjustment for BMI.

3.3.3. Prevalence and Characteristics of Glucose Intolerance

Of the 279 participants, 237 completed the GTT. Twenty-one percent (50/237) of women with PCOS had impaired fasting glucose (IFG), IGT, or DM2 compared with 4.8% (2/42) of controls. In the PCOS group, less than half (47.1% [33/70]) of women with MetS were normoglycemic as opposed to 92.2% (154/167) without MetS (Table 5). After BMI-adjustment there were higher rates of IFG, IGT, and DM2 in the PCOS group than controls ($P=0.03$) and in PCOS-MetS ($P=0.001$) than PCOS-noMetS. Similarly, the PCOS and the PCOS-MetS groups exhibited higher levels of fasting insulin, 2-hour insulin, 2-hour glucose, and HOMA-IR than controls and women with PCOS-noMetS, respectively ($P<0.001$). Following the OGTT, 2-hour insulin ($P=0.01$) and glucose ($P=0.001$) concentration remained significantly higher in the PCOS-MetS group compared with the PCOS-noMetS group after BMI adjustment.

Table 5. Demographic, anthropometric, clinical, and laboratory characteristics of women with PCOS and controls without PCOS at the time of PCOS diagnosis (n=279)

Measure (measurement unit)	**PCOS (n=237)**	**Control (n=42)**	**P value***	**PCOS with MetS (n=70)**	**PCOS without MetS (n=167)**	**P value***
Age (year)	27.7±4.6	26.5±4.1	0.11	28.8±4.3	27.3±4.6	0.02
Ethnicity (n [%])			0.74			0.73
Caucasian	195 [82.3]	38 [90.5]		55 [78.6]	140 [83.8]	
Asian	30 [12.7]	3 [7.1]		10 [14.3]	20 [12.0]	
Indigenous	4 [1.7]	1 [2.4]		1 [1.4]	3 [1.8]	
African	3 [1.3]	0		1 [1.4]	2 [1.2]	
Latin American	5 [2.1]	0		3 [4.3]	2 [1.2]	
Menstruation						
Menarche (age in year)	12.6±2.3	12.6±1.3	0.88	12.1±0.3	12.8±1.6	0.02[a]
Shortest interval between bleeding (d)	43.82±67.7	23.8±5.5	0.06	49.8±73.0	37.5±58.5	0.16
Longest interval between bleeding (d)	180.2±300.1	46.8±33.2	0.005	169.2±200.9	156.0±303.4	0.74
Clinical measures						
Presence of hirsutism (n [%])	164 [69.2]	0	<0.001[a]	54 [77.1]	110 [65.9]	<0.001[a]
Acne vulgaris severity (n [%])						
Mild	102 [43.0]	21[50.0]	0.34	31 [44.3]	71 [42.5]	0.05
Moderate	52 [21.9]	7 [16.7]		15 [21.4]	37 [22.2]	
Severe	13 [5.5]	0		8 [11.4]	5 [3.0]	
Presence of acanthosis nigricans (n [%])	123 [51.9]	3 [7.1]	<0.001[a]	49 [70.0]	74 [44.3]	<0.001[a]
Family history of DM2 (n [%])	164 [69.2]	27 [64.3]	0.16	48 [68.6]	116 [69.5]	0.64
Family history of CVD and/or hypertension (n [%])	166 [70.0]	30 [71.4]	0.38	52 [74.3]	114 [68.3]	0.26
Anthropometric measures						
Weight (kg)	87.1±24.4	65.0±12.3	<0.001	105.6±21.8	79.1±21.0	<0.001

BMI (kg/m^2)	32.2±8.6	23.6±3.9	<0.001	38.9±6.9	29.2±7.6	<0.001
MetS prevalence (n [%])	70 [29.5]	2 [4.8]	<0.001[a]	–	–	–
MetS components						
FPG (mmol/L)	5.0±0.6	4.8±0.3	0.05	5.4±0.9	4.8±0.3	<0.001[a]
WC (cm)	103.1±20.1	84.7±11.8	<0.001	118.7±15.9	96.3±18.0	<0.001
Systolic BP (mmHg)	116.8±10.0	111.3±8.2	0.002	122.0±11.1	114.7±8.8	<0.001[a]
Diastolic BP (mmHg)	76.4±11.8	74.8±18.6	0.49	81.9±16.5	74.2±8.3	<0.001[a]
TG (mmol/L)	1.3±0.8	0.8±0.4	0.001	2.0±0.9	1.0±0.73	<0.001[a]
HDL-C (mmol/L)	1.3±0.3	1.6±0.4	<0.001	1.0±0.2	1.4±0.3	<0.001[a]
Other lipid profile measures						
TC (mmol/L)	4.6±0.9	4.4±0.7	0.20	4.9±0.9	4.5±0.9	0.001[a]
LDL-C (mmol/L)	2.9±0.8	2.5±0.6	0.03	3.0±0.7	2.7±0.8	0.006
TC/HDL-C	3.8±1.2	2.9±0.8	<0.001[a]	4.9±1.0	3.3±1.0	<0.001[a]
Glycemic control measures						
Normoglycemic (n [%]) IFG and/or IGT[†] (n [%]) DM2 (n [%])	187 [78.9] 42 [17.7] 8 [3.4]	40 [95.2] 2 [4.8] 0	0.01[a]	33 [47.1] 29 [41.4] 8 [11.4]	154 [92.2] 13 [7.8] 0	<0.001[a]
Fasting insulin (µIU/mL)	14.3±13.7	5.0±2.7	<0.001	19.2±11.3	10.7±13.0	<0.001
2-hour OGTT[†] insulin (µIU/mL)	79.6±64.8	35.8±19.7	<0.001	114.6±71.5	54.1±48.4	<0.001[a]
2-hour OGTT[†] glucose (mmol/L)	6.2±2.0	4.8±1.1	<0.001	7.5±2.0	5.6±1.4	<0.001[a]
HOMA-IR	2.4±2.4	0.8±0.4	<0.001	3.4±2.2	1.9±2.4	<0.001
HbA1c (%)	5.1±1.0	5.0±0.3	0.29	5.4±0.7	4.0±1.0	0.001
hsCRP (mg/L)	4.1±4.5	0.8±1.0	0.003	6.8±6.1	3.5±7.8	0.003
Endocrine parameters						
LH/FSH ratio	2.2±1.6	1.2±0.8	<0.001[a]	2.0±0.1	2.2±1.8	0.23
Total testosterone (nmol/L)	2.0±1.2	1.6±1.7	0.04	2.1±1.3	2.0±1.2	0.35
SHBG (nmol/L)	38.7±25.4	61.3±26.9	<0.001a	26.5±12.5	43.6±27.5	<0.001[a]

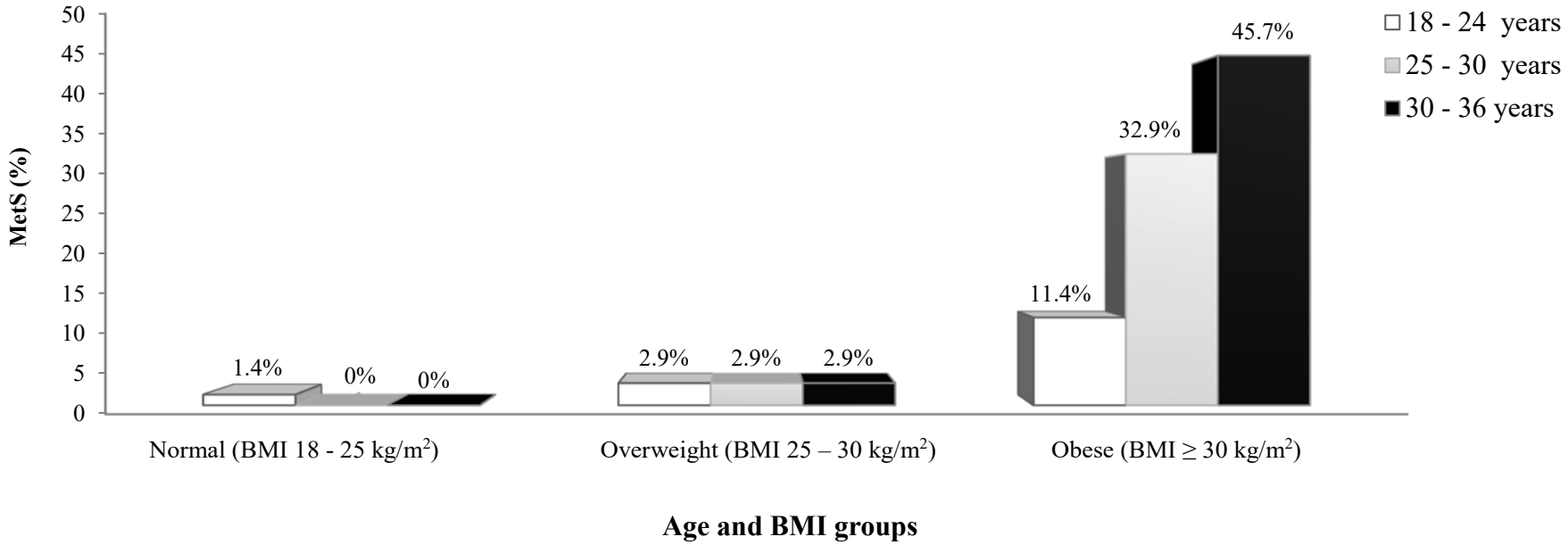
PCOS, polycystic ovary syndrome; MetS, metabolic syndrome; DM2, type 2 diabetes; CVD, cardiovascular disease; BMI, body mass index; FPG, fasting plasma glucose; WC, waist circumference; BP, blood pressure; TG, triglyceride; HDL-C, high–density lipoprotein cholesterol; TC, total cholesterol LDL-C, low-density lipoprotein cholesterol; IFG, Impaired fasting glucose; IGT, impaired glucose tolerance; OGTT, oral glucose tolerance test; HOMA-IR, homeostatic model assessment of insulin; HbA1c, glycated hemoglobin; hsCRP, highly sensitive C–reactive protein; LH, luteinizing hormone; FSH, follicle stimulating hormone; SHBG; sex–hormone binding globulin.

Values are mean±SD except indicated otherwise. *Student t-test and chi-squared test were used for comparisons of means and proportions between the two groups of women with PCOS and controls without PCOS, and among women with PCOS between the two groups with and without MetS. [†]n=225.

[a]P value≤0.05 when adjusted for BMI between the two groups using analysis of covariance for continuous variables and logistic regression for categorical variables.

Figure 2. The Prevalence of MetS across BMI and age ranges in women with PCOS (n=237)

The prevalence of MetS was different across the three BMI and age categories (P<0.001). Unlike non-obese BMI classes, differences in the prevalence rate of MetS between age categories remained significant (P<0.01) in obese women with PCOS (BMI≥30 kg/m^2). White bars, women with 18–24 years of age; grey bars, women with 25–30 years of age; Black bars, women with 30–36 years of age. Abbreviations: MetS: metabolic syndrome; BMI, body mass index; PCOS, polycystic ovary syndrome.



3.4. Discussion

Our study is unique in characterizing the metabolic profile of women diagnosed with PCOS who demonstrate a higher diagnostic threshold for the polycystic ovary (PCO). The diagnosis of PCOS utilized the 2006 diagnostic criteria and also complied with the 2013 recommendation for the PCO. In 2013 the AE-PCOS Task Force increased PCO threshold from ≥12 to ≥25 follicles 2-9 mm diameter (Dewailly et al., 2014). Many women without PCOS had polycystic ovaries according to the 2006 criterion. Our study also evaluated a subset of women diagnosed with PCOS who expressed menstrual cyclicity. Many researchers and clinicians have expressed concern that women with menstrual cyclicity did not have PCOS. However, a higher threshold for the PCO and hyperandrogenicity may increase confidence in the diagnosis of PCOS when the higher PCO threshold and hyperandrogenicity coexist. We prospectively recruited women with PCOS and healthy aged-matched controls in Saskatchewan, Canada. Recruits from 2 studies were pooled to increase our ability to identify their characteristics of PCOS.

We adopted ethno-specific adjustments to determine abdominal obesity and hirsutism. The prevalence rate of MetS was 29.5% in women with PCOS, nearly 6-fold higher than the age-matched control group without PCOS. Our findings align with previous evidence about the high prevalence rates of MetS in western populations with PCOS (Apridonidze et al., 2005; Ehrmann et al., 2006).

Glucose homeostasis abnormalities including acanthosis nigricans, insulin resistance, estimated by HOMA-IR and exaggerated insulin and glucose responses to OGTT were common in women with PCOS compared with age-matched controls. Traditional CVD risk factors were seen in the PCOS group including higher levels of BMI, SBP, hsCRP, abdominal obesity, and worse dyslipidemia with lower HDL-C, and higher TG, LDL-C, and TC/HDL-C compared with controls.

The features of MetS were noted in many women with PCOS. Insulin and glucose responses to OGTT were more abnormal compared with the MetS-free women; TC and TC/HDL-C ratio were higher in women with both PCOS and MetS compared with MetS-free women with PCOS, even after matching for BMI. Hyperinsulinemia secondary to IR stimulate ovarian theca compartment dysregulation and dysfunction of endothelial cells, leading to hyperandrogenicity. Hyperandrogenism underlies the cutaneous manifestations of PCOS,

including androgen-dependent hirsutism and acne vulgaris, through pre-established mechanisms (E Diamanti-Kandarakis & A Dunaif, 2012). The severity of hirsutism and severity of acne vulgaris in women with PCOS were more pronounced in women with MetS. Hirsutism and acne can be exacerbated by higher levels of IR and BMI. Acanthosis nigricans was present in more than half of women with PCOS and in 70% of women who had both PCOS and MetS conditions. Decreased SHBG and increased total testosterone concentrations consistent with the endocrine profile of PCOS. After BMI adjustment, lower levels of SHBG were observed in the PCOS-MetS group when compared with the PCOS MetS-free group. Our observations may be due to the complex interplay among deteriorated insulin sensitivity, deregulation of hypothalamic-pituitary-adrenal and ovarian axes, chronically stimulated LH secretion, and ovarian and adrenal androgen hyper-responsiveness (Indran et al., 2016).

Most (70.9%) women with PCOS were obese or overweight, consistent with the findings of previous studies (Barber et al., 2006; Norman et al., 2002). A unique aspect of the present study was the assessment of the influence of age and BMI categories on MetS in women with PCOS. We observed a progressive increase in the prevalence rate of MetS with age and BMI in the PCOS population, but not in controls. Unlike non-obese BMI classes, we observed an interaction between age and obesity (BMI≥30 kg/m^2) in women with PCOS in the prevalence rate of MetS, indicating adverse effects of aging on the risk of MetS in obese women with PCOS.

Age and BMI have been recognized as independent risk factors for MetS (Barber et al., 2006). Interestingly, after adjustment for BMI, women with PCOS had higher prevalence rates of MetS, glucose metabolism alterations (IFG/IGT/DM2) and levels of TC/HDL-C compared with age-matched controls. The observed metabolic perturbations in the PCOS group may be due to peripheral IR and hypersecretion of insulin as common driving forces for the overlapping metabolic abnormalities of PCOS with MetS (E Diamanti-Kandarakis & A Dunaif, 2012).

Women with PCOS exhibited worsened individual components of MetS compared with controls, most prominently increased WC and decreased HDL-C levels and corresponds to previous studies (Apridonidze et al., 2005; Soares et al., 2008). In addition to low HDL-C levels, women with PCOS exhibited elevated LDL-C, TG, and TC/HDL-C, hsCRP and SBP levels compared with controls. The high prevalence rate of MetS, IR, impaired glucose tolerance, central adiposity, and dyslipidemia have been recognized as detrimental cardiovascular risk and DM2 factors by the AE-PCOS Society (Wild et al., 2010). Beyond the classic risk markers,

women with PCOS displayed non-traditional surrogate cardiometabolic risk markers including hyperandrogenism and chronic inflammation (Chiu et al., 2017). Hyperandrogenism can increase the risk of premature atherosclerosis in women with PCOS by inducing oxidative stress, exacerbating chronic inflammation, promoting endothelial dysfunction, stimulating the activity of the intrarenal renin-angiotensin system, deregulating appetite, and increasing carotid intima-media thickness (Chen et al., 1992; Chiu et al., 2017; Diamanti-Kandarakis et al., 2006; Fenkci et al., 2003; Kravariti et al., 2005; Luque-Ramírez et al., 2007). Elevated CRP level, an indicator for low-grade chronic inflammation, is a reliable and independent predictor of CVD and DM2 (Duleba & Dokras, 2012). Inflammatory markers, such as hsCRP, can directly induce excess ovarian hyperandrogenism and promote atherosclerotic processes and endothelial cell inflammation independent of the cascade of molecular events that characterize metabolic aberration in PCOS (González et al., 2006; Ortega et al., 2014; Piotrowski et al., 2005). In the present study, 73.8% of women with PCOS had abdominal obesity, indicated by increased WC, similar to previous reports (Norman et al., 2002). Obesity is a chronic proinflammatory and prooxidant state that induces molecular alterations involved in the mechanisms of anovulation, hyperandrogenism, and metabolic disruptions in PCOS (Abdelhadi et al., 2013; González, 2015; González et al., 2013; González et al., 2012; Vendrell et al., 2004; Vincent & Taylor, 2005). Accumulation of visceral fat leads to excessive production of proinflammatory cytokines (mainly tumor necrosis factor-α and interleukin-6), non-esterified fatty acids, glycerol, and retinol-binding protein; increases the secretion of leptin, resistin, and visfatin; and decreases the production of adiponectin, which can lead to the development of IR (Kahn et al., 2006; Vendrell et al., 2004).

Our findings support the notion that reproductive-age women with PCOS have a substantial prevalence rate of MetS and exhibit adverse cardiometabolic and DM2 risk profiles. It is imperative to screen for metabolic abnormalities, including IGT, DM2, and dyslipidemia, for all women with PCOS, especially in women with increased BMI and age, at the time of diagnosis and regular follow up visits. The American College of Obstetricians and Gynecologists and the Endocrine Society recommended screening for IGT and DM2, using a standard 2-hour OGTT, and for dyslipidemia with a lipid profile for all women with PCOS ("American College of Obstetricians and Gynecologists. Polycystic ovary syndrome. ACOG Practice Bulletin no.

108," 2009; Legro et al., 2013). However, screening for metabolic abnormalities is underutilized by clinicians and is not a standard practice.

Study limitations included the potential for recall bias regarding obtaining the medical history related to menstruation patterns and the familial history of disease; and our inability to match the PCOS and control groups for BMI and sample size. Our PCOS group had higher BMI and WC than controls consistent with the anthropometric characteristic of women with PCOS. Thus, our results may have relevant implications for clinical practice.

Given the paucity of evidence, future studies are warranted to determine the magnitude of metabolic abnormalities associated with PCOS. Disclosure of a pattern of impaired glucose metabolism and early metabolic aberrations may motivate and encourage the affected women to adopt healthy lifestyle behaviours, such as diet, physical activity, and use of appropriate medical therapies to improve their condition. Early recognition of metabolic abnormalities can provide clinicians with preventive and management options, including lifestyle modifications and pharmaceutical modalities, such as insulin-sensitizing agents, to control the signs and symptoms of PCOS and to prevent or delay the long-term comorbidities associated with PCOS, including CVD and DM2.

As Objective i of the thesis, the contribution of the clinical and biochemical aberrations associated with PCOS to CVD and DM2 cardiometabolic risk profiles were evaluated. The results of the present study are important to convey the risk factors for women with PCOS. Controversy surrounds the optimal diet composition to mediate PCOS-associated reproductive disruptions. Chapter 4 of this thesis represents the first component of the thesis Objective ii – to compare the influence of a pulse-based diet with the standard TLC diet on reproductive health outcome measures in women with PCOS who also participated in an aerobic exercise program and received education and healthcare counselling about PCOS and lifestyle modification.

CHAPTER 4
EFFECTS OF THE LIFESTYLE INTERVENTION ON REPRODUCTIVE HEALTH OUTCOMES IN WOMEN WITH PCOS

Kazemi M, Pierson RA, McBreairty LE, Chilibeck PD, Zello GA, Chizen DR.

Insulin and ovarian morphology changes in polycystic ovary syndrome: a pulse-diet and exercise randomized controlled trial*

**format and structure of the original manuscript have been revised to provide a better flow within the thesis and be consistent with the format of previous chapters.*

Abstract

Background and objective: Controversy surrounds the optimal diet composition to mediate PCOS-associated reproductive disruptions. The purpose of current work was to compare the influence of a pulse-based diet (i.e. a low-glycemic index diet including lentils, beans, split peas, and chickpeas) with the standard TLC diet, on insulin response to the standard OGTT, ultrasonographic markers of ovarian and uterine morphology, hyperandrogenism, and menstrual regularity in PCOS.

Methods: Participants and Intervention: Ninety-five women with PCOS (18-35y) enrolled in a 16-week lifestyle changes program; 30 assigned to receive the pulse-based diet and 31 in TLC diet group completed the study. All women participated in an aerobic exercise program and received education and counselling about PCOS and lifestyle modification.

Results: Women in the pulse-based diet group had a greater reduction in total area under the curve (AUC) for insulin response to OGTT (mean change±SD: -121.0±229.9 vs -27.4±110.2 μIU/mL/min; $P=0.05$) than the TLC diet group. AFC and ovarian volume in the right ($P<0.00001$; $P<0.001$) and left ovaries ($P<0.001$; $P<0.0001$), testosterone levels ($P=0.05$), average ($P<0.01$) and longest ($P<0.001$) intervals between menses decreased in both groups. Endometrial thickness ($P=0.02$) and pattern ($P<0.001$) increased in both groups. Sex-hormone binding globulin levels increased ($P=0.0001$) in both groups with a tendency for a group-by-time interaction ($P=0.07$).

Conclusions: Both *ad libitum* lifestyle interventions improved ultrasonographic markers of ovarian and uterine morphology, hyperandrogenism, and menstrual bleeding intervals. The pulse-based diet was more effective for decreasing total insulin AUC in women with PCOS.

Keywords: Transvaginal ultrasound; follicle; hyperandrogenism; calorie restriction; reproduction; endocrine

4.1. Introduction

PCOS is a heterogenous endocrinopathy and the worldwide leading cause of anovulatory infertility among reproductive-age women with a prevalence of up to 18% (Carmina & Lobo, 1999; March et al., 2010). Most women with PCOS present with hyperinsulinemia, IR, hyperandrogenism, oligo-amenorrhea, enlarged, and PCO. Ultrasonographic examination of the PCO reveals an “increased number of pre-antral and antral follicles” distributed peripherally around or scattered through a bright echodense stroma due to disruptions at multiple stages of folliculogenesis (Azziz et al., 2006; Stephen Franks et al., 2008; Jonard & Dewailly, 2004).

Lifestyle modifications, comprised of dietary, exercise, and cognitive behavioural therapies, are recommended as the first-line strategy to mediate reproductive health outcomes in PCOS ("Practice Committee of American Society for Reproductive Medicine, Society for Reproductive Endocrinology and Infertility. Optimizing natural fertility: a committee opinion," 2013). However, a dietary composition optimized to ameliorate the reproductive sequelae of PCOS is less clear. A pulse-based diet has the potential to improve the metabolic changes associated with PCOS that culminate in reproductive problems. Pulses, that is, split-peas, beans, lentils, and chickpeas, are high in fiber, contain complex carbohydrates with a low GI are low in fat, contain high-quality protein, have low sodium content, and are a significant source of vitamins and minerals, such as iron, zinc, folate, calcium, magnesium, and potassium (Mudryj et al., 2014). Chronic consumption of pulses in other populations has been associated with decreased postprandial blood glucose and insulin concentrations, decreased lipids, and reduced obesity (Ha et al., 2014; McCrory et al., 2010; Sievenpiper et al., 2009). Insulin resistance and hyperinsulinemia are the key pathophysiological underpinning to

exacerbate PCOS reproductive sequelae (E Diamanti-Kandarakis & A Dunaif, 2012). The TLC diet, endorsed by the NCEP ATP III, is nutritionally balanced, with high fiber levels, reduced saturated fat and cholesterol, and options to include LDL-C lowering dietary components such as viscous fiber and plant stanol/sterol esters ("The NCEP ATP III final report," 2002). The TLC diet was considered, for our study, as a healthy control diet with the potential to improve PCOS reproductive abnormalities. We were unable to find a study involving women with PCOS where either a pulse-based or TLC diet were evaluated and where calorie restriction was not an integral component of the dietary alteration. We hypothesized that using diets with no energy restriction and where all participants were encouraged to exercise regularly, reproductive health outcome measures would improve when women with PCOS consumed a low-GI, pulse-based diet when compared to a standard TLC diet. Further, we hypothesized the pulse-based diet is more effective compared to the TLC diet after 6 and 12 months post-intervention to maintain improvements in reproductive outcome measures.

4.2. Materials and Methods

4.2.1. Study Design and Protocol

A multi-disciplinary, single-blind, metformin-stratified, parallel group randomized controlled trial design was conducted between April 2011 and June 2016 with women diagnosed with PCOS, in Saskatoon, SK, Canada at an academic research center. The primary objective of the trial was the assessment of reproductive outcomes, and the secondary outcome was an evaluation of MetS risk. Women were randomly allocated to receive either a pulse-based diet or the TLC diet after exclusion criteria were applied (McBreairty et al., 2017). As a standard of care, all women were enrolled in an aerobic training program and received education and counselling about PCOS and the value of lifestyle modifications in the management of the syndrome.

Participants were randomly allocated into one of the two diet groups after following the TLC diet for two weeks. Randomization was done with a computer-generated allocation schedule performed by an investigator who was not involved in obtaining, entering, or analyzing participant data. Randomization was stratified based on the current use of metformin, using a fixed block size of four and a permuted block design, using a computer random-number

generator. Participants were notified of diet allocation via email (and were not blinded). Although the participants were not blinded to the diet assignment, they were blinded to the hypothesis that the low-GI pulse-based diet would be more effective than the TLC diet to improve the risk of MetS. The allocation sequence was concealed from the dietitian who educated women about the TLC diet at baseline, as well as from individuals performing exercise training and data entry. The participants and group allocations were coded, and the investigators collecting and analyzing data were blinded to group assignment.

The pulse-based diet included soups, salads, and main course meals prepared with yellow split peas, green lentils, red split lentils, chickpeas, pinto, black, and kidney beans. Two meals (i.e., lunch and dinner) were supplied daily for participants in the pulse-based diet group. Each meal contained approximately 90 g of split peas or 225 g of chickpeas or beans or 150 g of lentils (cooked weight). The amount of dietary pulses in each meal was based on the amounts that have been beneficial for reducing blood glucose and lipid concentrations in prior investigations (Abeysekara et al., 2012; Schäfer et al., 2003; Shutler et al., 1989).

Each participant received a 1.5-hour, individualized dietary consultation session by a registered dietitian who was blinded to the intervention assignment. During the session, all women received a guide booklet outlining TLC diet guidelines. The guidelines were developed by the NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults ("The NCEP ATP III final report," 2002). A second dietitian who was not blinded to the randomization provided dietary consultation sessions after all women followed the TLC diet for two weeks. Women who were randomized to the pulse-based diet group were counselled to follow the TLC diet recommendations for breakfast and snacks. Women who were randomized to the TLC diet were instructed to consume minimal intake of dietary pulses, and consume low-fat cuts of meat and poultry and low fat or skim dairy as the main sources of protein, according to the TLC diet guidelines ("The NCEP ATP III final report," 2002). Caloric restriction was not part of the protocol design. Dietary intake was assessed using 24-hour dietary recall. Dietary intake data were analysed using the ESHA Food Processor SQL Software (version 7.02, ESHA Research, Salem, OR).

Each participant received standardized aerobic exercise training and was counselled to exercise for a minimum of 5 d/wk for 45 min/d of low-impact aerobic activity at an intensity between 60-75% of maximal heart rate reserve. Exercise consisted of brisk walking, training on

elliptical, cycling, and rowing machines, or stationary bike jogging, or any other aerobic exercise depending on the participants' preferences. Leisure time physical activity (i.e., physical activity outside the intervention) was evaluated using the Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985).

As a standard of care, before randomization to the diet groups each participant received two sessions of education and counselling for approximately four hours by a gynaecologist, MSc and PhD researchers who were knowledgeable about reproductive endocrinology and clinical nutrition. Topics included the human menstrual cycle, ovarian morphology in normal ovulating women, polycystic ovarian morphology, criteria used to diagnose PCOS and pathophysiology of PCOS. Other issues included determinants of health risk, complications and associated comorbidities of PCOS, medical, and diet and exercise lifestyle options to manage PCOS.

The trial protocol was approved by the Biomedical Research Ethics Board at the University of Saskatchewan. Before participants were enrolled in the study, consent was obtained in writing. All procedures were conducted in compliance with the World Medical Association Declaration of Helsinki, the Guidelines of the International Conference on Harmonization on Good Clinical Practice, and the Tri-Council Policy Statement on the Ethical Conduct for Research Involving Humans. We adhered to the CONSORT guidelines for reporting on randomized clinical trials (Schulz et al., 2010). The trial was registered at ClinicalTrials.gov (https://clinicaltrials.gov/, NCT01288638. Lifestyle Intervention for Polycystic Ovary Syndrome: Pulse-Based Diet and Exercise).

4.2.2. Participants

Recruitment was carried out by local newspaper advertisements, online bulletin posts, flyers available in physician offices, and placement of posters at the University of Saskatchewan and Royal University Hospital in Saskatoon, Canada. Three hundred twenty-four women responded to the recruitment advertisement. Ninety-five women, 18 to 35 years of age, who met the diagnostic criteria for PCOs were eligible for inclusion and were enrolled in the study.

After informed consents were obtained, women were enrolled if they met the following inclusion criteria: were between 18 to 35 years of age, had irregular periods, unwanted facial and/or body hair growth, and infertility. Women with the following conditions were excluded: used hormonal and/or fertility medications within the last 3 months of recruitment; took anti-seizure or anti-psychotic medications which are known to induce IR and PCO; diagnosed with

untreated hyperprolactinemia or thyroid disease; or, exhibited excessive adrenal androgen production confirmed by a diagnosis of congenital adrenal hyperplasia, Cushing's syndrome, or an adrenal tumor. Further, women with medical or dietary conditions that were limited in exercising or consumption of a pulse-based diet (allergies or intolerances) were excluded.

The diagnosis of PCOS was made according to the 2006 AE-PCOS Society criteria (Azziz et al., 2006) and complied with the 2014 AE-PCOS Society guidelines for polycystic ovarian morphology (a threshold of ≥25 antral follicle counts) (Dewailly et al., 2014). The diagnosis of MetS was made according to the 2005 International Diabetes Federation in collaboration with the American Heart Association/National Heart, Lung, and Blood Institute criteria (Alberti et al., 2009). Barrier contraceptive methods and a negative pregnancy test were required.

Women were screened for glucose intolerance and IR before and after the lifestyle intervention using a standard 2-hour 75-g OGTT. Blood samples were taken before, and following oral ingestion of a standard drink containing 75 g of glucose (Trutol 75; Thermo Scientific Inc., East Providence, RI, USA), every 30 minutes for 2 hours, using an intravenous catheter placed in the distal arm as previously described (McBreairty et al., 2017). Initial blood samples were collected between 8:00 and 9:00 am, after fasting overnight for 10 to 12 hours. Blood was collected in 6 mL EDTA and 10 mL empty collection tubes at baseline and at 30, 60, 90, and 120 mins following the consumption of the glucose drink. Plasma and serum samples were obtained by hospital staff by aliquoting samples into cryogenic vials which were frozen at -80 °C for future analysis of biochemical markers including glucose, lipid, and endocrine parameters as described in the biochemical analysis section below.

4.2.3. Ultrasonography Procedures and Measurements

Two experienced ultrasonographers performed transvaginal ultrasound scans using a standardized protocol for collecting ultrasonographic images and cine loops of the ovaries. Ovaries were scanned from inner to outer margins in the longitudinal and transverse planes using a Voluson E8 Expert (GE Medical Systems, Zipaf, Austria) and a three-dimensional 6-12-MHz transvaginal transducer. Scans were obtained during days 1-5 of women's menstrual bleeding cycles, with the first day of bleeding defined as day 1, or randomly if there was an absence of a dominant follicle (≥10 mm) or corpus luteum in women reporting irregular or absent cycles. Digital cine loops through each ovary (DICOM file format) and static images of the largest

cross-sectional view of each ovary (JPEG file format) were recorded for off-line analysis. Ovaries were examined in transverse and sagittal planes, and images were captured as real-time cine-loop recordings and 3-dimensional images. A single observer analyzed the stored ultrasound scans using Santesoft DICOM Editor software (Emmanouil Kanellopoulus, Athens, Greece) for the OV and AFC. Reliable follicle counts were achieved for each ovary by imposing a grid system onto the viewing window as previously described (Lujan et al., 2010; Lujan et al., 2013). The corresponding level of intra-observer agreement for AFC was 0.95, based on an interclass correlation coefficient analysis of follicle counts on 35 case files. OV was estimated using the equation for a prolate spheroid: ð/6 (transverse diameter) × (anteroposterior diameter) × (longitudinal diameter), when no follicles ≥10 mm in diameter were present.

4.2.4. Menstrual Bleeding Intervals

Intervals between menstrual bleeding (days) were recorded for the six months before the intervention and were tracked during the intervention via a self-report recall format. A menstrual bleeding interval was defined as the first day of bleeding to the start of a subsequent first day of bleeding. The shortest and the longest periods of time between bleeding intervals were recorded at baseline. Participants maintained a menstrual diary through the study and informed the researchers about the date on which menstrual bleeding began. The average length and the longest length between bleeding intervals were calculated.

4.2.5. Biochemical Analyses

Hormones and biochemicals evaluated included TT, estradiol, progesterone, FSH, LH, SHBG, and HbA1c; dehydroepiandrosterone sulfate, cortisol, prolactin, 17-hydroxyprogesterone, and thyroid hormones were measured to exclude endocrinopathies that mimicked the profile of PCOS. Plasma insulin and serum SHBG (Alpco Diagnostics, Salem, USA) were measured using high-sensitivity enzyme-linked immunosorbent assay, and plasma glucose (BioAssay Systems, Hayward, USA) by conventional colourimetric technique, using commercial kits. Serum TT by solid-phase, enzyme-labeled, chemiluminescent immunoassay was measured on Immulite 2000 Systems Analyzers (Siemens Healthcare Diagnostics Inc., Tarrytown, USA) with Roche kits (Roche Diagnostics Ltd., Basel, Switzerland). The remaining metabolites were analyzed using Roche Cobas Modular Analyzers and Roche kits, with HbA1c analyzed using the turbidimetric inhibition immunoassay, and serum FSH and LH using the electrochemiluminescence immunoassay. The HOMA-IR (Matthews et al., 1985) and free androgen index (FAI) (Clark et

al., 1975) were calculated as previously described. Fasting blood samples were taken when follicles ≥10 mm and corpora lutea were absent at ultrasonography done on days 1-5 of regular and predictable menstrual cycle lengths, or on random days for women with irregular/absent periods. Serial blood samples were taken pre-intervention, mid-intervention (9-weeks), and post-intervention (16-weeks), as well as at 6- and 12-months post-intervention. All samples were analyzed by the Saskatoon Health Region Laboratories immediately or during the first week of the collection after freezing at -80 °C. The intra- and inter-assay coefficients of variation were <6.8% consistent with good assay performance in both groups.

4.2.6. Statistical Analysis

Statistical analyses were carried out according to intention-to-treat principles with analyses carried out on all participants. Analyses were performed using SPSS for Windows (version 22.0; SPSS Inc., Chicago, IL, USA). Categorical variables were presented as numbers and percentages and continuous variables as mean±SD, except in figures, where mean±SEM was used for clarity. For between-group comparisons at baseline, Student's t-tests were used for continuous variables with chi-squared analyses with the Pearson and Fisher's Exact tests for categorical variables. To examine the differences in the outcome variables between women taking metformin compared with women not taking metformin, a three-factor ANOVA, with between-group factors (pulse-based vs TLC diet), and metformin (metformin users vs non-users) and a within-subjects factor of time (baseline and post-intervention) were used. If there were no metformin-group by time interactions, metformin groups were combined, and a two factor ANOVA was used to compare responses between groups (the pulse-based vs TLC diet groups) over all measurement time points (baseline, 16-week post-intervention, 6- and 12-month follow up) to increase the statistical power of the ANOVA. The area under the 2-hour response versus time curves (AUC) for insulin and glucose was determined using the trapezium rule (Tai, 1994). The incremental AUC, calculated as the increment in AUC over baseline concentrations, was used as summary measures of the postprandial insulin and glucose responses. The reliability analysis for the intra-observer agreement on ultrasound scan data was determined by Cohen's kappa statistic. Missing observations were assumed to be missing completely at random. Fasting blood samples and ultrasound scan data were collected nine weeks following the intervention (mid-intervention); mid-intervention data were carried forward for three women in the pulse-based diet group. Data collected at 9-week time-point were carried forward in place of any

missing data at the post-intervention time-point. To verify that mid-intervention data were an appropriate substitute for post-intervention, all analyses were also run using only participants who completed all testing time points (i.e., women who dropped out after mid-intervention were excluded from the secondary analysis). Long-term follow-up results of the study obtained at 6- or 12-months post-intervention were analysed in comparison to pre- and post-intervention data using repeated measure ANOVA as described above. For each ANOVA result that was significantly different 6- and 12-months post-intervention, pairwise comparisons were performed using a post hoc Bonferroni analysis to identify where the differences occurred. Changes in the mean fasting glucose from our previous pilot study (unpublished) on 28 women with PCOS were used to determine the required sample size for recruitment into the study. Changes in glucose levels were -0.18 and 0.14 mmol/L for the pulse-diet group and TLC group, respectively, with a population standard deviation of 0.46 and an effect size of -0.70. A sample size of 34 per group was determined to detect a significant difference in fasting glucose concentrations between groups at α=0.05 with 80% power, assuming an anticipated dropout rate of 32%. Mean estimates with corresponding 95% confidence intervals were calculated for each time-point. Results were considered significant at $P<0.05$.

4.3. Results

The participant flow through the phases of the clinical trial, along with losses and exclusions, is displayed in Figure 3. A total of 324 women responded to the recruitment advertisement; 95 were confirmed eligible and were enrolled in the study. Baseline characteristics of women enrolled in the study, including the use of metformin (1000-1500 mg/day) were comparable in both groups (Table 6). Baseline characteristics of women randomized to receive the intervention who did not complete the intervention were not different when compared with women who completed the intervention. Thirty women in the pulse-based diet group and 31 in the TLC diet group completed the 16-week intervention. The loss to follow up was similar between intervention groups (P=0.94). Two women refused to complete the OGTT following the intervention due to lack of time. The final analysis included 64 women (n=33 in the pulse-based and n=31 in the TLC diet groups). Final numbers analyzed for each outcome measure are presented in the outcome tables and figures. The level of compliance with exercise was 53.1±22.2 and 42.5±8.6 min/g/d over five d/wk for the pulse-based and TLC diet

groups, respectively (P=0.09). A mean diet adherence of 5.5±0.4 and 5.3±0.3 d/wk was reported in the pulse-based and TLC diet groups, respectively (P=0.12).

4.3.1. Metformin-Dietary Intervention Interactions

Eighteen women (38.3%) in the pulse-based and 20 [41.7%] in the TLC diet groups used metformin (Table 6). There were no group by time by metformin interactions for any of the evaluated outcome measures (data not shown) (P>0.05).

4.3.2. Anthropometric Measures

Following the intervention, both pulse-based diet and TLC diet groups exhibited lower BMI (P=0.01) and WC (P=0.02) over time; no group by time interaction was observed.

4.3.3. Endocrine Measures

Following the intervention, FPG (P<0.01), fasting plasma insulin (P<0.01), and HOMA-IR (P<0.001) decreased over time. There was a group by time interaction (P=0.05) for total insulin AUC with a greater decrease in the pulse-based diet group compared with the TLC diet group. Incremental insulin AUC (P=0.03), total (P<0.0001), and incremental (P<0.01) glucose AUC decreased over time without a group by time interaction. The LH/FSH ratio (P=0.03), TT (P=0.05), and SHBG (P<0.001) concentrations decreased over time in both groups, with a tendency for a group-by-time interaction for SHBG (P=0.07; Table 7).

4.3.4. Ultrasonographic Markers of Ovarian and Uterine Morphology

AFC in the left (P<0.001) and right ovaries (P<0.001), as well as OV in the left (P<0.0001) and right (P<0.00001) ovaries decreased over time without a group by time interaction (Table 7).

4.3.5. Menstrual Cyclicity

The average (P<0.01) and longest (P<0.001) period of time between spontaneous menstrual bleeds decreased over time in both groups without differences between groups (Table 7).

4.3.6. Endocrine measures following 6 and 12 months post-intervention

Thirty-two women completed the endocrine measures 6 months post-intervention including n=16 in the pulse-based diet group and n=16 in the TLC diet group. There were no group by time interactions for FPG (P=0.86) and fasting insulin (P=0.83) levels 6-months post-intervention. Results of pairwise post hoc analysis showed fasting insulin levels increased in both groups over time after 6 months of completing the intervention when compared to the 16-weeks

post-intervention timepoint (P=0.01); however, changes were not significant for FPG levels (P=0.08).

Twenty-five women completed the endocrine measures 12 months post-intervention including n=12 in the pulse-based diet group and n=13 in the TLC diet group. Both groups showed increased levels of FPG (P=0.04) and fasting insulin (P=0.02) over time, expressed as changes from baseline (Table 15). Results of the post hoc analyses showed there were no changes in the levels of FPG 12-months after the completion of the intervention (P=0.20) when compared to the 16-week post-intervention; however, the levels of insulin increased (P=0.05) in both groups 12 months following the completion of the intervention. Further details about changes in insulin and FPG levels over time have been presented in Chapter 5, Tables 11 and 12. The levels of LH/FSH ratio and TT did not change significantly 6 and 12 months post-intervention when compared to 16-weeks post-intervention (P>0.05). Further details about the level of adherence to the adopted healthy lifestyle practices in long-term have been presented in Chapters 5 and 7.

Figure 3. CONSORT 2010 standard RCT diagram

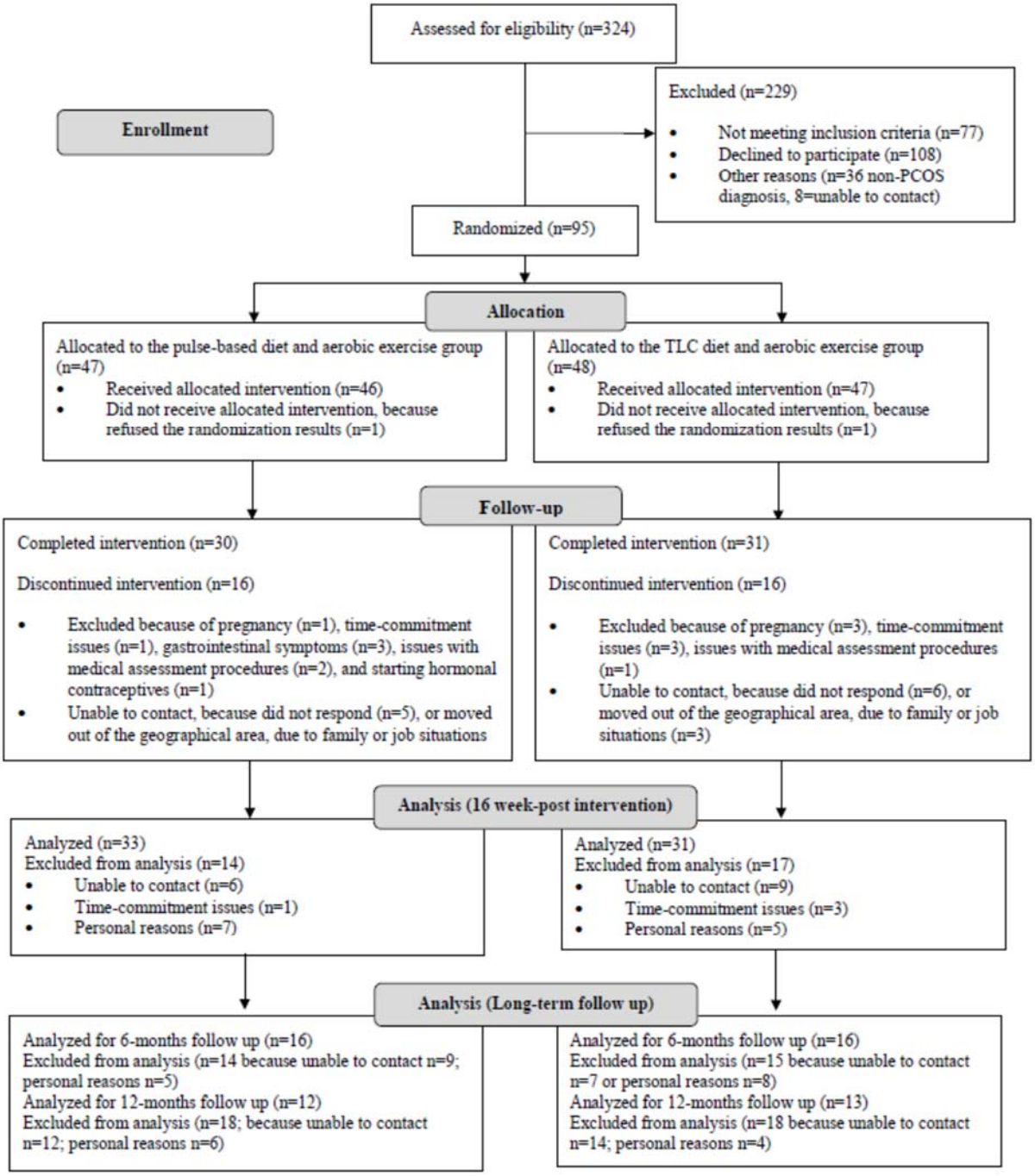


Table 6. Baseline characteristics of women with PCOS (n=95)

Measure	Pulse-based diet group	TLC diet group	P value*
Age (year)	27.0±4.6	26.9±4.4	0.91
Metformin therapy (n [%])	18 [38.3]	20 [41.7]	0.45
Anthropometric and physiologic measures			
Weight (kg)	87.36±23.9	92.24±24.5	0.33
Body mass index (kg/m^2)	32.52±8.4	33.35±9.0	0.65
Waist circumference (cm)	102.4±19.8	103.5±20.6	0.78
Menstruation			
Shortest interval between bleeding (d)	43.8±40.3	45.4±46.7	0.87
Longest interval between bleeding (d)	96.7±63.8	112.1±61.6	0.29
Total energy intake (kcal/d)	2165.1±774.3	2128.1±720.3	0.82
Leisure physical activity score (arbitrary units)	28.2±24.9	20.4±19.0	0.08
Insulin sensitivity measures			
Fasting plasma glucose (mmol/L)	5.2±1.4	5.3±1.3	0.68
Fasting insulin (μIU/mL)	13.0±10.0	15.6±12.2	0.25
HbA1c (%)	5.2±0.4	5.3±0.5	0.27
HOMA-IR index	3.0±2.3	4.0±4.0	0.16
Clinical presentations			
Presence of hirsutism† (n [%])	32 [68.1]	35 [72.9]	0.39
Presence of acanthosis nigricans (n [%])	23 [48.9]	18 [37.5]	0.18
Endocrine parameters			
LH/FSH ratio	2.2±1.1	2.6±1.7	0.32
Total testosterone (nmol/L)	1.5±0.5	1.7±1.1	0.41
Sex–hormone binding globulin (nmoL/L)	32.0±18.7	36.5±25.8	0.60
Free androgen index	5.8±3.7	7.6±7.6	0.17
Ultrasonographic markers			
Left ovary antral follicle counts	39.4±15.9	44.0±17.3	0.19
Right ovary antral follicle counts	43.8±21.0	45.6±18.0	0.67
Left ovary volume (cm^3)	10.2±6.1	10.0±4.6	0.81
Right ovary volume (cm^3)	12.9±6.9	11.5±6.0	0.30

Notes: Data are expressed as mean±SD except indicated otherwise. Numbers in each group for baseline characteristics were as follows: Pulse-based diet group=47; TLC diet group=48.

Abbreviations: PCOS, polycystic ovary syndrome; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin; LH/FSH, the ratio of luteinizing hormone to follicle stimulating hormone.

*Student t-test and chi-squared test were used for comparisons of means and proportions between groups. †Determined using the Ferriman–Gallwey Index, adjusted for ethnicity (Yildiz et al., 2010).

Table 7. Endocrine, ultrasonographic, and menstrual bleeding characteristics of women with PCOS pre- and post-intervention

	Pulse-based diet group			TLC diet group			P value	
	Baseline	16-weeks	Change	Baseline	16-weeks	Change	Time	Group x Time
Anthropometric and body composition measures								
Weight (kg)	89.9±27.0	84.4±26.8	-5.5±4.5	93.3±25.4	88.4±23.0	-4.9±15.8	<0.01	0.62
BMI (kg/m^2)	33.3±9.0	32.0±9.0	-1.3±1.4	34.0±9.8	32.2±8.6	-1.8±6.1	0 01	0.62
WC (cm)	103.9±19.8	99.5±18.0	-4.4±11.2	103.5±20.2	101.8±19.3	-1.7±7.6	0.02	0.30
Endocrine measures								
Fasting insulin (µIU/mL)	14.0±11.4	10.0±7.7	-4.0±9.7	15.7±12.4	12.7±10.3	-3.0±6.8	<0.01	0.60
HOMA-IR index	3.1±2.5	2.1±1.9	-1.0±2.1	4.2±4.4	2.9±3.6	-1.3±2.1	<0.001	0.66
Fasting insulin/glucose ratio	0.2±0.1	0.1±01	-0.0±0.1	0.2±0.1	0.1±0.1	-0.1±0.1	0.21	0.17
Total insulin AUC (µIU/mL/min)	326.9±266.5	205.9±106.7	-121.0±229.9	307.2±181.7	279.8±176.7	-27.4±110.2	<0.01	0.05
Incremental insulin AUC (µIU/mL/min)	49.8±45.7	32.5±22.4	-17.3±47.2	45.6±26.1	41.1±29.3	-4.5±22.1	0.03	0.19
LH/FSH ratio	2.6±1.1	1.8±1.2	-0.8±1.7	2.5±2.0	1.9±0.9	-0.6±2.2	0.03	0.69
Total testosterone (nmol/L)	1.7±0.6	1.5±0.5	-0.2±0.6	1.8±0.9	1.6±0.8	-0.2±0.6	0.05	0.98
Sex–hormone binding globulin (nmol/L)	24.0±11.6	40.0±23.1	16.0±20.5	27.6±19.6	36.5±22.5	8.9±12.4	0.0001	0.07
Ultrasonographic markers								
Left ovary antral follicle counts	40.9±14.1	32.2±12.3	-8.7±12.3	48.1±15.9	36.6±14.9	-11.5 ±16.5	<0.001	0.44

Right ovary antral follicle counts	44.4±20.4	33.4±15.1	-11.0±11.5	48.3±16.4	36.9±14.4	-11.4 ±19.4	<0.001	0.91
Left ovary volume (cm^3)	9.5±5.7	7.7±3.8	-1.8±4.5	11.7±4.7	8.5±3.8	-3.2±3.9	<0.0001	0.21
Right ovary volume (cm^3)	11.8±4.8	8.9±4.0	-2.9±3.5	12.9±6.4	10.7±4.8	-2.2±4.5	<0.00001	0.49
Menstruation								
Mean length between bleeding intervals (d)	60.0±49.3	47.1±37.1	-12.9±34.2	112.8±70.2	75.7±58.0	-37.1 ±51.3	<0.01	0.13
Longest intervals between bleeding cycles (d)	88.1±58.5	54.3±39.9	-33.8 ±51.3	138.6±65.2	84.0±56.8	-54.6 ±54.8	<0.001	0.30

Notes: Data are expressed as mean±SD. Numbers in each group for endocrine measures were as follows: Pulse-based diet group=20; TLC diet and group=22; Total testosterone: Pulse-based diet group=30; TLC diet group=29; SHBG: Pulse-based diet group=30; TLC diet group=29; Numbers in each group for sonographic measures were as follows: Pulse-based group=31; TLC diet group=31. Numbers in each group for menstruation measures were as follows: Pulse-based group=33; TLC diet group=31.

Abbreviations: PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; HOMA-IR, homeostatic model assessment of insulin; AUC, the area under the curve; LH/FSH ratio, the ratio of luteinizing hormone to follicle stimulating hormone.

4.4. Discussion

In the light of a state-of-art high-resolution ultrasound technology, reliable, innovative, and non-invasive examination of ultrasonographic features, and a well-defined PCOS population, the present study showed both pulse-based and TLC diets, when both groups exercised and received healthcare counselling, yielded substantial changes in ultrasonographic measures of ovarian reserve - including a reduction in AFC and OV; decreased TT; and reduced menstrual bleeding intervals. The severity of reproductive disruption and/or the likelihood of response to treatment in PCOS may be reflected by the changes in ovarian size, follicle number and the interval between menstrual bleeding (Jarrett & Lujan, 2017; Lujan et al., 2013). The pulse-based diet was more effective than the TLC diet to increase insulin sensitivity post-intervention, reflected by a reduction in total insulin AUC and a tendency toward a lower SHBG.

Our study evaluated the effects of two *ad libitum* diets with physical activity and lifestyle change counselling on ovarian morphology in PCOS. Prior studies have evaluated the effects of short-term physical activity, caloric restriction and low GI diets on overweight women. The loss of 5-10% weight had positive effects including a decrease of hyperandrogenism, IR, and fertility.(M. M. Huber-Buchholz et al., 1999; Marsh et al., 2010; Palomba et al., 2008; Thomson et al., 2008). Several mechanisms have been proposed for the positive effects of lifestyle interventions. .iThe regulation of gonadotropin secretion, neuroendocrine signalling, a decline in ovarian androgen secretion, increased insulin sensitivity and function, redistribution of body fat, especially central fat, recovery of spontaneous ovulation, and increased frequency of menstrual bleeding across age, BMI, and race/ethnicity categories appear following the lifestyle interventions(Bützow et al., 2000; Moran et al., 2011; Moran et al., 2007; Moran et al., 2009; Palomba et al., 2008). An increase in insulin sensitivity and decrease in insulin concentration are hypothesized to be the central physiological mechanism for changing ovarian morphology and function, and hyperandrogenism in both groups in our study, (E Diamanti-Kandarakis & A Dunaif, 2012). Although the pulse-based diet group experienced a greater decrease in total insulin AUC than the TLC diet group, a decline in the ultrasonographical features of ovarian morphology, hyperandrogenism, and menstrual regularity were comparable between groups. The lack of significant differences between

groups may be due to an insufficient reduction in total insulin AUC in the pulse-based diet group versus the TLC diet group.

Apart from inducing satiety and weight loss, several factors can explain the beneficial effects of a pulse-based diet on improving insulin response to OGTT and ovarian androgen biosynthesis, including high fiber content, low-GI, significant anti-oxidant content, and favourable micro- and macro-nutrient composition (McCrory et al., 2010; Mudryj et al., 2014; Sievenpiper et al., 2009). While our study goals were not focused on weight loss, our subjects achieved a 5% weight loss. We attributed the voluntary decrease in energy intake following education about lifestyle modification and increased physical activity, important ingredients to improve health, as explanations for the weight loss. Of note, because weight loss was not a stated goal or compensatory component of the present study, we believe that the study has not been biased by weight loss motivation as in most research. Also, our findings have broader generalizability across BMI categories.

Identification of abnormal morphological features in women with PCOS is required to guide innovative approaches, evaluate prognosis and derive tailored recommendations for the successful management of life-long reproductive and metabolic sequelae associated with the condition (Azziz et al., 2006; Homburg, 2006). Ultrasonographic features of ovarian morphology – specifically AFC, and to a lesser degree OV – have the diagnostic potential to reliably predict and detect the severity of reproductive, clinical, and metabolic abnormalities associated with PCOS (Jarrett & Lujan, 2017; Lujan et al., 2013). Enlarged ovaries and an excess of small antral follicles have been associated with follicle arrest and anovulation in PCOS (Adams et al., 1985; Stephen Franks et al., 2008) reflecting increased activation from the primordial pool (Webber et al., 2003), slower maturation (Maciel et al., 2004), or decreased atresia (Webber et al., 2007). The paucity of follicles >10 mm in diameter has been interpreted as a failure of selection, dominance, and ovulation (Franks et al., 1998). Aberrations in gonadotropin secretion (Franks et al., 1998) and impaired granulosa and theca cell function (Erickson et al., 1992; Gilling-Smith et al., 1994) likely inhibit the terminal follicular development and result in anovulation (Stephen Franks et al., 2008; Jonard & Dewailly, 2004). We observed a decrease in AFC and OV in women with PCOS post-

intervention. Importantly, in the present study, we used the latest criteria to diagnose the morphology of the PCO (Dewailly et al., 2014), which has been shown to yield a stringent cutoff to inform the severity of endocrine disturbances in women with PCOS (Clark et al., 2014). Decreased AFC is correlated with a modulated local ovarian hyperinsulinemia and androgen production, an increased sensitivity of granulosa cells to FSH, a systemic reduction in insulin levels and decreased IR, and weight loss , all features that could synergistically lead to increased menstrual cyclicity (Ding et al., 2006; Dunn et al., 1981). In our study, both intervention groups reported decreased intervals between spontaneous menstrual bleedings or improvements from irregular cycles to the shorter duration between bleeding that were not classified as regular menstrual cycles but did have a cyclic pattern (33 to 50 days). It is difficult to fully appreciate differences between the present study and previous investigations as previous reports were less clear regarding the ultrasonographic measures of the PCO. Our observations are in agreement with previous studies indicating increased menstrual regularity following lifestyle modification in PCOS populations (M. M. Huber-Buchholz et al., 1999; Marsh et al., 2010; Thomson et al., 2008). A decreased interval between menstrual bleeds may translate into the resumption of menstrual cyclicity in most women. The decreased interval between menstrual bleeding can be mapped by ultrasonography to reveal spontaneous ovulation with longer than normal follicular phase lengths, normal luteal phase dynamics and therefore less inherent risk for unopposed endometrial exposure to estrogen and long-term risk of uterine cancer. Our study is limited regarding reporting the resumption of spontaneous ovulation rates by the use of insufficient markers to confirm ovulation (Jarrett & Lujan, 2017). However, women with shorter intervals between menstrual bleeding did report fewer features of abnormal bleeding, with short limited flow seen after ovulation.

Contrary to our hypothesis, women in both intervention groups tended to revert to baseline levels for fasting insulin levels in long-term; however, women maintained their improvements in the levels of TT and LH/FSH ratio after the completion of the intervention. In the present study, we used supervised diet exercise programs combined with regular healthcare counselling, which were not continued after the trial period. Our participants had sub-optimal adherence to healthy lifestyle practices in 6- and 12-month

follow up phases reflected by lower frequency of pulse consumption, less favourable diet quality, and decreased physical activity as described in Chapters 5 and 7. Successful and sustainable adherence to healthy lifestyle change programs has been challenging with less favourable and mixed results in the context of PCOS (Domecq et al., 2013). Our observations reinforce the importance of healthy eating practices, increased physical activity, and reduce sedentary behaviour with continual monitoring, frequent assessment, and feedback from a multidisciplinary healthcare team to transfer the benefits of healthy lifestyle behaviours to usual daily activities after initial interventions in women with PCOS.

Limitations of the study included the potential for recall and self-reporting bias in reporting menstrual bleeding patterns; a high attrition rate; which is expected when voluntary participation is requested over prolonged time intervals, and when participants are asked to make marked lifestyle changes such as diet and exercise; technical and financial barriers to the biochemical assessment of TT by liquid or gas chromatography-tandem mass spectrometry and financial limitations to evaluate other markers such as inflammation, dyslipidemia and metabolism (Rosner et al., 2007).

In a diet intervention without energy restriction where aerobic exercise was recommended, and healthcare counselling was provided, both pulse-based and TLC diets showed similar favourable changes of ultrasonographic markers of ovarian morphology, hyperandrogenism, and menstrual regularity. The pulse-based diet was more effective for decreasing total insulin AUC in women with PCOS and had a tendency toward a greater increase in SHBG levels. Our results support the position that multi-dimensional lifestyle modifications are crucial in the management of PCOS and corroborate the potential of an *ad libitum* low-GI pulse-rich diet to improve reproductive features in young women with PCOS.

The second component of my thesis Objective ii was to compare the effect of a pulse-based diet to the TLC diet on cardio-metabolic outcome measures in women with PCOS. Next chapter (Chapter 5) represents changes in the risk factors of cardiometabolic disease in response to the lifestyle intervention program.

CHAPTER 5

EFFECTS OF THE LIFESTYLE INTERVENTION ON CARDIO-METABOLIC RISK IN WOMEN WITH PCOS

Kazemi M, McBreairty LE, Chizen DR, Pierson RA, Chilibeck PD, Zello GA.

Cardio-metabolic disease risk in women with polycystic ovary syndrome after an *ad libitum* pulse-based diet in a multidisciplinary randomized controlled trial*

**format and structure of the original manuscript have been revised to provide a better flow within the thesis and be consistent with the format of previous chapters.*

Abstract

Background and objective: Lifestyle modifications are recommended as the first-line approach in the management of PCOS. However, the optimal diet composition to mediate advantageous cardio-metabolic outcomes for women with PCOS remains unclear. The study objective was to compare the effect of a low-glycemic index pulse-based diet, containing lentils, beans, split peas, and chickpeas, to the NCEP's TLC diet, chosen because of LDL-C lowering effects, on cardio-metabolic risk measures in women with PCOS.

Design: Ninety-five women with PCOS (18-35 y) were randomized to receive either the pulse-based diet or TLC diet, without inducing calorie restriction, for 16 weeks. All women participated in an aerobic exercise program and received education and counselling about PCOS and lifestyle modification.

Results: Thirty women in the pulse-based diet and 31 in TLC diet group completed the study. The pulse-based diet group had a greater reduction in total AUC for insulin response to a standard oral glucose tolerance test (mean change ± SD: -121.0±229.9 vs -27.4±110.2 μIU/mL/min; P=0.05) than the TLC diet group. The pulse group exhibited lower diastolic BP (DBP; -3.6±6.7 vs -0.2±6.7 mmHg, P=0.03), TG (-18.1±51.9 vs 7.1±42.3 mg/dL, P=0.04), LDL-C (-10.7±17.0 vs -2.2±15.6 mg/dL, P=0.05), and TC/HDL-C ratio (TC/HDL-C; -0.4±0.4 vs 0.1±0.4, P<0.001), as well as a greater increase in HDL-C (2.0±7.4 vs -2.8±6.2 mg/dL, P<0.01) when compared to the TLC diet group. Body weight (P<0.01), wWC (P=0.02), percent body fat

(P<0.001), SBP (P<0.01), HOMA-IR (P<0.0001), incremental glucose (P<0.0001) and insulin (P=0.03) AUC, total glucose AUC (P<0.01), and TC (P<0.01) decreased in both groups.

Conclusions: A pulse-based diet was more effective than the TLC diet at improving many key risk factors for cardio-metabolic disease in women with PCOS.

Keywords: Exercise; insulin; lifestyle; lipid; metabolic syndrome; pulse foods; Therapeutic Lifestyle Changes diet

5.1. Introduction

PCOS is the most common endocrinopathy and the leading cause of anovulatory infertility among reproductive-age women worldwide, with a prevalence of up to 18% (Carmina & Lobo, 1999; March et al., 2010). Women with PCOS exhibit many metabolic abnormalities including peripheral IR and compensatory hyperinsulinemia**,** impaired glucose metabolism, dyslipidemia, HTN, and abdominal adiposity. These features are components of metabolic syndrome (MetS), which is defined as a complex of five interrelated risk factors for CVD and DM2. Compared to other clinical presentations associated with PCOS, metabolic aberrations represent critical contributors to increased rates of long-term morbidity and mortality (Carmina & Lobo, 1999; E Diamanti-Kandarakis & A Dunaif, 2012; Wild et al., 2010).

Lifestyle modifications comprised of dietary, exercise and behavioural therapies are recommended as first-line approaches in the management of PCOS. Optimizing body weight, engaging in regular physical activity, and learning cognitive behavioural skills are key strategies for PCOS. The most favourable dietary composition to facilitate metabolic changes in women with PCOS has been debated (Moran, Ko, et al., 2013). The positive effects of weight loss upon women with PCOS have been reported after short-term hypocaloric dietary interventions; however, a PCOS-optimized dietary composition has not been addressed. A pulse-based diet has the potential to improve the metabolic sequelae associated with PCOS. Pulses, that is, split-peas, beans, lentils, and chickpeas, are high in fiber, contain complex carbohydrates with a low GI, are low in fat, contain high-quality protein, have low sodium content, and are a significant source of vitamins and minerals, such as iron, zinc, folate, calcium, magnesium, and potassium (Mudryj et al., 2014). Chronic consumption of pulses in other populations has been associated with positive metabolic effects such as lowering postprandial blood glucose and insulin concentrations,

decreasing hypercholesterolemia, BP and obesity (Ha et al., 2014; McCrory et al., 2010; Sievenpiper et al., 2009). The TLC diet, endorsed by the NCEP Adult Treatment Panel III, is an integral component of a non-pharmacologic healthful lifestyle habits program designed to decrease LDL-C concentrations in individuals with hypercholesterolemia. The TLC diet, endorsed by the NCEP Adult Treatment Panel III, is an integral component of a non-pharmacologic healthy lifestyle habits program designed to decrease LDL-C concentrations in individuals with hypercholesterolemia. The TLC diet is a nutritionally balanced diet, accomplished by increasing fiber consumption, decreasing saturated fat and cholesterol, and adding LDL-C lowering dietary options such as viscous fiber and plant stanol/sterol esters ("The NCEP ATP III final report," 2002). The TLC diet was considered, for our study, as a healthy control diet with the potential to improve PCOS metabolic abnormalities. We were unable to find a study involving women with PCOS where either a pulse-based or TLC diet were evaluated. We hypothesized that when there is no energy restriction, a low-GI, pulse-based diet is more effective than the standard TLC diet for improving multiple risk measures of cardiometabolic disease in women with PCOS. Further, we hypothesized the pulse-based diet is more effective compared to the TLC diet after 6 and 12 months post-intervention to maintain improvements in cardio-metabolic outcome measures.

5.2. Materials and Methods

5.2.1. Study Design and Protocol

Details of the RCT design and protocol have been elaborated in Chapter 4, page 69.

5.2.2. Participants

Details of study subjects have been presented in Chapter 5, page 71.

5.2.3. Study Procedures

A standardized medical history and physical examination was performed to obtain a menstrual, medical and endocrine history, demographics, lifestyle factors, anthropometric, physiologic, and clinical measures as described previously (McBreairty et al., 2017). Weight was measured in light clothing using a mechanical weight scale (model 160KL; Health-O-Meter Inc., Bridgeview, IL, USA) to the nearest 0.1 kg. Height was measured without shoes using a portable stadiometer (Seca 208; Vogel and Halke, Hamburg, Germany) to the nearest millimeter. BMI was calculated using the formula: [body weight (kg)/(height squared) (m^2)]. Waist circumference

(WC) was measured following the World Health Organization Waist Circumference Expert Consultation on Waist Circumference protocol, with a measurement taken to the nearest millimeter (*World Health Organization. Waist circumference and waist-hip ratio: report of a WHO Expert Consultation, Geneva, 8–11 December 2008*). Body composition was evaluated from whole-body scans measured by dual-energy X-ray absorptiometry (QDR Discovery Wi; Hologic Inc., Bedford, MD, USA) using QDR software for Windows XP (QDR Discovery, Hologic Inc.). The coefficients of variation for total body fat mass, lean tissue, and trunk fat mass were 3.0%, and 0.5%, 5.0% respectively. BP was measured after a 10-minute rest period, in the supine position in the right arm using a standard manual mercury sphygmomanometer and a stethoscope (Littmann Master Classic, 3M Health Care, St. Paul, MN, USA), with both feet on the floor and the arm supported at heart level. BP was recorded to the nearest two mmHg. Transvaginal ultrasound examinations were performed using a Voluson E8 Expert (GE Medical Systems, Zipaf, Austria) and a three-dimensional 6-12-MHz transvaginal transducer, during days 1-5 of women's bleeding cycle, with the first day of bleeding defined as day 1, or at a time when no dominant follicle or ovulation gland was detected in women reporting irregular or absent cycles.

Hormones and biochemicals evaluated included total testosterone, estradiol, progesterone, FSH, LH, SHBG, TC, LDL-C, HDL-C, TC/HDL-C ratio, TG, hsCRP, and HbA1c; dehydroepiandrosterone sulfate, cortisol, prolactin, 17-hydroxyprogesterone, and thyroid hormones were measured to exclude endocrinopathies that mimicked the profile of PCOS. Fasting blood samples were taken when follicles ≥10 mm and corpora lutea were absent at ultrasonography done on days 1-5 of regular and predictable menstrual cycle lengths, or on random days for women with irregular/absent periods. Serial blood samples were taken pre-intervention, mid-intervention (9-weeks), and post-intervention (16-weeks), as well as at 6- and 12-months post-intervention. All samples were analyzed by the Saskatoon Health Region Laboratories immediately or during the first week of the collection after freezing at -80 °C.

5.2.4. Hormonal and Biochemical Analyses

Plasma insulin (Alpco Diagnostics, Salem, NH, USA) and serum SHBG (Alpco Diagnostics, Salem, NH, USA) were measured using high-sensitivity enzyme-linked immunosorbent assay (ELISA), and plasma glucose (QuantiChrom, DIGL-100, BioAssay Systems, Hayward, CA, USA) by routine colorimetric technique, using commercial kits. Serum

total testosterone (by solid-phase, enzyme-labeled, competitive chemiluminescent immunoassay) was measured on Immulite 2000 Systems Analyzers (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) with Roche kits (Roche Diagnostics Ltd., Basel, Switzerland). The remaining compounds were analyzed using Roche Cobas Modular Analyzers and Roche kits, with HbA1c analyzed using the turbidimetric inhibition immunoassay, serum SHBG using the solid-phase, enzyme-labeled competitive chemiluminescent immunoassay, hsCRP using a particle-enhanced immunoturbidimetric assay, serum TG and cholesterol using the colourimetric assay, and serum FSH and LH using the electrochemiluminescence immunoassay. Insulin resistance was estimated by the HOMA-IR method using the formula fasting plasma insulin (μIU/mL) × fasting plasma glucose (mg/dL)/405. FAI was calculated as total testosterone (nmol/L) / SHBG (nmol/L) × 100. The intra- and inter-assay coefficients of variation were 5.1% to 10.3% and 6.7% to 16.6% for insulin respectively, <3% for glucose, 1.2 and 1.2% for HbA1c, 2.1 and 3% for cholesterol, 3.3 and 3.0% for HDL-C, 2.1 and 3.0% for LDL-C, 2.1 and 2.5% for TG, 1.1 and 3.6% for hsCRP, 3.9 and 1.9% for FSH, 3.5 and 0.8% for LH, 4.2 and 1.3% for SHBG, and 6.8 and 5.4% for testosterone, consistent with good assay performance in both intervention groups.

5.2.5. Statistical Analysis

Details of the statistical analysis have been elaborated in Chapter 4, page 74 and 75.

5.3. Results

The flow-diagram of participants' progress through the phases of the clinical trial according to the CONSORT statement, along with losses and exclusions, is displayed in Figure 4. A total of 324 women responded to the recruitment advertisement; 95 were confirmed eligible and were enrolled in the study. Baseline data are presented in Table 8. Baseline characteristics, including the use of metformin (1000-1500 mg/g/d) were comparable in both groups (Table 8). The average age of women was 27.0±4.5 years, and average BMI was 32.9±8.7 kg/m^2. Most participants (74.7%; 71/95) were Caucasian. At baseline, the pulse-based and TLC diet groups did not differ regarding age, ethnicity, anthropometric and body composition measures, family history of DM2/CVD and/or HTN, insulin sensitivity measures, lipid profile, clinical and endocrine features of PCOS. There were no differences between groups at baseline for total energy intake and leisure time physical activity (Table 8). Baseline characteristics of women randomized to receive the intervention who did not complete the intervention were not different

when compared with women who completed the intervention. Thirty women in the pulse-based diet group and 31 in the TLC diet group completed the intervention. There were no differences between baseline characteristics of women who completed the study (Table 9). Baseline characteristics of women who did not complete the 16-week intervention were not different from those who completed the intervention (P>0.05; data not shown). The final analysis included 61/95 women. The percentages of women who did not complete the study intervention were similar between groups (P=0.94). Two women who completed the diet refused to complete the post-intervention OGTT due to lack of personal time. Incomplete questionnaire data (24-hour dietary recall, n=16 and physical activity record n=4) were due to errors in collection, lack of time, or refusal by participants. Final numbers analyzed for each outcome measure are presented in the outcome tables and figures. The level of compliance with exercise and dietary interventions have been presented in Chapter 4, pages 75 and 76.

5.3.1. Metformin-Dietary Intervention Interactions

There were no group by time by metformin interactions for any of the evaluated outcome measures (data not shown). No differences were observed between metformin users and nonusers in the pulse-based and TLC diet groups regarding baseline and changes in anthropometric and body composition measures, physiologic measures, insulin sensitivity, and serum plasma lipid concentrations.

5.3.2. Anthropometric, Body Composition and Blood Pressure Measures

Following the intervention, both pulse-based diet and TLC diet groups exhibited lower BMI (P=0.01) and WC (P=0.02), and trunk fat mass (P<0.001) over time; no group by time interaction was observed. Similarly, there was a time main effect for total body mass (P<0.01) and total %body fat (P<0.01) with both decreasing across groups, without a group by time interaction (Table 9). SBP decreased following the intervention across groups (time main effect; P<0.01). There was no difference between groups concerning a change in SBP post-intervention. There was a group by time interaction for DBP (P=0.05) with a greater decrease in the pulse-based diet compared to the TLC diet group (Table 9).

5.3.3. Insulin and Glucose Responses to OGTT

Details of the glucose and insulin responses of participants to intervention have been described in Chapter 4, page 76. Insulin responses of the pulse-based diet and the TLC diet groups to OGTT

are shown in Figure 5, A. Glucose response to the OGTT and the ratio of plasma insulin to plasma glucose concentrations are shown in Figures 5, B and C, respectively.

5.3.4. Lipid Profile

There was a group by time interaction for TG ($P=0.04$), HDL-C ($P<0.01$), LDL-C ($P=0.05$), and TC/HDL-C ratio ($P<0.001$). The pulse-based diet group exhibited greater reductions in TG, LDL-C, TC/HDL-C ratio, and a greater increase in HDL-C concentrations when compared with the TLC diet group (Table 9). There was a time main effect (decrease) for TC ($P<0.01$). Further, to verify that mid-intervention data were an appropriate substitute for post-intervention, women who dropped out after mid-intervention were excluded from the analysis. Analyses of results using data only from participants who completed all testing time points showed a similar group by time interaction for TG ($P<0.01$), HDL-C ($P<0.01$), LDL-C ($P=0.05$), and TC/HDL-C ratio ($P<0.0001$). The pulse-based diet group showed greater reductions in TG, LDL-C, TC/HDL-C ratio, and a greater increase in HDL-C concentrations when compared with the TLC diet group (data not shown). Similarly, there was a time main effect (decrease) for TC ($P<0.01$) for women who completed all testing time points.

The prevalence rate of MetS decreased from baseline in the pulse-based (from 36.7%; 11/30 to 30.0%; 9/30) and TLC diet (37.9%; 11/29 to 34.5%; 10/29) groups. When compared to pre-intervention, changes in the prevalence rate of MetS were no differences between groups in the post-intervention ($P=0.78$).

5.3.5. Physical Activity and Dietary Intake

During the intervention women in the pulse-based diet and TLC diet groups voluntarily reduced their average daily energy intake from baseline (-565.2±667.0 vs -556.4±696.3 kcal/g/d, respectively; time main effect; $P<0.0001$). There were no differences for changes in the energy intake from baseline between the pulse-based and TLC diet groups ($P=0.97$). Total carbohydrate, total fat, dietary cholesterol, saturated fat, trans fat, monounsaturated fat, polyunsaturated fat, and total protein intakes decreased from baseline during the intervention (time main effect, $P\leq0.05$, Table 13). The pulse-based diet group exhibited a greater decrease in dietary cholesterol intake during intervention when compared to the TLC diet group ($P<0.001$). The pulse-based diet group had a tendency toward a greater decrease in trans-fat intake during intervention from baseline than the TLC diet group ($P=0.06$). The pulse-based diet group consumed a higher amount of dietary fiber than the TLC diet group during the intervention ($P<0.01$). The pulse-based diet

group had a greater consumption of low-GI foods compared with the TLC diet group during the intervention (P<0.01).

Results of pairwise post hoc analysis of dietary intake of women who completed the long-term follow up phases of the study showed both the pulse-based and TLC diet groups maintained the reductions in total energy intake achieved during the intervention period 6 (mean energy intake: 1762±288 vs 1687±386 kcal/d; P=0.34) and 12 months (mean energy intake: 1735±358 vs 1749±324 kcal/d; P=0.20) after the completion of the intervention. Both groups maintained their levels of dietary cholesterol, saturated fat, trans fat, monounsaturated fat, polyunsaturated fat, and total protein intakes between 6 and 12 months follow up timepoints and during the intervention (P>0.05). When compared to the intervention period, the pulse-based diet group exhibited a tendency toward decreased dietary fiber intake versus the TLC diet group 6 months after the completion of the intervention (-18.2 vs 4.5 g/d; P=0.07). Both the pulse-based (-10.1 g/d) and TLC diet (-5.0 g/d) groups consumed a lower amount of dietary fiber 12 months after the completion of the intervention when compared to the intervention period (P=0.02). Unlike the 12-month follow-up timepoint (P=1.00), both the pulse-based and TLC diet groups had a tendency toward decreased intakes of soluble fiber (-0.2 vs -0.7 g/d P=0.06) between the intervention period and 6 months post-intervention. Further, the consumption of low-GI foods decreased in both groups 6 (P<0.01) and 12 months (P<0.01) after the completion of the intervention.

Leisure time physical activity score increased in both groups during intervention from baseline in both of the pulse based (31.6±25.5 to 37.7±28.6 arbitrary units) and TLC (24.0±20.2 to 33.6±23.8 arbitrary units) (P<0.01); no differences were observed between groups (P=0.53). Results of the post hoc analysis on women who completed the long-term follow-up phases of the study showed decreased scores of leisure time physical activity in both the pulse-based and TLC diet groups 6 months after the completion of the intervention when compared to the intervention period (-16.6±22.7 vs -7.3±20.8 units; P=0.008). Both the pulse-based and TLC diet groups showed a trend toward decreased scores of leisure-time physical activity 12 months after the completion of the intervention when compared to the intervention period (-16.1±25.4 vs -12.4±18.5 units; P=0.08).

5.3.6. Side Effects

Three volunteers reported four side effects in the pulse-based diet group during the intervention. The side effects were upset stomach (n=2), flatulence (n=1) and bloating (n=1). One participant reported a combination of upset stomach and bloating, one participant experienced only upset stomach, and one participant reported flatulence. The side effects were rated as mild-moderate in severity, were classified as "possibly" related to the intervention and led to withdrawal of participants from the study.

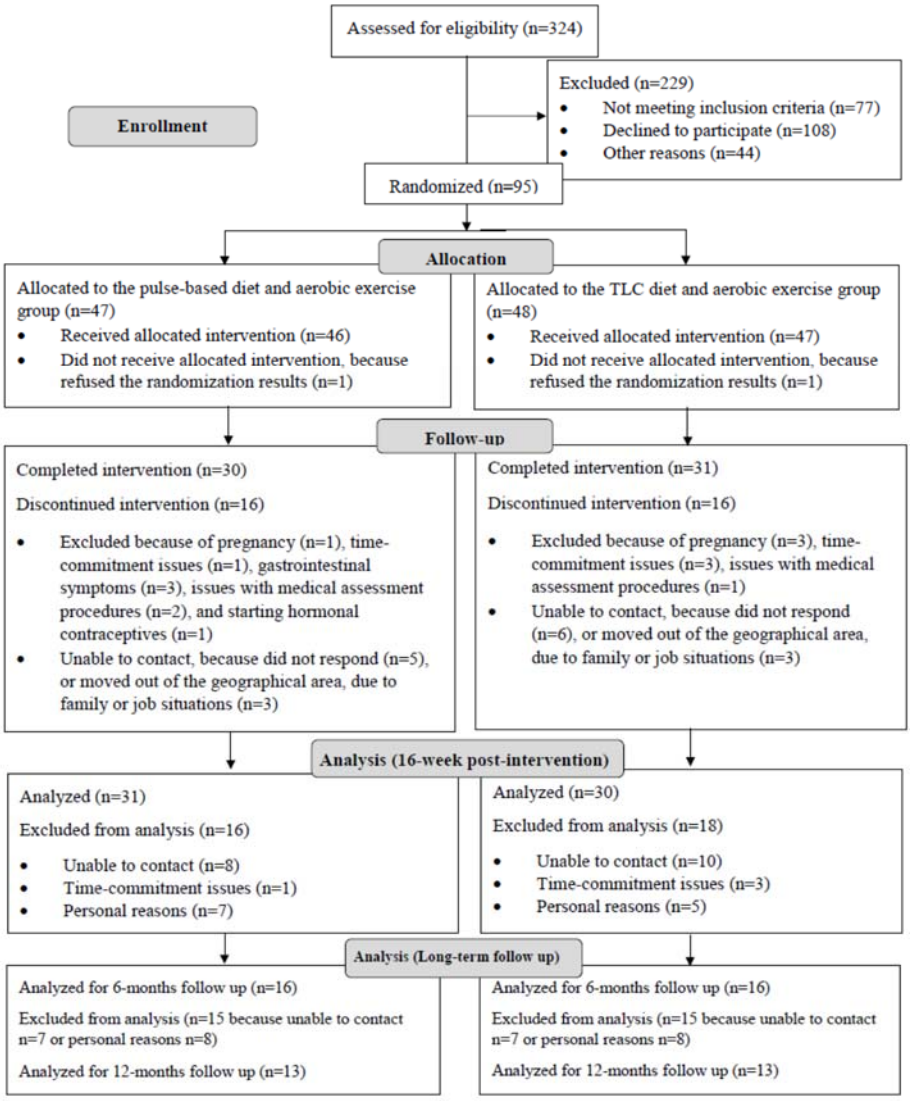
5.3.7. Clinical and biochemical measures following 6 and 12 months post-intervention

Thirty-two women completed the endocrine measures 6 months post-intervention including n=16 in the pulse-based diet group and n=16 in the TLC diet group. Results of the post hoc analyses showed an increase in body weight (P<0.0001) and BMI (P=0.006), expressed as changes from 16-week post-intervention, 6 months following the completion of the intervention. However, WC levels (P=1.00), total body fat mass (P=0.17), and total body fat% (P=0.90) remained unchanged between the two time points. There were no group by time interactions for FPG (P=0.86) and fasting insulin (P=0.83) levels 6-months post-intervention. Results of the post hoc analyses showed fasting insulin levels increased in both groups over time after 6 months of completing the intervention when compared to 16-week post-intervention timepoint (P=0.01). Both groups had an increased level of TC 6 months following the intervention expressed as changes from 16-week post-intervention (time main effect; P=0.03). There were no differences in the changes of HDL-C levels 6-month following the intervention when compared to 16-week post-intervention (P=1.00). Both groups had a tendency toward increased levels of LDL-C 6 months post-intervention (P=0.07). The reduction in the levels of TC/HDL-C that was observed following the intervention, maintained 6 months post-intervention (P=0.34; Table 10).

Twenty-five women completed the clinical and biochemical measures 12 months post-intervention including n=12 in the pulse-based diet group and n=13 in the TLC diet group. Results of the pairwise post hoc analyses showed no changes between the 16-week post-intervention and the long-term follow up time point at 12 month following the completion of the intervention in body weight (P=0.17), BMI (P=0.37), total body fat mass (P=1.00), total body fat% (P=1.00), and SBP (P=0.07). Both groups showed increased levels of FPG (P=0.04) and fasting insulin (P=0.02) over time (Table 11). There were no changes in the levels of FPG 12-months after the completion of the intervention when compared to post-intervention (P=0.20); however, the levels of insulin increased (P=0.05) in both groups 12 months following the

completion of the intervention, expressed as changes from the 16-week post-intervention. Similarly, 12 months after the completion of the intervention the levels of TC increased when compared to the post-intervention levels (P=0.005); however levels of HDL-C (P=0.30), LDL-C (P=0.70), and TC/HDL-C (P=0.09) remained unchanged between the 16-week post-intervention and 12-month follow up timepoints for both groups. Further details have been presented in Tables 10 and 11.

Figure 4. Flow diagram of the randomized controlled trial.



In the "follow-up" section – "completed the intervention represented" – women who completed the 16-week lifestyle intervention; – "discontinued the intervention" – represented women who dropped out of the study before completing the 16-week lifestyle intervention. In the analysis section, the number of subjects that were analyzed in the pulse-based and TLC diet groups included women who completed the 16-week lifestyle intervention and women who dropped out of the study before completing the 16-week intervention period, but their last observation data which were collected at 9-weeks post-intervention were carried forward to 16-week time point according to the intention-to-treat principle.

Table 8. Baseline characteristics of women with PCOS by intervention group (n=95)

	Pulse-based diet group	**TLC diet group**	**P value***
Age (year)	27.0±4.6	26.9±4.4	0.91
Ethnicity (n [%])			0.69
Caucasian	35 [74.5]	36 [75.0]	
Asian	8 [17.0]	10 [20.8]	
Indigenous	1 [2.1]	0	
African	1 [2.1]	0	
Latin American	2 [4.3]	2 [4.2]	
Metformin Tx (n [%])	18 [38.3]	20 [41.7]	0.45
Anthropometrics and body composition measures			
Weight (kg)	87.36±23.9	92.24±24.5	0.33
BMI (kg/m^2)	32.52±8.4	33.35±9.0	0.65
WC (cm)	102.4±19.8	103.5±20.6	0.78
Total body fat mass (kg)	36.1±13.0	39.6±16.7	0.26
Total body fat (%)	41.3±7.2	42.3±8.5	0.54
Total body lean mass (kg)	46.4±7.3	48.8±9.5	0.18
Physiologic measures			
SBP (mmHg)	115.3±8.2	117.3±10.6	0.33
DBP (mmHg)	76.4±7.5	76.8±9.2	0.81
Pulse rate (beats/min)	73.7±14.0	74.4±14.2	0.82
Total energy intake (kcal/g/d)	2165.1±774.3	2128.1±720.3	0.82
Leisure physical activity score† (arbitrary units)	28.2±24.9	20.4±19.0	0.08
Family history of DM2 (n [%])	34 [77.3]	32 [76.2]	0.55
Family history of CVD and/or HTN (n [%])	32 [72.7]	34 [81.0]	0.26
Insulin sensitivity measures			
Fasting plasma glucose (mmol/L)	5.2±1.4	5.3±1.3	0.68
Fasting insulin (μIU/mL)	13.0±10.0	15.6±12.2	0.25
HbA1c (%)	5.2±0.4	5.3±0.5	0.27
HOMA-IR index	3.0±2.3	4.0±4.0	0.16
Fasting insulin/glucose ratio	0.2±0.1	0.2±0.1	0.55
Lipid profile			
TC (mmol/L)	4.7±1.0	4.5±0.7	0.38
TG (mmol/L)	1.4±0.8	1.3±0.7	0.81
HDL-C (mmol/L)	1.3±0.3	1.3±0.4	0.80
LDL-C (mmol/L)	2.8±0.9	2.6±0.7	0.31
TC/HDL-C ratio	3.8±1.2	3.7±1.3	0.84
Prevalence of MetS‡ (n [%])	18 [38.3]	16 [33.3]	0.67
Presence of hirsutism§ (n [%])	32 [68.1]	35 [72.9]	0.39
Endocrine parameters			
LH/FSH ratio	2.2±1.1	2.6±1.7	0.32

Total testosterone (nmol/L)	1.5±0.5	1.7±1.1	0.41
Sex–hormone binding globulin (nmoL/L)	32.0±18.7	36.5±25.8	0.60
Free androgen index	5.8±3.7	7.6±7.6	0.17
hsCRP (mg/L)	4.0±3.8	5.5±6.6	0.18

Data are expressed as mean±SD except indicated otherwise. Numbers in each group for baseline characterises of all women who were enrolled in the study were as follows: pulse-based diet and aerobic exercise group=47; TLC diet and aerobic exercise group=48.

Abbreviations: PCOS, polycystic ovary syndrome; TLC, Therapeutic Lifestyle Changes; Tx, therapy; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM2, type 2 diabetes; CVD, cardiovascular disease; HTN, hypertension; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC/HDL-C, ratio of total cholesterol/high-density lipoprotein cholesterol; LH/FSH, ratio of luteinizing hormone to follicle stimulating hormone; SHBG, sex–hormone binding globulin; hsCRP, highly sensitive C–reactive protein.

*Student t-test and chi-squared test were used for comparisons of means and proportions between groups. †Determined using the Godin Leisure-Time Exercise Questionnaire (Godin & Shephard, 1985). ‡Determined according to the 2005 International Diabetes Federation in collaboration with the American Heart Association/National Heart, Lung, and Blood Institute criteria (Alberti et al., 2009). §Determined using the Ferriman–Gallwey Index, adjusted for ethnicity (Yildiz et al., 2010)

Table 9. Anthropometric, body composition, physiologic, insulin sensitivity, and lipid outcomes at baseline and after intervention

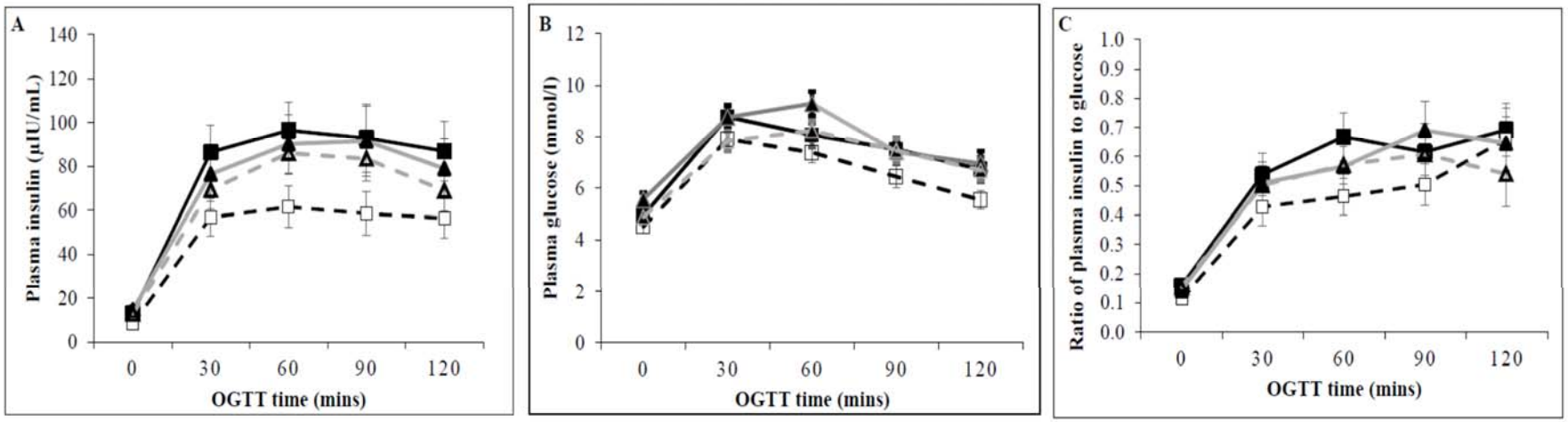
	Pulse-based diet group			**TLC diet group**			**P value**	
	Baseline	16-weeks	Change	Baseline	16-weeks	Change	Time	Group x Time
Anthropometric and body composition measures								
Weight (kg)	89.9±27.0	84.4±26.8	-5.5±4.5	93.3±25.4	88.4±23.0	-4.9±15.8	<0.01	0.62
BMI (kg/m^2)	33.3±9.0	32.0±9.0	-1.3±1.4	34.0±9.8	32.2±8.6	-1.8±6.1	0 01	0.62
WC (cm)	103.9±19.8	99.5±18.0	-4.4±11.2	103.5±20.2	101.8±19.3	-1.7±7.6	0.02	0.30
Total body fat mass (kg)	36.3±13.5	34.6±13.8	-1.7±2.4	40.5±15.0	37.5±15.3	-3.0±7.5	<0.01	0.41
Trunk fat mass (kg)	16.0±13.8	14.9±13.3	-1.1±2.0	19.3±14.0	17.3±13.5	-2±3.9	<0.0001	0.25
Total body fat (%)	41.1±7.2	40.1±7.8	-1.0±2.0	41.4±8.7	40.4±8.5	-1.0±2.4	<0.01	0.96
Total body lean mass (kg)	47.5±8.1	46.8±8.1	-0.7±2.2	49.5±9.1	49.7±9.3	0.2±14.1	0.22	0.09
Physiologic measures								
SBP (mmHg)	116.0±7.3	112.6±10.3	-3.4±8.3	117.9±10.5	112.8±9.9	-5.1±8.1	<0.001	0.45
DBP (mmHg)	77.2±7.4	73.6±8.0	-3.6±6.7	77.1±9.4	76.9±9.8	-0.2±6.7	0.03	0.05
Pulse rate (beats/min)	73.6±14.4	74.0±14.3	0.4±10.0	73.0±12.5	73.0±14.1	0.0±11.8	0.89	0.89
Insulin sensitivity measures								
FPG (mmol/L)	5.0±1.5	4.6±1.3	-0.4±1.7	5.6±1.4	4.8±1.6	-0.8±1.5	<0.01	0.38
Fasting insulin (µIU/mL)	14.0±11.4	10.0±7.7	-4.0±9.7	15.7±12.4	12.7±10.3	-3.0±6.8	<0.01	0.60
HbA1c (%)	5.3±0.4	5.2±0.4	-0.1±0.3	5.3±0.5	5.3±0.4	0.0±0.3	0.18	0.71
HOMA-IR index	3.1±2.5	2.1±1.9	-1.0±2.1	4.2±4.4	2.9±3.6	-1.3±2.1	<0.001	0.66
Fasting insulin/glucose ratio	0.2±0.1	0.1±0.1	-0.0±0.1	0.2±0.1	0.1±0.1	-0.1±0.1	0.21	0.17
Total insulin AUC (µIU/mL/min)	326.9±266.5	205.9±106.7	-121.0±229.9	307.2±181.7	279.8±176.7	-27.4±110.2	<0.01	0.05

Incremental insulin AUC (μIU/mL/min)	49.8±45.7	32.5±22.4	-17.3±47.2	45.6±26.1	41.1±29.3	-4.5±22.1	0.03	0.19
Total glucose AUC (mmol/L/min)	32.3±9.6	26.8±5.3	5.5±10.4	33.7±5.8	29.7±7.1	-4.0±6.3	<0.01	0.77
Incremental glucose AUC (mmol/L/min)	6.7±1.9	6.2±1.4	-0.5±2.3	7.2±1.5	6.4±1.5	-0.8±1.5	<0.0001	0.51
Lipid profile								
TC (mmol/L)	5.0±1.0	4.6±0.8	-0.4±0.5	4.4±0.8	4.3±0.8	-0.1±0.5	<0.01	0.12
TG (mmol/L)	1.5±0.8	1.3±0.7	-0.2±0.6	1.3±0.7	1.3±0.8	0±0.5	0.36	0.04
HDL-C (mmol/L)	1.3±0.3	1.4±0.3	0.1±0.2	1.3±0.4	1.2±0.3	-0.1±0.2	0.64	<0.01
LDL-C (mmol/L)	2.9±0.4	2.7±0.8	-0.2±0.4	2.6±0.7	2.5±0.6	-0.1±0.4	<0.01	0.05
TC/HDL-C	4.0±1.2	3.6±1.1	-0.4±0.4	3.7±1.3	3.8±1.3	0.1±0.4	0.01	<0.001
hsCRP (mg/L)	4.2±3.8	3.9±4.8	-0.3±3.4	5.0±6.4	5.0±8.2	0.0±4.2	0.78	0.72

Data are expressed as mean±SD. Numbers in each group were as follows: pulse-based diet group=31 (30 for insulin sensitivity data); TLC diet group=30 (29 for insulin sensitivity data).

Abbreviations: TLC, Therapeutic Lifestyle Changes; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; AUC, Area under the curve; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC/HDL-C, ratio of total cholesterol/high-density lipoprotein cholesterol.

Figure 5. Plasma insulin and glucose response to a standard 75-g OGTT before and after 16-week of intervention combined with combined with aerobic exercise and healthcare counselling in the pulse-based diet and TLC diet groups



Solid dark lines represent women in the pulse-diet group at baseline (n=30). Solid light lines represent women in the TLC diet group at baseline (n=29). Dotted dark lines represent women in the pulse-diet group after intervention (n=30). Dotted light lines represent women in the TLC diet group after intervention (n=29). Insulin time course in response to OGTT (A); glucose time course in response to OGTT (B); ratio of plasma insulin to glucose time course in response to OGTT (C). Groups were comparable at baseline. Data are expressed as mean±SEM. Abbreviations: OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; TLC, Therapeutic Lifestyle Changes.

Table 10. Clinical and biochemical characteristics of participants who completed the 6-months follow up phase of the study at baseline, 16-weeks, and 6 months post-intervention

	Pulse-based diet group				**TLC diet**		**P value**	
	Baseline	16-weeks	6 months	Baseline	16-weeks	6 months	Time	Group x Time
Anthropometric and body composition measures								
Weight (kg)	81.6±13.5	78.0±12.6	79.6±13.4	95.8±21.1	92.0±20.5	94.8±18.1	<0.00001	0.43
BMI (kg/m^2)	30.4±6.2	29.0±5.6	31.2±7.2	35.1±9.1	33.9±8.9	34.7±8.8	<0.0001	0.30
WC (cm)	98.0±13.7	94.6±13.6	94.9±14.2	108.2±20.6	105.5±17.7	105.5±17.1	<0.005	0.95
Total body fat mass (kg)	32.3±10.7	29.8±10.1	30.3±9.9	42.7±14.3	40.3±14.3	41.5±14.9	<0.001	0.67
Total body fat (%)	38.9±7.8	37.3±8.1	37.4±7.6	43.8±8.2	42.3±8.0	43.0±8.1	0.001	0.52
Total body lean mass (kg)	46.5±4.4	45.7±4.7	46.7±4.9	51.0±8.4	51.1±8.5	51.4±8.6	0.18	0.42
Physiologic measures								
SBP (mmHg)	115.1±8.2	112.0±10.7	111.7±8.8	120.9±10.5	114.9±9.4	117.8±10.8	0.01	0.49
DBP (mmHg)	75.4±7.4	71.3±7.1	73.6±7.8	80.8±6.9	80.6±5.0	76.7±8.2	0.11	0.01
Pulse rate (beats/min)	73.1±13.7	70.1±13.9	69.5±15.3	72.8±15.8	73.6±13.2	76.8±9.6	0.78	0.27
Insulin sensitivity measures								
FPG (mmol/L)	5.3±1.7	4.6±1.6	4.9±0.2	5.5±1.5	4.6±1.8	5.3±0.9	0.08	0.86
Fasting insulin (µIU/mL)	13.5±12.8	9.8±8.7	13.3±11.2	14.6.1±3.6	10.4±11.9	16.7±9.1	0.002	0.83
Lipid profile								
TC (mmol/L)	4.7±1.1	4.4±0.8	4.6±0.8	4.4±0.8	4.2±0.8	4.4±0.7	<0.01	0.90

TG (mmol/L)	1.4±0.9	1.3±0.8	1.3±0.7	1.2±0.6	1.2±0.6	1.3±0.6	0.68	0.86
HDL-C (mmol/L)	1.2±0.3	1.3±0.4	1.3±0.4	1.3±0.4	1.2±0.3	1.2±0.2	0.98	0.02
LDL-C (mmol/L)	2.8±1.0	2.4±0.8	2.6±0.8	2.5±0.7	2.4±0.6	2.6±0.5	0.008	0.35
TC/HDL-C	4.0±1.5	3.6±1.4	3.6±1.2	3.5±0.8	3.5±1.0	3.6±0.8	0.005	0.02
hsCRP (mg/L)	3.2±3.4	2.4±2.8	2.9±4.1	5.2±7.3	6.3±10.8	5.6±7.4	0.99	0.22

PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high–density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, highly sensitive C–reactive protein.

Data are expressed as mean±SD. Numbers in each group for anthropometric, body composition, and physiologic measures were as follows: Pulse-based diet group=20; TLC diet group=19. Numbers in each group for insulin sensitivity measures, lipid profile, and hsCRP were as follows: Pulse-based diet group=16; TLC diet group=16.

Table 11. Clinical and biochemical characteristics of participants who completed the 12-months follow up phase of the study at baseline, 16-weeks, and 12 months post-intervention

	Pulse-based diet group				**TLC diet group**		**P value**	
	Baseline	16-weeks	12 months	Baseline	16-weeks	12 months	Time	Group x Time
Anthropometric and body composition measures								
Weight (kg)	84.0±14.6	78.9±11.7	80.4±14.0	96.1±24.6	92.8±24.9	95.9±27.4	0.01	0.53
BMI (kg/m^2)	31.6±6.0	30.0±5.5	30.7±6.8	35.5±10.9	34.5±10.6	35.3±11.2	0.02	0.74
WC (cm)	98.7±11.6	94.4±9.3	93.6±11.4	107.6±24.6	104.3±20.9	105.5±21.7	0.13	0.66
Total body fat mass (kg)	32.2±11.1	29.4±9.2	29.8±9.9	42.1±16.2	40.9±16.5	41.3±16.9	0.003	0.34
Total body fat (%)	39.4±8.0	37.7±7.2	38.2±7.6	44.6±8.4	43.9±8.0	43.6±7.9	0.01	0.45
Total body lean mass (kg)	45.6±3.5	45.4±3.9	45.2±4.3	48.7±10.2	48.4±1.0	49.4±1.0	0.49	0.50
Physiologic measures								
SBP (mmHg)	112.4±8.1	109.1±10.9	113.8±8.3	120.9±10.9	112.1±9.4	116.7±10.8	0.02	0.17
DBP (mmHg)	76.4±6.4	73.2±7.5	76.0±7.6	79.5 ± 7.8	77.7±6.1	77.7±6.3	0.12	0.64
Pulse rate (beats/min)	75.8±16.2	71.5±12.7	70.2±10.2	72.9±17.4	73.6±14.5	76.6±18.0	0.85	0.50
Insulin sensitivity measures								
FPG (mmol/L)	5.2±1.1	4.3±0.8	4.9±0.6	5.5±1.4	4.7±1.9	5.3±0.5	0.04	0.98
Fasting insulin (µIU/mL)	16.3±15.3	11.4±11.2	14.1±9.6	18.0±17.3	11.9±12.6	16.5±10.0	0.02	0.90
Lipid profile								
TC (mmol/L)	4.8±1.1	4.5±0.9	5.0±1.1	4.8±0.5	4.5±0.5	4.7±0.7	0.001	0.43
TG (mmol/L)	1.5±0.8	1.2±0.5	1.4±0.6	1.2±0.6	1.2±0.5	1.4±0.6	0.41	0.38
HDL-C (mmol/L)	1.1±0.2	1.3±0.4	1.3±0.3	1.4±0.4	1.3±0.2	1.4±0.4	0.22	0.02
LDL-C (mmol/L)	3.0±1.1	2.6±0.8	2.9±1.0	2.7±0.5	2.6±0.4	2.5±0.7	0.02	0.15

TC/HDL-C	4.4±1.3	3.8±1.2	3.7±0.9	3.6±0.8	3.5±0.7	3.6±0.8	0.003	0.005
hsCRP (mg/L)	4.5±4.0	5.0±7.6	5.8±4.4	5.9±7.7	7.1±11.4	8.0±14.4	0.48	0.95

PCOS, polycystic ovary syndrome; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; HDL-C, high–density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; hsCRP, highly sensitive C–reactive protein.

Data are expressed as mean±SD. Numbers in each group for anthropometric, body composition, and physiologic measures were as follows: Pulse-based diet group=19; TLC diet group=17. Numbers in each group for insulin sensitivity measures, lipid profile, hsCRP, and endocrine measures were as follows: Pulse-based diet group=12; TLC diet group=13.

5.4. Discussion

The most significant finding from our PCOS study was that without inducing energy restriction, a low-GI pulse-based diet was more effective at decreasing total insulin AUC, concentrations of LDL-C, TG, TC/HDL-C ratio, DBP, and increasing concentration of HDL-C than the TLC diet. Improved lipid profiles were noteworthy as the control TLC diet is recommended to elicit LDL-C lowering effects in subjects at risk for CVD and DM2 ("The NCEP ATP III final report," 2002); the pulse-based diet was even more effective in the lowering effect on LDL-C. We showed greater favourable effects with the pulse-based diet than the heart-healthy TLC diet. Similar improvements in both dietary groups were seen with glucoregulation, BMI, WC, %body fat, SBP, and TC in women with PCOS. The current study was a unique multi-dimensional lifestyle changes program designed to evaluate the potential of a low-GI pulse-rich diet, without energy restriction, to improve the cardio-metabolic and DM2 risk profiles in young women with PCOS.

Our observations are in agreement with previous studies regarding the positive effects of dietary pulses upon risk indicators of MetS, CVD, and DM2 (Abeysekara et al., 2012; Anderson & Major, 2002; Ha et al., 2014; Jayalath et al., 2014; Sievenpiper et al., 2009). There was a greater reduction from baseline of TC by 4%, LDL-C by 8.2%, and TC/HDL-C ratio by 12.7% in the pulse diet group compared with the TLC diet group; by extrapolation, an estimated risk reduction of 8-12% in future major cardiovascular events may be realized (Cholesterol Treatment Trialists' (CTT) Collaborators et al., 2012; Manson et al., 1992). Results of two meta-analyses of 26 RCTs (n=1037) (Ha et al., 2014) and 10 RCTs (n=268) (Bazzano et al., 2011) established evidence for a modest reduction in LDL-C (-0.17 to -0.21 mmol/L) and TC (-0.31 mmol/L), following the consumption of pulses over 3-16 weeks across age, BMI classes, and metabolic phenotypes. Hypocholesterolemic effects of pulses appear to be multifactorial and can be attributed to their fiber content and antioxidant capacity. In our study, the pulse-based diet group had higher dietary fiber intake than the TLC diet group during the intervention (i.e. an increase in fiber intake of ~10 versus 1 g/d, respectively). Approximately 60% of the fiber in pulses is insoluble, and 31% is soluble. Both types of fiber in pulses modulate blood cholesterol by binding to bile acids in the intestine and preventing the reabsorption of bile acids and cholesterol (Brown et al., 1999; Galisteo et al., 2008; Kishimoto et al., 1995). Gut microbiota in the colon ferment dietary fiber and produce short-chain fatty acids. Short-chain fatty acids are

energy and signalling molecules with prebiotic effects involved in the retention of beneficial bacterial flora, regulation of hepatic gluconeogenesis, lipogenesis, and lipid storage (Kishimoto et al., 1995; Koh et al., 2016; van Bennekum et al., 2005). Tannins, flavonoids, polyphenols, phytates, and saponins found in pulses have antioxidant effects (Mudryj et al., 2014). The inhibition of oxidation of LDL-C and modulation of TC concentrations are attributed to dietary pulses (Mudryj et al., 2014).

Following the intervention, the pulse-based diet group had a greater decrease in total insulin AUC during an OGTT when compared to the TLC diet group. An increase in insulin sensitivity in response to a pulse-based diet may be due to relatively slow digestibility and thus low GI of pulses. Low GI of the pulse-rich foods has been attributed to complex carbohydrate profile, protein composition, protein-starch matrix, and anti-nutrient factors of pulses including phytates, saponins, lectins, and tannins (McCrory et al., 2010; Mudryj et al., 2014; Sievenpiper et al., 2009; Thorne et al., 1983). The direct effect of pulses on glucoregulation, recommended dosage of pulses, and optimal duration of pulse consumption needed to prevent insulin resistance, impaired glucose tolerance and DM2 is less clear (Jacobs et al., 1998; Jang et al., 2001; Swennen et al., 2006).

In addition to improving lipid and insulin profiles, the pulse-based diet group exhibited a 4.4% greater reduction in DBP when compared to the TLC diet group. A reduction of this change of DBP is estimated to reduce the risk of myocardial infarction by 9-13% (Manson et al., 1992). Jayalath et al. performed a meta-analysis on eight isocaloric trials (n=554 participants) and showed pulse consumption (~162 g/d) over ten weeks reduced mean arterial BP by -0.75 mmHg in middle-aged subjects (Jayalath et al., 2014). A diet rich in pulses shares many nutritional characteristics with the Dietary Approaches to Stop Hypertension eating plan (Sacks et al., 2001b) and the Mediterranean diet (Willett et al., 1995) that are endorsed for the prevention and treatment of HTN. Pulses can confer BP-lowering effects by increasing dietary intakes of low-GI foods, dietary fiber, plant protein, potassium, and magnesium, as well as by decreasing sodium intake through pre-established mechanisms (Aburto et al., 2013; Altorf–van der Kuil et al., 2010; Jee et al., 2002; Mudryj et al., 2014; Tielemans et al., 2013).

Lifestyle management research in PCOS has been focused primarily upon short-term calorie restriction designed to achieve weight loss. In overweight and obese women with PCOS, weight loss has been considered effective to improve the prognosis for long-term health (Marsh

& Brand-Miller, 2005). However, weight loss through calorie restriction often has not been attainable or sustainable (Legro, 2017). Innovative PCOS-specific diets have been largely overlooked as a means to achieve persistent, long-term lifestyle change. The optimal dietary recommendations for obese and non-obese women with PCOS has been debated. Our results align with the findings of Marsh et al. (Marsh et al., 2010) who found positive effects on insulin sensitivity with an *ad libitum* low-GI diet when compared with a macronutrient- and fiber-matched healthy diet. Increased insulin sensitivity was attributed to the consumption of low-GI foods and a modest weight loss (4-5% of baseline body weight). It should be noted that the low-GI diet of the Marsh et al. study had very few pulse-based foods, and in contrast to our study, no improvement in lipid profiles was observed. While our study goals were not focused on weight loss, our subjects achieved a 5% weight loss across both diet groups. The observed weight loss can be attributed to a voluntary decrease in energy intake following education about lifestyle modification and increase in physical activity. A 5-10% weight loss is considered clinically significant and has been associated with a substantial improvement in the metabolic profile and risk factors for CVD and DM2 (Knowler et al., 2002; Orchard et al., 2005).

The participation attrition rate was high (33.7%) in both groups in the current study. A high attrition rate (27-49%) in other RCTs focused on lifestyle changes in women with PCOS reported a progressive increase in dropouts corresponding with the duration of intervention (Crystal C. Douglas et al., 2006; Hoeger et al., 2004; Marsh et al., 2010; Turner-McGrievy et al., 2014b). Difficulties in sustaining a new lifestyle program, the time-consuming nature of the intervention, and competing family, work, and school obligations were among the reasons that contributed to attrition rate of our participants. During the study, we found regular communication for assessments, provision of education, encouragement, and feedback by a multidisciplinary healthcare team improved our participants' adherence to new lifestyle strategies specific to women with PCOS and likely decreased the rate of attrition; we postulate that more frequent, regular communication with participants as a deliberate study design feature may have increased long-term adherence and maintained long-term improvements over the 6 and 12 month follow-up intervals. We also postulate that providing pulse-based foods to participants may have left participants ill-equipped to prepare and include pulses in their meals following the 16-week intervention. In the long-term women in the pulse-based diet group maintained some of the beneficial impacts of the intervention on lipid profile, including HDL-C and TC/HDL-C

levels. However, both groups tended to regain weight and exhibited signs of deteriorated cardio-metabolic profile, reflected by increased fasting insulin and TC levels in long-term after the completion of the intervention. As elaborated in Chapters 4 and 7, these observations can be contributed to a sub-optimal adherence to healthy lifestyle practices including lower frequency of pulse consumption, less favourable diet quality, and decreased physical activity. The duration of our intervention might not have been adequate to sustain and maintain the newly adopted healthy lifestyle practices in our participants in long-term. Our observations reinforce the importance of long-term treatment and continual monitoring of patients by their healthcare providers.

Strengths of the study included diverse ethnicity sample with ~25% of subjects being non-Caucasian; a well-defined PCOS population; and use of ethno-specific adjustments when determining hirsutism to diagnose PCOS and abdominal obesity to identify MetS. Limitations included a high attrition rate, economic limitations against recruiting more subjects, overlooking an invitation to indigenous populations by advertising in the urban areas where they live, spending more time with follow-up communication to ensure long-term dietary and exercise adherence and potential for recall and self-reporting bias for physical activity and dietary intake. Although a 24-hour recall was the most accurate/least biased method for self-reporting dietary intake, our participants' dietary recall may have had a tendency toward underreporting and reactivity (Thompson et al., 2015). Our study was underpowered to detect FPG differences between groups post-intervention. Our results may be interpreted with caution in terms of generalization to all PCOS populations.

In conclusion, in a diet intervention without energy restriction where aerobic exercise was recommended as a part of a healthy lifestyle and healthcare counselling was provided, the pulse-based diet was more effective than the TLC diet for improving insulin response to OGTT, concentrations of TG, LDL-C, HDL-C, TC/HDL-C ratio, and DBP in women with PCOS. Our results support the position that lifestyle modifications are crucial in the management of PCOS and add another dimension to the existing evidence concerning the positive effects of pulse consumption on cardio-metabolic and DM2 risk profiles in women with PCOS.

In Chapters 4 and 5 the reproductive health and cardiometabolic risk outcome components of research Objective ii were addressed. We demonstrated the positive impacts of our multidimensional lifestyle change program comprised of dietary, exercise, education, and healthcare counselling on multiple clinical and biochemical outcomes associated with PCO

following a 16-week intervention. In the next Chapter, Chapter 6, the third component of Objective ii, effects of the intervention on HRQoL indices in women with PCOS, will be addressed.

CHAPTER 6

EFFECTS OF THE LIFESTYLE INTERVENTION ON QUALITY OF LIFE IN WOMEN WITH PCOS

Kazemi M, McBreairty LE, Pierson RA, Chilibeck PD, Gordon JJ, Serrao SB, Zello GA, Chizen DR.

Quality of life in a polycystic ovary syndrome randomized controlled pulse-diet trial, with exercise*

**format and structure of the original manuscript have been revised to provide a better flow within the thesis and be consistent with the format of previous chapters*

Abstract

Background and objective: PCOS is the most common endocrinopathy and cause of anovulatory infertility among reproductive-age women. Aside from metabolic and reproductive complications, PCOS is associated with compromised HRQoL which is often neglected. The objective of the present study was to compare PCOS-specific HRQoL indices among women with PCOS before and after receiving a pulse-based diet, a low glycemic index diet including split peas, lentils, beans, and chickpeas, or the TLC diet, alongside exercise, education and counselling.

Methods: Ninety-five women with PCOS (18-35 years) enrolled in a 16-week lifestyle changes trial were randomized to Pulse or TLC diets, without calorie restriction. All participants completed aerobic exercise and received education and counselling about PCOS and lifestyle modification. The HRQoL survey was administered before and after the education, counselling and diet intervention. The recruitment sample size was calculated with a study drop-out rate of 32% to achieve n=34 per diet group. The primary study outcome was to observe changes in HRQoL domains over time for each diet group.

Results: Of 95 enrolled women, 30 assigned to receive the pulse-based diet and 31 in TLC diet group completed the diet intervention. Ninety percent (55/61) of women completed the HRQoL survey pre- and post-intervention. Despite no caloric restriction, both lifestyle intervention groups achieved weight loss ($P<0.01$). There were no differences between groups

for all outcomes. The HRQoL scores of both groups increased in the domains of knowledge ($P<0.0001$), concerns about PCOS ($P<0.05$), healthcare satisfaction ($P<0.001$), and lifestyle practices comprised of physical activity ($P<.0001$) and healthy diets ($P<0.001$). Women had greater positive feelings and experiences about participating in the intervention ($P<0.01$). The greatest positive changes occurred in the healthy eating domain (effect size=0.68). Healthy lifestyle changes were regarded by participants as the most helpful and least anticipated management strategies for PCOS.

Conclusion: Both dietary interventions, without calorie restriction, in combination with aerobic exercise and healthcare counselling, yielded substantial improvements in all evaluated domains of HRQoL in women with PCOS.

Keywords: Aerobic exercise; Diet; Health-related quality of life, Lifestyle, Pulse-foods

6.1 Introduction

The PCOS is a common endocrinopathy among reproductive-age women with a prevalence of up to 18% (Carmina & Lobo, 1999; Knochenhauer, 1998; March et al., 2010). Most women with PCOS present with excess weight and metabolic complications, including insulin resistance, glucose intolerance, dyslipidemia, and predisposition to develop DM2 and CVD (Carmina & Lobo, 1999; Teede et al., 2006). Women with PCOS are affected by several psychological issues: altered self-perception, altered feminine identity, eating disorders, anxiety, depression, social phobia, and suicidal attempts (Deeks et al., 2010b; Elsenbruch et al., 2003). Mental-health and health-related quality of life (HRQoL) are adversely affected in many women with PCOS (Deeks et al., 2010a; Rasgon et al., 2003). HRQoL is defined as the "physical, psychological, and social domains of health", described as "distinct areas influenced by a person's experiences, beliefs, expectations, and perceptions" (Testa & Simonson, 1996). Healthcare providers can assess HRQoL, design individually-tailored therapies to control PCOS symptoms, and evaluate the effectiveness of treatment, that are relevant from patients' perspectives (Crosby et al., 2003; Wilson & Cleary, 1995).

There is limited evidence to demonstrate positive effects of lifestyle modification on HRQoL in women with PCOS. A pulse-based diet (i.e., a low-GI diet including split peas,

lentils, and chickpeas) has a favourable nutritional composition. Chronic pulse consumption has been shown to modify postprandial glucose and insulin levels, and potentially reduce the risk of DM2 and CVD (Ha et al., 2014; Sievenpiper et al., 2009). The TLC diet has been considered a standard healthy diet with the capacity to improve lipid profiles. Increased insulin sensitivity alleviated metabolic disruptions, and weight management, can exert psychological benefits and positively impact HRQoL of women with PCOS (Harris-Glocker et al., 2010; McCook et al., 2005; Thomson et al., 2010). We hypothesized that independent of calorie restriction, a pulse-based diet would be more effective than the TLC diet in improving HRQoL in women with PCOS who had participated in an exercise program and received healthcare counselling.

6.2. Materials and Methods

6.2.1. Study Design and Protocol

Details of the study design, randomization, dietary and exercise interventions, and ethics have been presented in Chapter 4, page 69. A brief description about the education and healthcare counselling provided to the study subjects follows.

Before randomization to the diet groups, each participant received two four-hour sessions of counselling as a standard of care. Counselling topics included the human menstrual cycle, ovarian morphology in normal ovulating women, polycystic ovarian morphology, criteria used to diagnose PCOS and pathophysiology of PCOS. Other topics included risk determinants, complications, and associated comorbidities of PCOS, as well as medical and lifestyle (i.e., diet and exercise) options to manage PCOS. The counselling was delivered by a gynaecologist, an MSc and a PhD researcher. All personnel had specialization in reproductive endocrinology and clinical nutrition. Before randomization, and after the initial HRQoL survey, each participant received an individualized dietary consultation session by a registered dietitian for approximately 1.5 hours. During the dietary consultation session, all women received a guide booklet outlining TLC diet guidelines.

The HRQoL survey was given to the participants after the diagnosis of PCOS, but before the start of the counselling and intervention, and was repeated at the end of the intervention. Compliance with diet and exercise regimens was monitored by self-administered food and exercise daily logs completed by participants throughout the intervention.

6.2.2. Participants

Details of study subjects have been presented in Chapter 4, page 71.

6.2.3. HRQoL Survey

Participants' HRQoL was assessed using a researcher-devised and validated PCOS-specific survey described elsewhere (Colwell et al., 2010). The survey was administered at baseline and after the completion of the 16-week intervention. The survey was revised to be compatible with the administration at baseline and after the completion of the intervention. The survey was used to evaluate quantitative changes in HRQoL scores after participating in the study in the domains of knowledge, concerns, and understanding about PCOS, documentation and tabulation of participants' lifestyle behaviours, and evaluation of participants' satisfaction with prior healthcare services. Also, the survey was used qualitatively as a means to gauge the participants' feelings and experiences about their PCOS diagnosis, and their attitude and comfort level toward participation in the study.

The HRQoL survey of 82 items was devised to gather quantitative and qualitative information. Quantitative information was obtained using 7 domains: health concerns (12 items), healthcare satisfaction (5 items), knowledge about PCOS (23 items), healthy lifestyle behaviours (6 items about active living, and 8 items about eating a healthy diet), feelings and experiences about PCOS diagnosis (7 items), and feelings and experiences about participating in the lifestyle intervention (13 items). Raw data were compiled and reviewed for accuracy. A 5- or 6-point Likert scale was used to measure the extent to which each participant agreed with a suggested statement and the level of active engagement in certain lifestyle behaviours, respectively. The highest scores represented the optimal function or highest level of agreement and the lowest reflected maximum level of impairment or lowest level of agreement. The mean score of all items within a domain provided a domain score; lower scores indicated a greater negative impact. Qualitative information was obtained using eight open-ended questions. Participants were asked to share their feelings, experiences, emotions, comprehension, and comments about their condition and participation in the trial. Following the trial intervention, participants were asked to disclose the most surprising and most helpful issues perceived as a result of study participation. The HRQoL survey was completed primarily by a self-administered online survey. When participants did not have internet access, the survey was completed on paper. A summary of the HRQoL survey and the mean scores of groups for each survey item is presented in Appendix A.

6.2.4. Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were presented as mean ± SD and categorical variables as numbers and percentages. Baseline differences between continuous variables were determined by t-tests and categorical variables by chi-squared tests. An ANOVA test was used to assess baseline differences in the pulse-based diet, TLC diet, and the group who were randomized but failed to complete the HRQoL survey following the intervention. A mixed model ANOVA was used to compare changes in the scores of HRQoL domains over time (time effect) between groups (the pulse-based and TLC diets), and to examine differences in the scores of HRQoL domains over time (group by time interaction). An average effect size was calculated to estimate the magnitude of improvement in each domain over time following the intervention. Effect sizes of 0.10, 0.30 and 0.50 were deemed small, medium and large effect sizes, respectively (Aron et al., 2006). Results were considered significant at $P<0.05$.

6.3. Results

6.3.1. Demographic, Anthropometric, and Clinical Characteristics

The flow-diagram of subjects' progress through the phases of the clinical trial according to the Consolidated Standards of Reporting Trials statement is shown in Figure 6. Of 95 enrolled participants, 30 women in the pulse-based diet group and 31 women in the TLC diet group completed the intervention trial. Fifty-five (n=55; 90%) of the 61 women who completed the intervention trial also completed both initial and post-intervention HRQoL surveys: 28 in the pulse-based diet group and 27 in the TLC diet group.

Sociodemographic, anthropometric, and clinical characteristics of the women included in the analysis are shown (Table 12). The average age of women was 26.4±4.9 years, and average BMI was 33.3±8.9 kg/m^2. Participants were Caucasian (67.3%, 37/55), worked full-time (50.9%, 28/55), had a university degree (49.1%, 27/55), and were single (36.4%, 20/55). At baseline, pulse-based and TLC diet groups did not differ regarding age, ethnicity, weight, BMI, WC, sexual orientation, menarche, menstruation patterns, clinical features of PCOS, and number of pregnancies and children. Baseline characteristics of women randomized to receive the intervention who did not complete the HRQoL survey post-intervention (n=40) were not different when compared with women who completed the intervention. The level of compliance with exercise and dietary interventions have been presented in Chapter 4, pages 75 and 76.

Following the intervention, both groups exhibited a reduction in body weight (pulse-based diet group –3.8±1.3 kg, TLC diet group –5.9±2.7 kg; time main effect P=0.005): no differences were observed between groups regarding weight loss (P=0.53).

6.3.2. HRQoL Survey

Results of the comparisons of HRQoL domains are presented in Figure 7. At baseline, pulse-based diet and TLC diet groups were comparable in the domains of health concerns and knowledge about PCOS, healthcare satisfaction, active living, eating a healthy diet, and feelings and experiences toward participation in a lifestyle intervention study. Scores for all domains increased over time with no group by time interaction (Figure 7). The largest mean increases in time-effects occurred in the domains of healthy eating (P=0.0001, effect size=0.68), knowledge about PCOS (P<0.0001, effect size=0.48), physical activity (P<0.0001, effect size=0.37), healthcare satisfaction (P=.0001, effect size=0.35), feelings and experiences about participating in the lifestyle intervention (P=0.004, effect size=0.14), and health concerns (P=0.02, effect size=0.11). Of note, there were no differences in the evaluated domains at baseline among women who did not complete the HRQoL survey post-intervention and women who completed the intervention.

Analysis of results from women who completed the survey (n=55) showed most of our participants (58.2% [32/55]) were first diagnosed with PCOS as a result of participation in the study, and 49.1% (27/55) had been unaware of having male-pattern excess hair growth until being examined and informed during the pre-study diagnosis. Eighty percent (44/55) of participants were overweight or obese (BMI≥25 kg/m^2), and 70.9% (39/55) stated excess weight to be frequent among their first-degree relatives. At baseline, 76.4% (42/55) of our participants felt a need to lose weight for health reasons. However, inquiry about feelings and experiences about weight loss revealed that only 22.9% (8/35) were motivated to lose weight, and 77.1% (27/35) reported feelings of “frustration”, “anger”, “pressure”, “stigma”, “embarrassment”, “intimidation”, “desperation”, “depression”, and “sadness”, mainly due to multiple failed attempts at weight loss in the past.

Over one-third (34.3% [12/35]) of responders reported to have suffered from being “just told by doctors to lose weight”, had “lack of knowledge” and had no “tools” to lose weight. A majority of the responders (85.7% [30/35]) complained about the lack of “guidance” and “support from doctors”, which led to doubt and skepticism about the existence of an effective

and sustainable weight-control strategy for PCOS. One participant stated, “if it was easy, I [would have] had done it already”. One participant stated weight loss to be “unhealthy”, “risky”, and “inadequate”. When asked the same questions after the intervention, 32.5% (13/40) of responders reported feelings of “frustration”, “hopelessness”, and “stress” about weight loss, while 67.5% (27/40) reported to feel “motivated” and “good” to “lose more weight”. Overall, the motivated women reported to be more “confident” and “determined” to follow the newly adopted lifestyle strategies, had “the solution in their “hands”, and were “…coached on proper eating and exercise” during the study, while admitting weight management to be “important” and a “lifelong challenge”. When inquired about the implemented medical procedures during the intervention, 91.1% (41/45) of responders described the transvaginal ultrasound examination as a “positive” and “interesting” experience, and expressed their gratitude for having informative conversations about polycystic appearing ovaries on the ultrasound machine screen with the study gynecologist performing ultrasound examinations, despite feeling mild levels of pressure (37.8% [17/45]) and pain (4.4% [2/45]) by some of the responders during the procedure. Likewise, 97.4% (37/38) of responders reported the physical examination to be a “respectful”, “comfortable”, and “informative” experience. One participant reported feeling “embarrassment” when her body hair was examined. Women expressed their gratitude for being listened to, respected, engaged, and informed about PCOS during the PCOS diagnosis and healthcare counselling sessions. The responders felt that being informed about development and progress toward treatment goals during the study’s medical procedures promoted their levels of engagement and motivation during the intervention.

Figure 8 illustrates the information received from the lifestyle intervention study that women found most helpful (A) and most surprising (B). The most helpful information obtained (50.9% [28/55]) was related to management of PCOS symptoms with dietary and exercise modification and to physiological mechanisms through which PCOS can increase the risk of DM2, CVD, and endometrial cancer (12.7% [7/55]). Participants were most surprised to learn the importance of dietary and exercise modification on PCOS management (38.2% [21/55]), and secondly, to receive a positive diagnosis of PCOS and comprehend that their symptoms have been related to PCOS (12/55 [21.8%]). There were no statistical differences between the intervention groups for all the information obtained in the domain of feelings and experiences toward PCOS diagnosis.

Figure 6. CONSORT 2010 standard RCT diagram

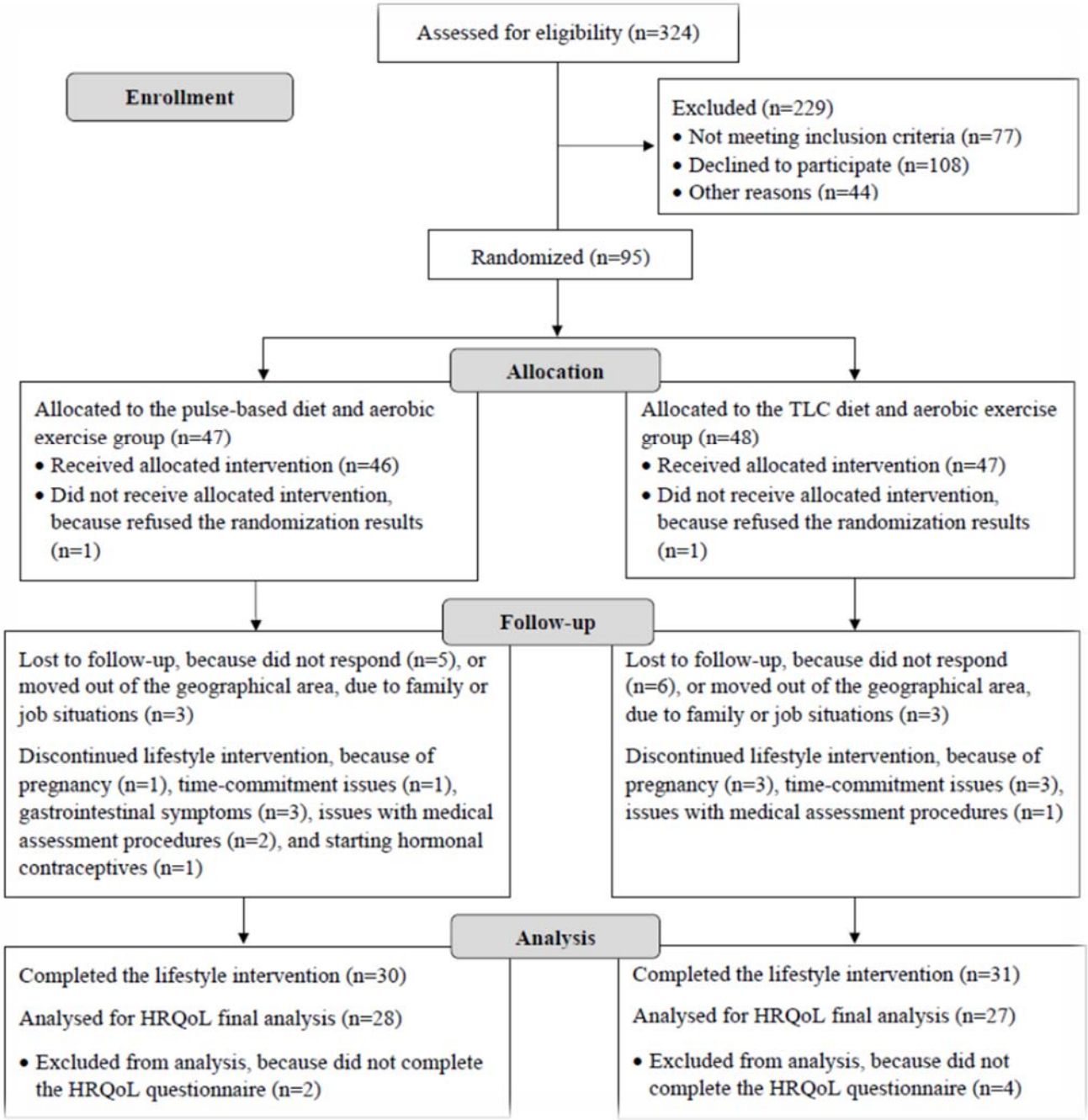


Table 12. Baseline sociodemographic, anthropometric, and clinical characteristics of women with PCOS (n=55)

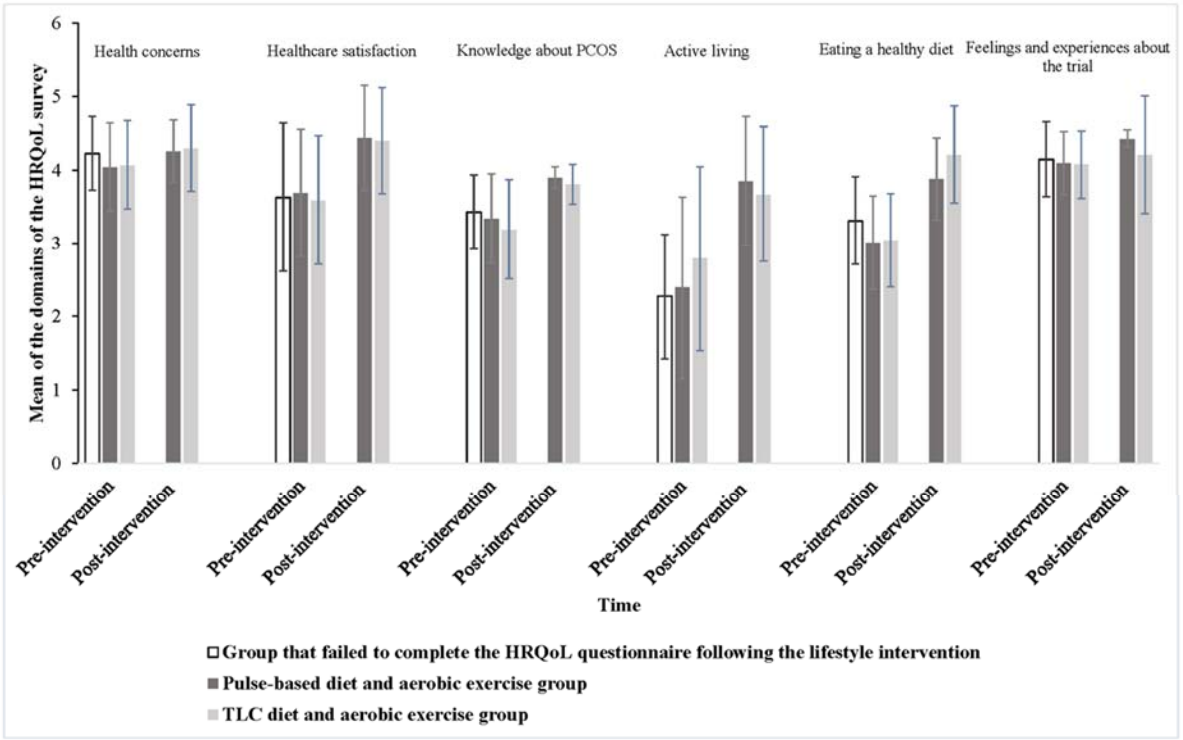
Measure (measurement unit)	**Pulse-based diet group**	**TLC diet group**	**P value***
Age (years)	26.2±4.8	26.7±5.2	0.76
Ethnicity (n [%])			
Caucasian	19 [67.8]	18 [66.7]	
Asian	7 [25.0]	8 [29.6]	0.78
Indigenous	1 [3.6]	0	
Latin American	1 [3.6]	1 [3.7]	
Anthropometric measures			
Weight (kg)	86.0±20.1	95.2±25.1	0.14
BMI (kg/m^2)	32.0±7.8	34.7±9.8	0.26
Waist circumference (cm)	102.1±18.7	103.4±21.1	0.81
Sexual orientation (n [%])			
Heterosexual	26 [92.9]	25 [92.6]	0.68
Bisexual	2 [7.1]	2 [7.4]	
Menstruation			
Menarche (year)	12.9±1.8	12.6±1.7	0.55
Shortest interval between bleeding (days)	75.4±148.8	62.8±99.2	0.74
Longest interval between bleeding (days)	129.1±143.8	168.9±303.3	0.55
Clinical presentations			
Hirsutism† (n [%])	20 [71.4]	18 [66.7]	0.46
Acne vulgaris (n [%])	19 [67.9]	19 [70.4]	0.54
Acanthosis nigricans (n [%])	17 [60.7]	18 [66.7]	0.43
Marital status (n [%])			
Single	9 [32.1]	11 [40.7]	0.20
Married/Common-law	16 [57.2]	16 [59.3]	
Divorced/Separated	3 [10.7]	0	
Number of pregnancies (n [%])			
0	19 [67.9]	24 [88.9]	
1	4 [14.3]	1 [3.7]	0.27
2	3 [10.7]	1 [3.7]	
3	2 [7.1]	1 [3.7]	
Highest level of education (n [%])	11 [39.3]	10 [37.0]	0.44
High school	2 [7.1]	5 [18.5]	
College/Technical school			
University degree	15 [53.6]	12 [44.5]	
Employment status (n [%])			
Full-time	13 [46.4]	15 [55.6]	

Part-time	1 [3.6]	1 [3.7]	0.51
Student	13 [46.4]	8 [29.6]	
Unemployed	1 [3.6]	3 [11.1]	

Abbreviations: PCOS, polycystic ovary syndrome; TLC, Therapeutic Lifestyle Changes; BMI, body mass index.

Notes: Values are mean±SD except indicated otherwise. *Student t-test and chi-squared test were used for comparisons of means and proportions between the groups. †Hirsutism was defined based on the Ferriman–Gallwey Index, adjusted for ethnicity (Yildiz et al., 2010).

Figure 7. Mean scores of the five domains of the HRQoL survey at baseline and after the 16-week of intervention.



Dark bars represent all women in the pulse-diet group (n=28); light bars represent all women in the TLC diet group (n=27); white bars represent women who failed to complete the HRQoL survey following the intervention (n=40). HRQoL domains of women who did not complete the intervention were compared with women in the pulse and TLC diet groups to determine whether women who dropped out of the study had lower scores in any of the domains of the quality of life. Groups were comparable at baseline. Improvements over time were significant ($P<0.05$) in all domains as determined by repeated measure ANOVA, with no group by time interaction. Scores are mean±SD. A 5-point Likert scale was used to measure the extent to which the participant agreed with a suggested statement, and a 6-point scale measured how frequently the participant engaged in certain lifestyle behaviours. A higher change reflects a greater improvement in each domain. HRQoL, health-related quality of life; PCOS, polycystic ovary syndrome; TLC, Therapeutic Lifestyle Changes.

Figure 8. Information received about PCOS following the intervention that participants found most helpful (A) and most surprising (B, next page; n=55).

PCOS, polycystic ovary syndrome; CVD, cardiovascular disease; DM2, diabetes type 2; MetS, metabolic syndrome; TLC, Therapeutic Lifestyle Changes.

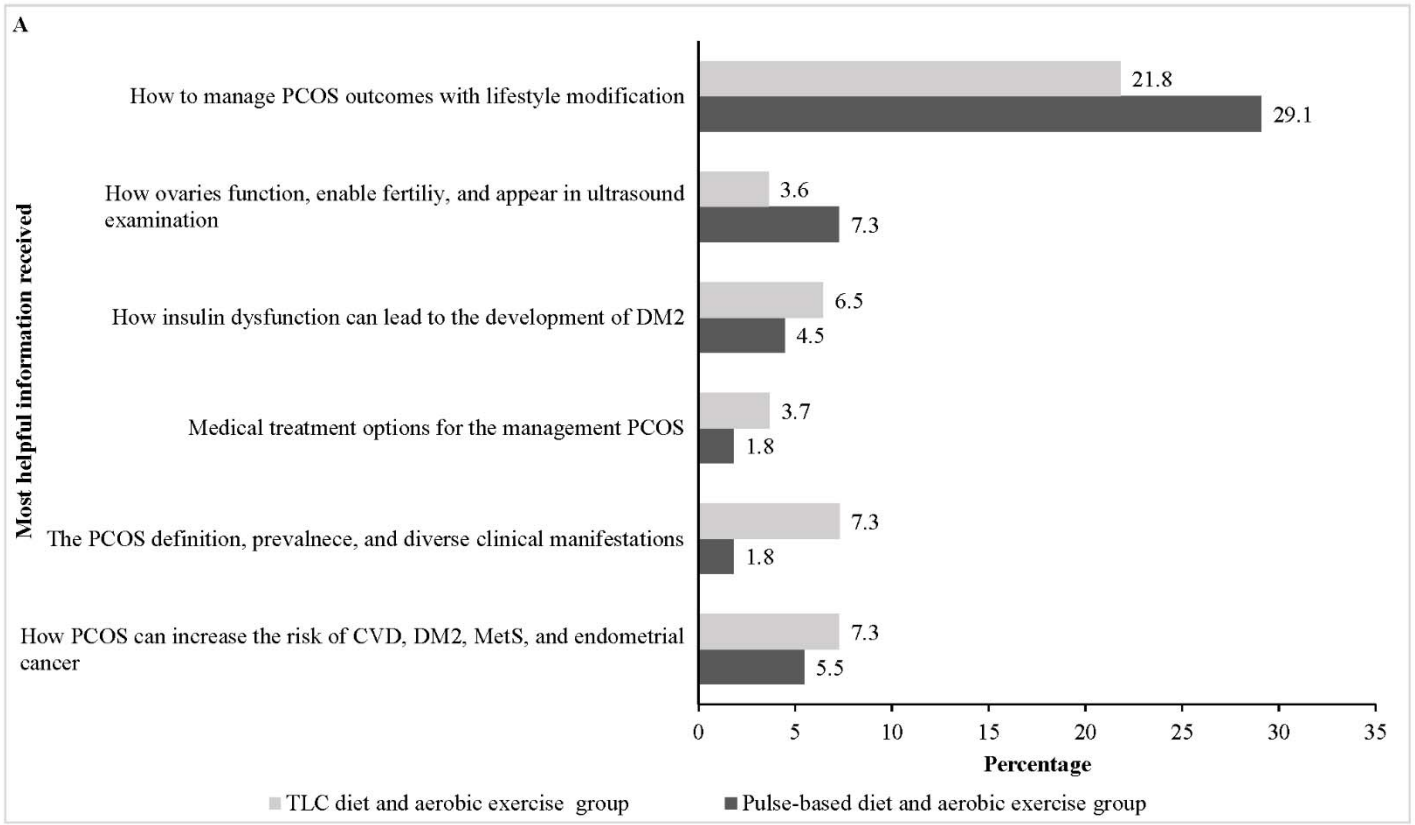
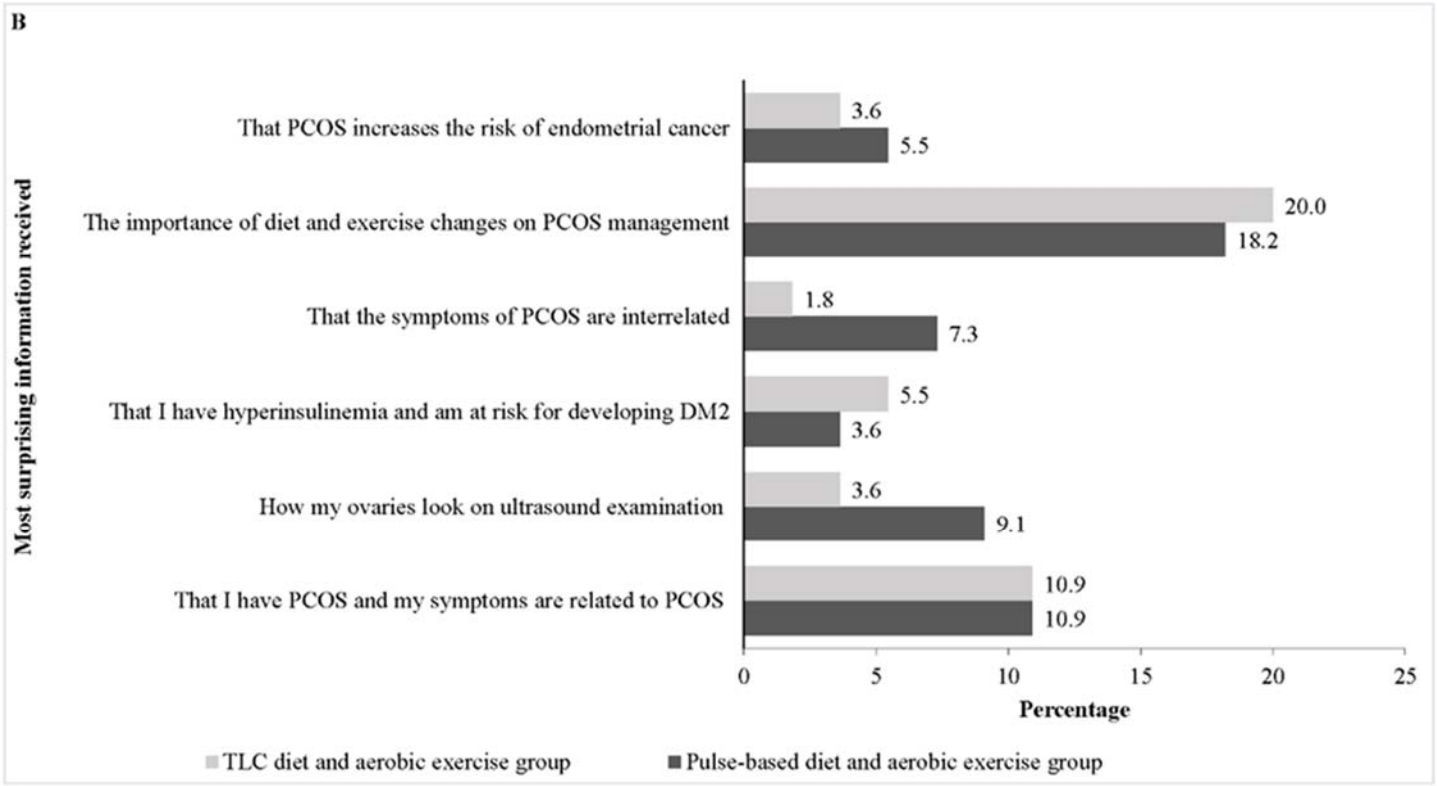


Figure 8. Continued.



6.4. Discussion

All evaluated domains of HRQoL: knowledge and concerns about PCOS; healthcare satisfaction; and lifestyle behaviours (i.e., physical activity and healthy eating) improved after women with PCOS engaged in the pulse-based and TLC diet interventions. Before the intervention, women were highly troubled by lack of information about effective, sustainable weight-control strategies for PCOS. It was very helpful to receive detailed, patient-centered lifestyle counselling sessions and education about the mechanisms and methods through which healthy eating behaviours and physical activity could improve insulin sensitivity and diminish PCOS associated health complications. Women identified dietary and physical activity recommendations as the least anticipated and most helpful information to effectively manage PCOS. The clinical implication of our study is that HRQoL improved after a multidisciplinary healthcare team delivered medical, dietary, and exercise counselling after diagnosis and study visits. Women felt empowered to manage their condition after understanding reasons for PCOS symptoms and how many acts of daily living influence improvement or deterioration of health.

Compromised HRQoL and high psychological morbidity in women with PCOS has been well documented (Elsenbruch et al., 2003; Hahn et al., 2005; McCook et al., 2005). Obesity is an important concern to women with PCOS that negatively impacts HRQoL (Susanne Hahn et al., 2006; Hahn et al., 2005; McCook et al., 2005). At baseline, over 75% of our participants had feelings of exasperation, guilt, sadness, stigma, shame, and anger about excess weight and ongoing difficulties with weight loss. Women reported a negative body image, lowered confidence and reduced quality of life following multiple unsuccessful attempts at weight loss. Persistent feelings of futility, in turn, may lower motivation to adopt healthy lifestyle changes and adhere to medical treatments. Following the intervention, our participants exhibited the greatest positive changes in the domains of healthy eating, knowledge about PCOS, and physical activity participation. The second highest positive changes were observed regarding healthcare satisfaction, feelings and experiences about participating in a research study, and health concerns about PCOS. Our observations align with the findings of Thomson et al., where women showed improvements in HRQoL and less depression after participation in a lifestyle intervention involving an energy-restricted high protein diet alone or in combination with aerobic exercise (Thomson et al., 2010). In contrast, our intervention focused on the education of women about

the development and management of PCOS, as well the promotion of physical activity and healthy eating practices without calorie restriction. Our pulse-based diet was a low fat, high fiber diet with a low glycemic index. The TLC diet was nutritionally balanced with increased fiber, decreased saturated fatty acids and cholesterol, and contained low-density lipoprotein cholesterol lowering dietary options: viscous fiber and plant stanol/sterol esters ("The NCEP ATP III final report," 2002). While study goals were not focused on calorie restriction or weight loss, both intervention groups lost weight post-intervention, improved motivation to make positive changes in their lifestyle habits and gained the confidence to continue to lose weight by implementing the learned healthy lifestyle patterns. Our findings revealed that education is integral when promoting the consumption of healthy food items, improving dietary composition, and sustaining long-term healthy eating behaviours and did not require focusing on short-term energy restriction for weight loss.

Strengths of our study methods included identifying a well-defined PCOS population; educating women about the pathophysiology and diagnosis of PCOS; enabling women to understand why recommended management strategies help; using a comprehensive PCOS-specific HRQoL survey; BMI- and age-matched treatment groups: RCT design. Because improvements in health for women with PCOS have been demonstrated when both exercise and dietary changes are achieved, we sought to include exercise for all participants with the allocation to a dietary intervention. We were therefore able to encourage two modalities of lifestyle change that would potentially improve quality of life during the study. We were limited however in ability to differentiate whether the combination of exercise and diet or either modality alone or whether the counselling sessions were integral and responsible for improvements in quantitative QOL indices. Limitations included potential recall and self-reporting bias and high participant drop-out rate. We were ethically unable to ask if “dropouts” were poorly motivated to complete the intervention. Although some results may need to be interpreted with caution, an improvement in HRQol was realized overall for both dietary groups as a result of participating in the diet and exercise interventions.

The present study supports the importance of lifestyle interventions and education to improve HRQoL in women with PCOS. The positive HRQoL outcomes in the present study may be attributed to educating women about improving a range of healthy eating practices, the favourable nutritional composition of both *ad libitum* pulse-based and TLC diets and

encouraging aerobic exercise via healthcare counselling. Our findings reinforce the urgency to educate women using a multidisciplinary approach, through regular, detailed, individualized counselling, to provide knowledge about PCOS and motivate healthy lifestyle practices. Empowering women with feasible and sustainable lifestyle and medical therapies to improve health will help women to adopt effective strategies to cope with PCOS. By identifying and minimizing barriers to long-term lifestyle modification, women's relationship with multidisciplinary healthcare providers will also improve (Brady et al., 2009; Y. Li et al., 2011). Our study results reinforce the need for physicians and allied health professionals to acknowledge the importance of providing education and counselling to improve healthy lifestyle behaviours of women with PCOS and focus not only the physical symptoms but also on ways to improve the overall quality of life of women with PCOS.

CHAPTER 7

EFFECTS OF THE LIFESTYLE INTERVENTION ON DIETARY INTAKE AND PULSE CONSUMPTION IN WOMEN WITH PCOS

Kazemi M, McBreairty LE, Chizen DR, Pierson RA, Chilibeck PD, Zello GA.

Participating in a pulse-based diet and exercise randomized clinical trial affects dietary intake and pulse consumption behaviours in women with polycystic ovary syndrome*

**format and structure of the original manuscript have been revised to provide a better flow within the thesis and be consistent with the format of previous chapters*

Abstract

Background and Objectives: Controversy surrounds the optimal diet composition to mediate favourable health-related outcomes for women with PCOS. In Canada, pulse consumption is low despite its health benefits. We hypothesized that a nutritionally balanced, low-glycemic index, pulse-based diet containing lentils, beans, split peas, and chickpeas would increase insulin sensitivity and function; thereby, improving metabolic and reproductive sequelae associated with PCOS. In a secondary outcome analysis of a randomized controlled trial, we 1) compared the effects of a pulse-based diet with the National Cholesterol Education Program TLC diet on dietary patterns in women with PCOS; and 2) evaluated the effects of participating in a pulse-based versus standard TLC dietary intervention study on knowledge, attitude, and consumption of pulses in women with PCOS using a self-administered Pulse Consumption Survey.

Methods: Dietary intake were assessed in women randomly assigned to 1 of 2 diets without energy restriction in a controlled, prospective clinical intervention using serial 24-hour dietary recalls. Ninety-five women with PCOS (18-35y) enrolled in a 16-week lifestyle changes program; 30 women assigned to the pulse-based diet group and 31 women in the TLC diet group completed the study. All women participated in an aerobic exercise program and received education and counselling about PCOS and lifestyle modification.

Results: Both the pulse-based (2273 vs 1707 kcal) and TLC diet (2211 vs 1654 kcal) groups voluntarily reduced their average daily energy intake from baseline ($P<0.001$). Dietary cholesterol intake decreased, expressed as change from baseline, in the pulse-based diet group compared to the TLC diet group (-228.8 vs 3.2 mg/d, respectively; $P<0.001$). Dietary intakes, expressed as change from baseline, increased for fiber (10.5 vs 0.0 g/d), folate (259.5 vs -48.9 μg/d), magnesium (73.1 vs -8.0 mg/d), and iron (1.9 vs -8.6 mg/d) in the pulse-based diet group compared to the TLC diet group (all $P<0.05$). The pulse-based diet group tended toward a greater decrease in the change of trans-fat intake from baseline than the TLC diet group (-0.9 vs -0.1 g/d; $P=0.06$). Following the intervention, women in the pulse-based diet group exhibited higher scores in the domain of knowledge about the nutritional composition of pulses, recommended servings of legumes based on Canada Food Guide, environmental, and economic benefits of pulse consumption when compared with the TLC diet group ($P<0.05$). Both groups exhibited increased scores in the domain of attitudes about the palatability, accessibility, preparation, and affordability of pulse foods over the 16 weeks ($P<0.01$). Changes in scores in the domain of attitude toward pulses were not different between groups over time ($P=0.11$). Following the intervention, women in the pulse-based diet group exhibited a greater frequency of pulse consumption compared with the TLC diet group (93% vs 68%; $P<0.01$).

Conclusion: Participating in a pulse-based diet intervention resulted in more favourable diet quality and greater knowledge about the health benefits of pulses versus the TLC diet intervention. Increased knowledge about pulses and a higher frequency of pulse consumption in women who were randomized to the pulse-based diet group were attributed to a greater awareness of pulses through education and receiving pulses as meals over a 16-week intervention.

Keywords: Polycystic ovary syndrome; lifestyle; Therapeutic Lifestyle Changes diet; pulse-foods; glucose; Dietary Reference Intake

7.1. Introduction

PCOS is a heterogenous endocrinopathy and the leading cause of anovulatory infertility among reproductive-age women worldwide with a prevalence of up to 18% (Carmina & Lobo, 1999; March et al., 2010). Many women with PCOS present with

adverse CVD and DM2 risk indicators including hyperinsulinemia, compensatory IR, excess weight, and hyperandrogenism (Azziz et al., 2009; E. Diamanti-Kandarakis & A. Dunaif, 2012).

Lifestyle modifications, comprised of dietary, exercise, and cognitive behavioural therapies, are recommended as the first-line strategy to mediate health-related outcomes in PCOS ("Practice Committee of American Society for Reproductive Medicine, Society for Reproductive Endocrinology and Infertility. Optimizing natural fertility: a committee opinion," 2013). Low-GI calorie-restricted diets have been demonstrated to induce weight loss, ameliorate IR, and improve health-benefits in overweight and obese women with PCOS (Barr et al., 2013; Marsh et al., 2010; Turner-McGrievy et al., 2014a). However, calorie restricted dietary interventions are limited in terms of sustainability in the long-term and their applicability to women with normal weight. There is a paucity of evidence about a PCOS-optimized dietary composition to ameliorate PCOS health outcomes. As the underlying premise of the RCT in the present thesis, we hypothesized a nutritionally balanced pulse-based diet containing lentils, beans, split peas, and chickpeas would increase insulin sensitivity and function, thereby improving metabolic and reproductive sequelae associated with PCOS. Pulses are high in fiber, contain complex carbohydrates with a low GI, are low in fat, contain high-quality protein, have low sodium content, and are a significant source of vitamins and minerals, such as iron, zinc, folate, calcium, magnesium, and potassium (Mudryj et al., 2014). In Canada, pulse consumption is low despite its health benefits. Only 13% of Canadians consume pulses on any given day (Mudryj et al., 2014). Chronic consumption of pulses in other populations has been associated with decreased postprandial blood glucose and insulin concentrations, hypercholesterolemia, and obesity in non-PCOS populations (Ha et al., 2014; McCrory et al., 2010; Sievenpiper et al., 2009). The TLC diet is a nutritionally balanced diet, accomplished by increasing fiber consumption, decreasing saturated fat and cholesterol, and adding LDL-C lowering dietary options such as viscous fiber and plant stanol/sterol esters ("The NCEP ATP III final report," 2002). The TLC diet was considered, for our study, as a healthy control diet with the potential to mediate health-outcomes in women with PCOS.

We were unable to find a study involving women with PCOS where either a pulse-based or TLC diet were evaluated. For the purposes of the present study, we hypothesized participation in a pulse-based diet would result in greater improvements in dietary intake and pulse consumption behaviours versus the TLC diet in women with PCOS. In a secondary outcome analysis of a randomized controlled trial, we evaluated the effects of participating in a pulse-based versus standard TLC dietary intervention study on changes in dietary intake (macronutrients, micronutrients, GI and GL), knowledge, attitude, and consumption of pulses in women with PCOS. This study also determined the barriers to pulse consumption in this population.

7.2. Materials and Methods

7.2.1 Study Design and Protocol

Details of the RCT design and protocol have been elaborated in Chapter 4, page 69.

7.2.2 Participants

Details about the study participants have been presented in Chapter 4, page 71.

7.2.3 Dietary Assessment

Dietary intake was assessed using serial 24-hour dietary recalls through a self-administered method. Dietary recalls were obtained at baseline and monthly during the 16-week intervention period. To standardize reporting portion sizes, we used a photo album that illustrated different portion sizes. All women were instructed about filling out the dietary recalls during dietary counselling sessions. Dietary intake data were analyzed using the ESHA Food Processor SQL Software (version 7.02, ESHA Research, Salem, OR). For mixed meals, nutrients were calculated by meal components. GI was calculated according to the UN Food and Agriculture Organization/WHO ("World Health Organization. Preventing and managing the global epidemic," 1998) as described elsewhere (Graff et al., 2013). The GI values were determined using the International Table of Glycemic Index Values and Glycemic Load (Atkinson et al., 2008) using white glucose as the standard reference. For various foods reported in the recalls, the best matched GI was assigned by manually reviewing the table as previously used in several publications (Beulens et al., 2007; Egan et al., 2011; Jenkins et al., 2002). The GL of a serving of each food was calculated as ([g of carbohydrate from food item × GI value of the food item]/100) (Atkinson et al., 2008; Salmerón et al., 1997). The obtained dietary outcomes

were presented in comparison with the National Institute of Health Dietary Reference Intakes ("Nutrient Recommendations: Dietary Reference Intakes (DRI)," 2011)

7.2.4. Pulse Consumption Survey

The Pulse Consumption Survey (PCS) was completed by participants before healthcare counselling and randomization and after finishing the dietary interventions and exercise training as previously described (McBreairty et al., 2017). As elaborated in Chapter 6, all women received education and counselling about PCOS and healthy lifestyle behaviours to manage their condition.

The PCS has been developed by our research group. The survey was a modified version of the previously validated Diet Approaches to Increase Lentils in Youth (DAILY) questionnaire (Phillips et al., 2014). The PCS was designed to determine pulse consumption in the population as well as to assess knowledge, attitudes, and barriers related to dietary pulses and pulse foods consumption. The survey had 3 parts; part 1 included 21 questions to assess attitude about the palatability, accessibility, preparation, and affordability of pulse foods; part 2 included 2 questions to determine the frequency and barriers of consumption; and part 3 included 10 questions to assess the participants' knowledge about the nutritional composition of pulses, recommended servings of legumes based on Canada's Food Guide, environmental, and economic benefits of pulse consumption. The PCS is presented in Appendix B.

7.2.5. Statistical Analysis

Details of the statistical analysis have been elaborated in Chapter 4, page 74 and 75. An average effect size was calculated to estimate the magnitude of improvement in each part of the PC survey over time following the intervention. Effect sizes of 0.10, 0.30 and 0.50 were deemed small, medium and large effect sizes, respectively (Aron et al., 2006). Results were considered significant at $P<0.05$.

7.3. Results

The CONSORT flow diagram of the study has been presented in Figure 5, Chapter 5. Of a total of 324 women responded to the study recruitment advertisement, 95 were enrolled in the study. Thirty women in the pulse-based diet group and 31 in the TLC diet group completed the 16-week intervention. There were no significant differences in the clinical or biochemical features in women with PCOS enrolled in either arm of the trial. Details of the baseline

characteristics of participants have been presented in Table 13. Only 44 women responded to the serial 24-hour dietary recalls during the intervention: n=21 in the pulse-based diet group and n=23 in the TLC diet group. Final numbers analyzed for each outcome measure are presented in Table 13. The level of compliance with exercise and dietary interventions have been presented in Chapter 4, pages 75 and 76.

7.3.1. Dietary Intake

Both diet groups voluntarily reduced their average daily energy intake from baseline ($P<0.001$, Table 13). There were no differences for changes in the energy intake from baseline between the pulse-based and TLC diet groups ($P=0.97$). The percentage of energy intake from carbohydrates showed a greater increase in the pulse-based diet group (9.6% [47.6% to 57.2%]) than the TLC diet group (3.0% [50.2% to 53.2%]) expressed as changes from baseline ($P=0.05$); however, the pulse-based diet group (-16.7) exhibited a greater decrease in dietary GI levels from baseline when compared to the TLC diet group (-3.8; Table 13). The percentage of energy intake from dietary fats decreased from baseline in both pulse-based (-4.9 [34.2% to 29.3%]) and the TLC diet (-4.4% [34.5% to 30.1%]) groups over time ($P<0.01$) without a group by time interaction ($P=0.88$). Changes in the percentage of energy intake from dietary proteins in the pulse-based (0.3% [16.8% to 16.5%]) and the TLC diet (1.8% [16.2% to 18.0%]) groups were comparable ($P=0.08$), without a group by time interaction ($P=0.07$). Dietary cholesterol, saturated fat, trans fat, monounsaturated fat, and polyunsaturated fat intakes decreased from baseline during the intervention (time main effect, $P\leq0.05$; Table 13). The pulse-based diet group exhibited a greater decrease in dietary cholesterol intake during the intervention when compared to the TLC diet group ($P<0.001$). The pulse-based diet group had a tendency toward a greater decrease in trans-fat intake during the intervention from baseline than the TLC diet group ($P=0.06$). The pulse-based diet group consumed higher amount of dietary fiber than the TLC diet group during the intervention ($P<0.01$), and there was a time main effect (increase) for soluble fiber intake ($P=0.04$). Vitamin B3 and B5 intakes decreased over time in both groups ($P\leq0.05$). Dietary intakes, expressed as change from baseline, increased for folate, vitamin K, copper, magnesium, manganese, and iron in the pulse-based diet group compared to the TLC diet group ($P<0.05$, Table 13). The pulse-based diet had a greater decrease in dietary sodium intake when compared to the TLC diet group ($P<0.05$). The pulse-based diet exhibited a decrease in omega 3 fatty acids intake, expressed as changes from baseline when compared to the TLC diet group

(P=0.03, Table 13). There were no changes in the dietary intake of other nutrients in response to intervention (data not shown). Results of the post hoc comparisons of macronutrients between the intervention period and long-term follow up phases of the study have been presented in Chapter 5. When compared to the intervention period, the pulse-based diet group exhibited a tendency toward decreased dietary magnesium intake versus the TLC diet group 6 months after the completion of the intervention (-99.3 vs 23.5 mg/d; P=0.06). There were no changes in magnesium intake between the intervention period and 12 months after the completion of the intervention (P=0.35). Dietary intake of manganese, expressed as change from the intervention period, decreased in both the pulse-based and the TLC diet groups 6 (-2.6 vs -0.2 mg/d; P=0.02) and 12 months after the completion of the intervention (-3.0 vs -1.2 mg/d; P<0.001). Unlike the 12-month follow up timepoint (P=1.00), both the pulse-based (1400 mg/d) and the TLC diet (922 mg/d) groups exhibited increased intakes of sodium intake 6 months after the completion of the intervention when compared to the intervention period (P=0.01). The potassium intake of groups did not change 6 months after the completion of the intervention (P=0.27); however, both the pulse-based (-1338 mg/d) and TLC diet (-737 mg/d) groups decreased their intakes of potassium 12 months after the completion of the intervention from the intervention period (P=0.03). There were no changes in the dietary intake of other nutrients between intervention and long-term follow-up time points (data not shown).

7.3.2. PCS

Analysis of the results from the PCS at baseline showed a low frequency of pulse consumption in women with PCOS. Forty-three percent [26/61] of responders consumed 1-3 pulse meals per month and 27.9% [17/61] never or rarely ate pulse foods. The most frequent barriers associated with pulse consumption pertained to a lack of preparation knowledge (rank 1, 47.5% [29/61] agreed), time constraints (rank 2, 21.3% [13/61]), and a belief that pulses do not taste good (rank 3, 13.1% [8/61]). Most participants believed pulses would be too expensive to add to meals (85.2% [52/61] agreed), were motivated to consume pulses (65.6% [40/61] agreed) and needed more information about preparing pulse foods (78.7% [48/61] agreed). There were no differences between groups for all the measured outcomes at baseline (P>0.05).

Following the intervention, women in the pulse-based diet group exhibited higher scores in the part of knowledge about the nutritional composition of pulses, recommended servings of legumes based on Canada Food Guide, environmental, and economic benefits of pulse

consumption when compared with the TLC diet group (P=0.04; effect size=0.53). Both groups exhibited increased scores in the part of attitudes about the palatability, accessibility, preparation, and affordability of pulse foods over the 16 weeks (P<0.01; effect size=0.76). Changes in scores in the section on attitude toward pulses were not different between groups over time (P=0.11).

As expected, following the intervention, women in the pulse-based diet group exhibited a higher frequency of pulse consumption (defined as the consumption of ≥1 pulse meals per week) than the TLC diet group (93.3% [28/30] vs 67.7% [21/31]; P<0.01). However, results of the long-term follow phases of the study showed the frequency of pulse-food consumption decreased in both the pulse based and TLC diet groups when compared with the 16-week post-intervention timepoint (P<0.01); there were no differences in the frequency of pulse consumption between women who completed the 6- and 12-month follow up phases and were randomized to receive either the pulse-based and TLC diets 6 (43.7% [7/16] vs 37.5% [6/16]; P=0.45) and 12 (58.3% [7/12] vs 9/13 [69.2%]; P=0.69) months after the completion of the intervention.

Table 13. Dietary intakes at baseline and during the intervention

	Pulse-based diet group			TLC diet group			P value	
	Baseline	16-weeks	Change	Baseline	16-weeks	Change	Time	Group x Time
Diet								
Total energy intake (kcal/d)	2273±724	1707±27	-566±667	2211 ±536	1654±406	-557±696	<0.0001	0.97
Carbohydrate intake (g/d)	272.3±100.4	243.5±64.1	-28.8±93.4	281.5±95.1	221.2±71.8	-60.3±106.8	<0.01	0.30
Fat intake (g/d)	88.0±42.0	56.7±24.8	-31.3±43.6	84.6±27.5	55.3±19.8	29.3±37.0	<0.0001	0.87
Protein intake (g/d)	92.0±29.3	69.1±15.7	-22.9±24.9	87.3±25.7	75.3±25.7	-12.0±34.6	<0.001	0.24
Dietary fiber (g/d)	22.8±7.8	33.3±8.2	10.5±10.2	24.5±14.6	24.5±9.5	0.0±14.4	<0.01	<0.01
Cholesterol intake (mg/d)	357.0±184.9	128.2±114.0	-228.8±20.6.5	284.6±154.8	287.7±164.9	3.2±195.6	<0.01	<0.001
Saturated fat (g/d)	27.9±12.2	14.0±5.4	-13.9±13.1	24.7±15.7	17.4±9.9	-7.3±18.8	<0.0001	0.18
Trans fat (g/d)	1.3±1.9	0.4±0.5	-0.9±2.0	0.4±0.4	0.3±0.4	-0.1±0.5	0.04	0.06
Monounsaturated fat (g/d)	25.2±14.2	19.0±5.9	-6.2±13.5	22.1±12.5	16.9±8.4	-5.2±12.0	<0.01	0.78
Polyunsaturated fat (g/d)	13.4±12.0	9.9±3.3	-3.5±11.2	11.1±6.5	8.8±3.3	-2.3±7.0	0.05	0.68
Glycemic index (GI)	55.5±7.3	38.8±6.7	-16.7±10.3	54.3±7.7	50.5±8	-3.8±8.0	<0.0001	<0.01
Glycemic load (g)	142.1±59.8	96.3±25.3	45.8±56.7	162.7±58.1	101.3±26.2	61.3±49.3	<0.0001	0.43
Vitamin B3 (mg/d)	29.3±23.7	20.7±18.1	-8.6±17.4	26.3±23.4	20.7±9.9	-5.6±25.5	0.04	0.64
Vitamin B5 (mg/d)	9.1±13.6	7.7±8.7	-1.4±7.0	8.4±11.7	5.3±5.5	-3.1±7.6	0.05	0.47
Folate (µg/d)	380.0±205.1	639.5 ±266.0	259.5±286.6	344.0±260.0	295.1±159.5	-48.9±271.4	0.02	0.001
Vitamin K (µg/d)	76.4±41.1	146.9±83.2	70.5±84.7	91.1±121.7	102.0±107.4	10.9±80.3	<0.01	0.02
Copper (µg/d)	1542.2±1126.7	2068.3±918.2	526.1±960.5	1412.4±1169.4	1060.0±438.7	-352.4±1193.4	0.60	0.01
Manganese (mg/d)	2.9±1.4	5.0±1.8	2.1±2.0	3.5±2.6	3.0±1.1	-0.5±2.8	0.03	0.001
Magnesium (mg/d)	283.9±120.1	357.0±116.4	73.1±115.4	279.3±145.3	271.3±95.7	-8.0±140.7	0. 1	0.04

Iron (mg/d)	17.1±8.2	19.0±5.4	1.9±6.7	21.8±20.8	13.2±4.0	-8.6±21.8	0.16	0.03
Sodium (g/d)	3.7±2.2	1.7±0.5	-2.0±2.2	3.1±1.6	2.4±1.5	-0.7±2.1	<0.001	0.05
Omega 3 polyunsaturated fatty acids (g/d)	0.6±0.6	0.3±0.5	-0.3±0.6	0.5±0.7	0.7±0.9	0.2±0.8	0.99	0.03

Data are expressed as mean±SD. Numbers for dietary intake in each group were as follows: pulse-based diet group=23; TLC diet group=21. Numbers for leisure physical activity score in each group were as follows: pulse-based diet group=27; TLC diet group=30.

7.4. Discussion

The purpose of current study was to determine changes in dietary intake, knowledge, attitude, and frequency of pulse consumption after participating in an *ad libitum* pulse-based versus standard TLC dietary intervention in women with PCOS who exercised and received education and counselling about PCOS and healthy lifestyle behaviours. The most significant finding of the study was participating in a pulse-based diet intervention resulted in more favourable diet quality and greater knowledge about the health benefits of pulses versus the TLC diet intervention. To our knowledge, the present study is the first multi-dimensional lifestyle changes program to demonstrate the positive impacts of a pulse-based dietary intervention on dietary intakes and pulse consumption behaviours in women with PCOS.

The percentages of energy intake from macronutrients were within the acceptable macronutrient distribution range (AMDR) in our participants both at baseline and after the 16-week intervention. Our observations are consistent with previous reports on the proportion of energy intake from macronutrients in women with PCOS (Altieri et al., 2013; Crystal C Douglas et al., 2006; Shishehgar et al., 2016a; Toscani et al., 2011). Previous studies have shown worse dietary intakes in women with PCOS compared to healthy controls, characterized by increased consumption of high-GI foods, saturated fats, and a low intake of dietary fiber, despite similarities in the overall energy and nutrient intakes (Crystal C Douglas et al., 2006; Shishehgar et al., 2016a). By contrast, the mean dietary GI of our subjects (56.6) was interpreted as moderate at baseline (Frost & Dornhorst, 2012). Our participants had a marginally low (23.6 g/d) intake of dietary fiber at baseline when compared to the adequate intake (AI) of 28g/d ("Nutrient Recommendations: Dietary Reference Intakes (DRI)," 2011). Instead, our observations align with those of Barr et al. and Moran et al. on similarities between dietary GI and fiber intakes of women with PCOS and non-PCOS healthy controls (S. Barr et al., 2011; Moran, Ranasinha, et al., 2013).

Unlike the TLC diet group, women who were randomized to the pulse-based diet group achieved a low GI ($\leq$45) during the intervention. A low GI diet has been recommended to reduce the risk of CVD and DM2 in long-term (Barclay et al., 2008). The pulse-based diet group also showed a greater increase in the proportion of energy intake from dietary carbohydrates and a low GL, expressed as changes from baseline, which indicate an improvement in the type of carbohydrate intake. It is crucial to acknowledge a reduction in the amount of carbohydrate

intake *per se* may not be optimal or a practical strategy for all women with PCOS, including those with normal BMI, certain non-insulin resistant phenotypes, and women with obesity in long-term. Further, women in the pulse-based diet group exhibited a higher improvement in the type of dietary fat intake, reflected by a greater decrease in dietary cholesterol and a tendency toward a greater decrease in trans-fat intakes during intervention when compared to the TLC diet group. Dietary consumption of trans fats and excessive cholesterol intake have been shown to aggravate IR, dyslipidemia, hyperandrogenism, and proinflammatory state (Lefevre et al., 2005; Mozaffarian et al., 2004). Previous studies have shown the positive effects of PUFA intake on the metabolic health outcomes of PCOS including IR, abdominal adiposity, chronic-low inflammation, and dyslipidemia (Bahcecı et al., 2007; Diamanti-Kandarakis et al., 2006; Kasim-Karakas et al., 2004; Kirchengast & Huber, 2001; Velazquez et al., 2000). However, in our study, both groups showed decreased levels of PUFA intake during the intervention.

In the present study, decreased insulin levels and increased insulin sensitivity were hypothesized to be the key pathophysiological factors for improving health-outcomes associated with PCOS, even if a voluntary weight loss occurred (E. Diamanti-Kandarakis & A. Dunaif, 2012). Our study goals did not focus on calorie restriction; however, both intervention groups achieved a 5% weight loss post-intervention, attributed to a decrease in total energy intake, increased intake of low-GI foods, and increased physical activity, as described in Chapter 5. While both intervention groups exhibited decreased glucose and insulin responses to OGTT, the pulse-based diet group showed a greater decrease in total insulin AUC. Our results are similar to those of Marsh et al. (Marsh et al., 2010) on increased insulin sensitivity following an *ad libitum* low-GI diet versus a macronutrient-matched healthy diet. Apart from inducing satiety and weight loss, several factors can explain the beneficial effects of a pulse-based diet on improving glycemic response to OGTT including a high fiber content, low-GI, significant anti-oxidant content, and a favourable micronutrient and macronutrient composition (McCrory et al., 2010; Mudryj et al., 2014; Sievenpiper et al., 2009).

During the course of the intervention, we observed changes in the micronutrient intakes of groups, including achieving the recommended intakes of folate, magnesium, and iron in the pulse-based diet group and decreased iron intake in the TLC diet group in relation to the recommended RDA. However, it is difficult to derive a conclusion about the positive impact of the pulse-based diet on micronutrients adequacy based on the RDA values *per se*. There is a

significant potential to overestimate the dietary requirement of women who do not meet the recommended RDA cut-offs because the values have been defined to meet the recommendations of 97.5% of the North American population (*Dietary reference intakes: the essential guide to nutrient requirements*, 2006; "Nutrient Recommendations: Dietary Reference Intakes (DRI)," 2011). The sodium intake of our participants exceeded the American Heart Association recommendations (≤2400 mg/d) at baseline, consistent with the sodium intake of American women with PCOS (Crystal C Douglas et al., 2006). The mean sodium intake of the pulse-based diet group was still higher than the adequate intake (AI; 1500 mg/d) during the intervention; however, the pulse-based diet group experienced a greater decrease in dietary sodium intake during the intervention when compared to the TLC diet group. High sodium intake has been associated with increased glucocorticoid synthesis, IR, hyperglyceridemia, and lower adiponectin levels (Baudrand et al., 2014; Vedovato et al., 2004).

Compliance with healthy dieting habits has been challenging and poor in the context of PCOS especially in long-term studies (S. Barr et al., 2011; Marsh & Brand-Miller, 2005; Turner-McGrievy et al., 2015). In the present study, the compliance to the dietary interventions was increased by education about healthy eating behaviours and dietary counselling. Consumption of pulses among our participants was low at baseline. Our observation was similar that of Mudryi et al. reporting low intake of pulses in Canadian diet (Mudryj et al., 2014). Low consumption of pulse foods has been attributed to a lack of knowledge about the health benefits of pulses, negative feelings and experiences pertaining to pulse foods, including poor texture, flavour, gastrointestinal intolerance, and social norms of eating in Canada (Mudryj et al., 2012). Following the intervention, we observed increased knowledge about pulses and a higher frequency of pulse consumption in women who were randomized to the pulse-based diet group. These observations can be attributed to a greater awareness of pulses through education and consumption of pulse foods over the intervention period. Our observations reinforce the importance of patient-centred dietary interventions on improving the compliance to healthy lifestyle behaviours in women with PCOS, beyond the consumption of pulse foods.

Limitations of the study included the potential for recall bias (Thompson et al., 2015), and the Hawthorne effect (McCarney et al., 2007) in obtaining dietary information much like what happens in clinical practice. We used serial 24-hour dietary recalls as the most accurate and least biased instrument of reporting dietary intake. However, our participants' dietary recalls may

have a tendency toward random/systematic error, underreporting, and reactivity (Thompson et al., 2015). A fundamental limitation was a high attrition rate and poor response rate to the dietary recalls. Thereby our results may be skewed toward responders. As previously described in Chapter 5, a high drop-out rate is a common event in PCOS lifestyle change research (Hoeger et al., 2004; Marsh et al., 2010; Turner-McGrievy et al., 2015). Another limitation is the lack of a gold standard for GI values, and associated issues affecting GI, including the ripeness of fruits and vegetables, type, processing, and specific combination of foods. We acknowledge that the population studied represented ethnicities found in urban prairie cities in Canada. The indigenous population was largely underrepresented with respect to the prairie population, possibly due to recruitment geographically in an area without a large indigenous population. Therefore results cannot be generalized to an indigenous population.

In a dietary intervention without energy restriction, where aerobic exercise was recommended and healthcare counselling was provided, both pulse-based and TLC diet groups reduced their energy intake. The pulse-based diet was more efficient than the TLC diet in improving the overall diet quality and knowledge about pulses, attributed to a greater awareness of pulses through education and receiving pulses as meals over a 16-week multifactorial lifestyle changes program. In Chapter 8, a general discussion has been provided based on the results of Chapters 4 to 7.

CHAPTER 8
GENERAL DISCUSSION

The main objective the thesis was to compare the effects of a low-GI pulse-based diet with the standard TLC diet on multiple health outcomes of PCOS after a 16-week intervention when both groups exercised and received education and healthcare counselling about PCOS and lifestyle modification. Insulin resistance and hyperinsulinemia are key pathophysiologic contributors to PCOS disruptions which can be aggravated by concomitant obesity. Our findings reported in Chapter 3 further support the position that reproductive-age women with PCOS exhibit adverse cardio-metabolic and DM2 risk profiles, attributed to inherent IR, impaired insulin action, and obesity. These observations align with previous evidence about the substantial risk of CVD and DM2 in western populations with PCOS (Apridonidze et al., 2005; Ehrmann et al., 2006). Rationalized by the interplay between IR and obesity, work to date has provided insights into the short-term benefits of calorie-restricted low-GI diets on improving insulin sensitivity, glucoregulation, and weight management in PCOS (Barr et al., 2013; Marsh et al., 2010; Turner-McGrievy et al., 2014a). However, there is a paucity of evidence surrounding an optimal dietary composition to mediate favourable PCOS health outcomes in the long-term specifically across various BMI categories. We hypothesized that a nutritionally balanced *ad libitum* low-GI pulse-based diet containing lentils, beans, split peas, and chickpeas would increase insulin sensitivity; thereby, improving reproductive and cardio-metabolic sequelae associated with PCOS. In Chapter 4, we demonstrated the pulse-based diet was more efficient than the TLC diet to increase insulin sensitivity post-intervention, reflected by a reduction in total insulin AUC and a tendency toward a lower SHBG. Further, we reported decreased bilateral AFC, OV, TT, and increased menstrual regularity in both groups over time. These observations inform decreased severity of reproductive disruptions and/or the likelihood of response to treatment in PCOS (Jarrett & Lujan, 2017; Lujan et al., 2013), attributed to pre-established mechanisms (Bützow et al., 2000; Moran et al., 2011; Moran et al., 2007; Moran et al., 2009; Palomba et al., 2008) as discussed in Chapter 4. The most significant finding of the present study was that the pulse-based diet was more effective than the diet endorsed to decrease LDL-C, the TLC diet, ("The NCEP ATP III final report," 2002), to reduce LDL-C, TG, TC/HDL-C ratio, DBP, and increase HDL-C levels in women with PCOS (Chapters 5). Further, we observed

modifying dietary composition yielded similar improvements in glucoregulation, BMI, WC, SBP, and TG in both intervention groups, which can be translated to improved cardio-metabolic and DM2 risk profiles in women with PCOS. Hypocholesterolemic and BP lowering effects of pulses can be attributed primarily to their low-GI, high fiber content, favourable nutrient and antinutrient composition including low sodium content, high potassium content, and in part, attibuted to improvements in the **renin angiotensin** aldosterone system that secondary to decreased insulin resistance of our participants (Aburto et al., 2013; Altorf–van der Kuil et al., 2010; Jayalath et al., 2014; Jee et al., 2002; Kalupahana & Moustaid-Moussa, 2012; McCrory et al., 2010; Mudryj et al., 2014; Sievenpiper et al., 2009; Thorne et al., 1983; Tielemans et al., 2013) as elaborated in Chapter 5. Although negative impacts of PCOS on HRQoL have been documented, surprisingly, few studies have investigated the implications of lifestyle intervention for the improvement the HRQoL of women with PCOS. In Chapter 6, we showed women with PCOS were highly troubled with the lack of knowledge about PCOS and feasible strategies to manage their condition. Following the intervention, women in both groups exhibited improvements in all evaluated aspects of quality of life, including health concerns and knowledge about PCOS, healthcare satisfaction, active living, healthy dieting, and feelings and experiences about participation in the study. Women felt empowered about the management of their condition, through information received during education and counselling sessions. The positive HRQoL outcomes were attributed to educating women about improving a range of healthy eating practices, the favourable nutritional composition of diets, and encouraging aerobic exercise via healthcare counselling as described in Chapter 6. We showed that a lifestyle intervention comprised of diet, exercise and counselling has had a positive effect on HRQOL regardless of the dietary intervention. The outcome of the study revealed the importance for women with PCOS to understand the underlying reasons for their condition and ways to improve health to make meaningful long-term positive life changes. Analyses of results in women who completed the long-term follow up phase of the study showed that women experienced the greatest improvements in anthropometric, body composition, physiologic, insulin sensitivity, hormonal, and lipid outcomes at 16- weeks post intervention as opposed to 6- or 12-months after ithe ntervention. While some of the improvements maintained (HDL-C and TC/HDL-C levels), the overall metabolic profile of our participants exacerbated in long-term after the completion of the intervention, reflected by increased BMI, fasting insulin and TC levels. These observations

may be attributed to decreased levels of physical activity, lower frequency of pulse consumption, and less favourable dietary intakes, including decreased consumption of dietary fiber in both groups. Our observations highlight the importance of regular follow-up visits with a multidisciplinary team to monitor, support, motivate, and encourage women with PCOS to increase their adherence to the newly adopted healthy lifestyle practices. In Chapter 7, dietary intake and pulse consumption of intervention groups were described during the intervention, expressed as changes from baseline. Both groups voluntarily reduced their average daily energy intake from baseline. However, an analysis of the overall dietary intake of the pulse-based diet group revealed greater improvements in healthy dietary components than observed in the TLC diet group; expressed as changes from baseline, the pulse-based diet group had a greater decrease in dietary cholesterol intake, a tendency toward a greater decrease of trans-fat intake, and increased fiber, folate, magnesium, and iron intakes compared to the TLC diet group. As described in Chapter 7, knowledge and attitudes to eating pulses were evaluated in both. Women in the pulse-based diet group exhibited higher scores in the domain of knowledge about dietary pulses, which was attributed to a greater awareness of pulses through education and receiving pulses in their meals over 16 weeks. Both groups exhibited increased scores in the domain of attitudes about pulse foods over the 16 weeks.

A pulse recipe resource guide has been developed during the course of the study. The recipe resource guide, being both evidence-based and modified for palatability and acceptability for Canadian women, has the potential to be an effective tool both for the clinician as well as for the general public. The resource guide can be used for future research studies investigating the effects of pulse-based diets in various populations. The booklet can be used as a potential resource proactively to encourage beneficial eating habits among healthy populations and patients at risk for specific diseases and can be distributed to healthcare practitioners to promote pulse utilization and consumption.

8.1. Strengths and Limitations of the Study

Several RCTs have evaluated the effects of dietary and exercise interventions on various health descriptors in women with PCOS. Most studies had small sample sizes and were less comprehensive in the measures examined. Our RCT was unique, as it evaluated the effects of a comprehensive lifestyle changes intervention on multiple health-related outcome measures in

women with PCOS. Our study adopted a multi-dimensional approach comprised of dietary, exercise, education, and healthcare counselling strategies to evaluate multiple physiologic, metabolic, reproductive, HRQoL and dietary factors in response to treatment in women with PCOS. The diagnosis of PCOS and polycystic ovaries utilized advanced ultrasound technology and analysis methods to determine the effects of treatment on changes in ovarian morphology for the first time. Women's' medical histories and clinical examinations were completed by very few investigators to ensure uniformity and critical appraisal. Biochemical assessments were done at standard baseline menstrual cycle timelines to ensure uniformity of sampling. Participant education and counselling were likewise uniformly delivered by a small team of knowledgeable individuals. novel PCOS-specific survey was used to examine HRQoL indices before and following the intervention as opposed to less inclusive questionnaires such as the health-related quality-of-life questionnaire (Cronin et al., 1998). The present study had a diverse sample with 25.5% of subjects being non-Caucasian. We used ethno-specific adjustments when evaluating for hirsutism and abdominal obesity as related to MetS. Two dietitians assisted to improve the adherence to dietary interventions during the course of the study. Our first dietitian was blinded to group assignments to minimize the bias of dietary education for or against pulse-based diets to all women at baseline. Dietary compliance was improved by regular nutritional counselling by a second researcher dietitian during the intervention. Long-term follow up of lifestyle changes programs have been challenging and neglected in PCOS research. The present study included long-term monitoring of participants at 6- and 12-months post-intervention.

A number of limitations may be included in the interpretation of our results, including the potential for recall and/or self-reporting bias in obtaining physical activity, dietary intake and menstrual patterns. In our Canadian population, invitations for indigenous participation was limited by the geographical location of the study. While a high attrition rate as described in Chapter 5 was expected for long-term studies, this factor and economic realities of performing studies limited our ability to increase our study sample size.

8.2. Concluding Remarks

The present study results support the importance of lifestyle modifications in the management of PCOS and add another dimension to the existing knowledge concerning the positive effects of a low-GI pulse-rich diet on improving multiple health-related outcomes in

women with PCOS. While both *ad libitum* dietary interventions yielded substantial improvements in reproductive, cardio-metabolic, HRQoL outcome measures, the pulse-based diet was more effective than the TLC diet in decreasing insulin response to OGTT, decreasing risk factors for metabolic syndrome, cardio-metabolic disease and diabetes.in women with PCOS following a16-week intervention

8.3. Future Directions

Motivating women to incorporate beneficial lifestyle changes such as pulse-based diets and exercise into daily life needs to be the focus of future large studies to improve overall health for women with PCOS. To accomplish this goal, studies are needed to help women to understand how to incorporate pulses in their diets, as pulse consumption was foreign to participants with PCOS who volunteered for our study. Our attention was directed at providing prepared meals containing pulses to evaluate the beneficial effects of pulse-based diets upon women with PCOS. Long-term continuation of a pulse diet requires that participants start preparing their meals in order to have the skills to continue consuming a healthy diet that is suited to their palate. In Canada, although pulses comprise a large agricultural market share, Canadians do not consume pulses on a regular basis. Future research is required to address the mechanisms through which pulse consumption would mediate health benefits in women with PCOS.

It has been demonstrated in the past that consumption of a pre-prepared low-calorie food substitute is effective at short-term weight loss, only when the food substitute is consumed. Weight regain occurs immediately after restricted diets cease because there has been no incorporation of healthy eating habits into daily life activities. There is likely to be more long-term adherence to consumption of pulses and a higher probability for long-term health improvements when women are educated to incorporate low-GI nutrient-rich pulse consumption into their everyday diet. To address the high attrition rate and poor adherence to lifestyle change intervention programs, smaller and more gradual changes are needed over a longer study duration to improve long-term compliance. To address ethnic variations, diets need to be adjusted to incorporate ethnic palates. A focus towards healthy eating and optimal exercise are likely to result in long-term weight loss and thereby address BMI issues and risks for metabolic syndrome. The incorporation of cognitive behavioural modifications through education and

regular counselling with a multidisciplinary healthcare team are warranted for the successful and sustainable management of complications associated with PCOS in long-term.

REFERENCES

Abbott, D. H., Dumesic, D. A., & Franks, S. (2002). Developmental origin of polycystic ovary syndrome-a hypothesis. *J Endocirnol, 174*(1), 1-5.

Abdelhadi, O. A., Shepard, M. K., Sia, C., Garrett, T. J., & González, F. (2013). Activation of nuclear factor κB in response to cream ingestion is related to ovarian androgen hypersecretion in polycystic ovary syndrome. *Fertil Steril, 100*(3), S39.

Abeysekara, S., Chilibeck, P. D., Vatanparast, H., & Zello, G. A. (2012). A pulse-based diet is effective for reducing total and LDL-cholesterol in older adults. *Br J Nutr, 108*(Suppl S1), S103-S10.

Aburto, N. J., Hanson, S., Gutierrez, H., Hooper, L., Elliott, P., & Cappuccio, F. P. (2013). Effect of increased potassium intake on cardiovascular risk factors and disease: systematic review and meta-analyses. *BMJ, 346*, f1378.

ACOG committee on Practice Bulletins-Gynecology. ACOG practice bulletin no. 108: polycystic ovary syndrome. (2009). *Obstet Gynecol, 114*(4), 936-49.

Adams, J., Polson, D. W., Abdulwahid, N., Morris, D. V., Franks, S., Mason, H. D., Tucker, M., Price, J., & Jacobs, H. S. (1985). Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotropin releasing hormone. *Lancet, 326*(8469-8470), 1375-9.

Adashi, E. Y., Rock, J. A., Guzick, D., Wentz, A. C., Jones, G. S., & Jones, H. W., Jr. (1981). Fertility following bilateral ovarian wedge resection: a critical analysis of 90 consecutive cases of the polycystic ovary syndrome. *Fertil Steril, 36*(3), 320-5.

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J. C., James, W. P. T., Loria, C. M., & Smith, S. C. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation, 120*(16), 1640-5.

Alberti, K. G. M. M., Zimmet, P., & Shaw, J. (2006). Metabolic syndrome: a new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabetic Med, 23*(5), 469-80.

Alberti, K. G. M. M., & Zimmet, P. Z. (1998). Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med, 15*(7), 539-53.

Alberti, K. G. M. M., Zimmet, P. Z., & Shaw, J. (2007). International Diabetes Federation: a consensus on Type 2 diabetes prevention. *Diabet Med, 24*(5), 451-63.

Altieri, P., Cavazza, C., Pasqui, F., Morselli, A. M., Gambineri, A., & Pasquali, R. (2013). Dietary habits and their relationship with hormones and metabolism in overweight and obese women with polycystic ovary syndrome. *Clin Endocrinol (Oxf), 78*(1), 52-9.

Altorf–van der Kuil, W., Engberink, M. F., Brink, E. J., van Baak, M. A., Bakker, S. J., Navis, G., van't Veer, P., & Geleijnse, J. M. (2010). Dietary protein and blood pressure: a systematic review. *PLoS One, 5*(8), e12102.

American College of Obstetricians and Gynecologists. Polycystic ovary syndrome. ACOG Practice Bulletin no. 108. (2009). *Obstet Gynecol, 114*(4), 936-49.

Anderson, J. W., & Major, A. W. (2002). Pulses and lipaemia, short- and long-term effect: potential in the prevention of cardiovascular disease. *Br J Nutr, 88 Suppl 3*, S263-71.

Anderson, P. J., Critchley, J. A. J. H., Chan, J. C. N., Cockram, C. H., Lee, Z. S. K., Thomas, G. N., & Tomlinson, B. (2001). Factor analysis of the metabolic syndrome: obesity vs insulin resistance as the central abnormality. *Int J Obesity, 25*, 1782-8.

Apridonidze, T., Essah, P. A., Luorno, M. J., & Nestler, J. E. (2005). Prevalence and characteristics of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 90*, 1929-35.

Arai, T., Wang, N., Bezouevski, M., Welch, C., & Tall, A. R. (1999). Decreased atherosclerosis in heterozygous low density lipoprotein receptor-deficient mice expressing the scavenger receptor BI transgene. *J Biol Chem, 274*(4), 2366-71.

Archer, J. S., & Chang, R. J. (2004). Hirsutism and acne in polycystic ovary syndrome. *Best Pract Res Clin Obstet Gynaecol 18*(5), 737-54.

Aron, A., Aron, E., & Coups, E. (2006). *Statistics for Psychology*. New Jersey: Pearson Education Inc.

Asemi, Z., Samimi, M., Tabassi, Z., Shakeri, H., Sabihi, S.-S., & Esmaillzadeh, A. (2014). Effects of DASH diet on lipid profiles and biomarkers of oxidative stress in overweight and obese women with polycystic ovary syndrome: A randomized clinical trial. *Nutr, 30*(11–12), 1287-93.

Atkinson, F. S., Foster-Powell, K., & Brand-Miller, J. C. (2008). International Tables of Glycemic Index and Glycemic Load Values: 2008. *Diabetes Care, 31*(12), 2281-3.

Au, W.-S., Kung, H.-f., & Lin, M. C. (2003). Regulation of microsomal triglyceride transfer protein gene by insulin in HepG2 cells roles of MAPKerk and MAPKp38. *Diabetes, 52*(5), 1073-80.

Aubuchon, M., Laughbaum, N., Poetker, A., Williams, D., & Thomas, M. (2009). Supervised short-term nutrition and exercise promotes weight loss in overweight and obese patients with polycystic ovary syndrome. *Fertil Steril, 91*(4), 1336-8.

Aune, D., De Stefani, E., Ronco, A., Boffetta, P., Deneo-Pellegrini, H., Acosta, G., & Mendilaharsu, M. (2009). Legume intake and the risk of cancer: a multisite case–control study in Uruguay. *Cancer Cause Control, 20*(9), 1605-15.

Azziz, R. (2004). PCOS: a diagnostic challenge. *Reprod Biomed Online, 8*(6), 644-8.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H. F., Futterweit, W., Janssen, O. E., Legro, R. S., Norman, R. J., & Taylor, A. E. (2006). Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. *J Clin Endocrinol Metab, 91*(11), 4237-45.

Azziz, R., Carmina, E., Dewailly, D., Diamanti-Kandarakis, E., Escobar-Morreale, H. F., Futterweit, W., Janssen, O. E., Legro, R. S., Norman, R. J., Taylor, A. E., & Witchel, S. F. (2009). The androgen excess and PCOS society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil Steril, 91*(2), 456-88.

Azziz, R., Marin, C., Hoq, L., Badamgarav, E., & Song, P. (2005). Health care-related economic burden of the polycystic ovary syndrome during the reproductive life span. *J Clin Endocrinol Metab, 90*(8), 4650-8.

Azziz, R., Sanchez, L. A., Konchenhauer, E. S., Moran, C., Lazenby, J., Stephens, K. C., Taylor, K., & Boots, L. R. (2004). Androgen excess in women: experience with over 1000 consecutive patients. *J Clin Endocrinol Metab, 89*(2), 453-62.

Badawy, A., & Elnashar, A. (2011). Treatment options for polycystic ovary syndrome. *Int J Womens Health, 3*, 25-35.

Bahcecı, M., Aydemır, M., & Tuzcu, A. (2007). Effects of oral fat and glucose tolerance test on serum lipid profile, apolipoprotein, and CRP concentration, and insulin resistance in patients with polycystic ovary syndrome. *Fertil Steril, 87*(6), 1363-8.

Baillargeon, J. P., Iuorno, M. J., & Nestler, J. E. (2003). Insulin sensitizers for polycystic ovary syndrome. *Clin Obstet Gynecol, 46*(2), 325-40.

Baird, D. T., Corker, C. S., Davidson, D. W., Hunter, W. M., Michie, E. A., & Van Look, P. F. (1977). Pituitary-ovarian relationships in polycystic ovary syndrome. *J Clin Endocrinol Metab, 45*(4), 798-809.

Balen, A. H., Conway, G., Homburg, R., & Legro, R. (2005). *Polycystic ovary syndrome: a guide to clinical management*. London: Taylor & Francis.

Balfour, J. A., & McClellan, K. (2001). Topical eflornithine. *Am J Clin Dermatol, 2*(3), 197-201.

Banting, L. K., Gibson-Helm, M., Polman, R., Teede, H. J., & Stepto, N. K. (2014). Physical activity and mental health in women with polycystic ovary syndrome. *BMC Women's Health, 14*(1), 1-9.

Barber, T. M., McCarthy, M. I., Wass, J. A. H., & Franks, S. (2006). Obesity and polycystic ovary syndrome. *Clin Endocrinol (Oxf), 65*(2), 137-45.

Barclay, A. W., Petocz, P., McMillan-Price, J., Flood, V. M., Prvan, T., Mitchell, P., & Brand-Miller, J. C. (2008). Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. *Am J Clin Nutr, 87*(3), 627-37.

Barr, E. L., Zimmet, P. Z., Welborn, T. A., Jolley, D., Magliano, D. J., Dunstan, D. W., Cameron, A. J., Dwyer, T., Taylor, H. R., & Tonkin, A. M. (2007). Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance The Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation, 116*(2), 151-7.

Barr, S., Hart, K., Reeves, S., Sharp, K., & Jeanes, Y. M. (2011). Habitual dietary intake, eating pattern and physical activity of women with polycystic ovary syndrome. *Eur J Clin Nutr, 65*(10), 1126-32.

Barr, S., Reeves, S., Sharp, K., & Jeanes, Y. M. (2013). An isocaloric low glycemic index diet improves insulin sensitivity in women with polycystic ovary syndrome. *J Acad Nutr Diet, 113*(11), 1523-31.

Barry, J. A., Kuczmierczyk, A. R., & Hardiman, P. J. (2011). Anxiety and depression in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod, 26*(9), 2442-51.

Baudrand, R., Campino, C., Carvajal, C., Olivieri, O., Guidi, G., Faccini, G., Vöhringer, P., Cerda, J., Owen, G., & Kalergis, A. (2014). High sodium intake is associated with increased glucocorticoid production, insulin resistance and metabolic syndrome. *Clin Endocrinol (Oxf), 80*(5), 677-84.

Bazzano, L. A., Thompson, A. M., Tees, M. T., Nguyen, C. H., & Winham, D. M. (2011). Non-soy legume consumption lowers cholesterol levels: a meta-analysis of randomized controlled trials. *Nutr Metab Cardiovasc Dis 21*(2), 94-103.

Benson, S. (2008). Obesity, depression, and chronic low-grade inflammation in women with polycystic ovary syndrome. *Brain Behav Immun, 22*, 177-84.

Bergman, R. N., Finegood, D. T., & Ader, M. (1985). Assessment of Insulin Sensitivity in Vivo. *Endocr Rev, 6*(1), 45-86.

Bethea, S. W., & Nestler, J. E. (2008). Comorbidities in polycystic ovary syndrome: their relationship to insulin resistance. *Panminerva Med, 50*(4), 295-304.

Beulens, J. W., de Bruijne, L. M., Stolk, R. P., Peeters, P. H., Bots, M. L., Grobbee, D. E., & van der Schouw, Y. T. (2007). High dietary glycemic load and glycemic index increase risk of cardiovascular disease among middle-aged women: a population-based follow-up study. *J Am Coll Cardiol, 50*(1), 14-21.

Boudreaux, M. Y., Talbott, E. O., Kip, K. E., Brooks, M. M., & Witchel, S. F. (2006). Risk of T2DM and impaired fasting glucose among PCOS subjects: results of an 8-year follow-up. *Curr Diab Rep, 6*(1), 77-83.

Boulman, N., Levy, Y., Leiba, R., Shachar, S., Linn, R., Zinder, O., & Blumenfeld, Z. (2004). Increased C-reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease. *J Clin Endocrinol Metab, 89*(5), 2160-5.

Brady, C., Mousa, S. S., & Mousa, S. A. (2009). Polycystic ovary syndrome and its impact on women's quality of life: more than just an endocrine disorder. *Drug Healthc Patient Saf, 1*, 9-15.

Brassard, M., AinMelk, Y., & Baillargeon, J.-P. (2008). Basic infertility including polycystic ovary syndrome. *Med Clin North Am, 92*(5), 1163-92.

Brien, S. E., & Katzmarzyk, P. T. (2006). Physical activity and the metabolic syndrome in Canada. *Appl Physiol Nutr Metab, 31*(1), 40-7.

Brower, M. A., Jones, M. R., Rotter, J. I., Krauss, R. M., Legro, R. S., Azziz, R., & Goodarzi, M. O. (2015). Further investigation in Europeans of susceptibility variants for polycystic ovary syndrome discovered in genome-wide association studies of Chinese individuals. *J Clin Endocrinol Metab, 100*(1), E182-6.

Brown, A. J., Setji, T. L., Sanders, L. L., Lowry, K. P., Otvos, J. D., Kraus, W. E., & Svetkey, P. L. (2009). Effects of exercise on lipoprotein particles in women with polycystic ovary syndrome. *Med Sci Sports Exerc, 41*(3), 497-504.

Brown, J. (1978). Pituitary control of ovarian function-concepts derived from gonadotrophin therapy. *Aust N Z J Obstet Gynaecols, 18*(1), 47-54.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr, 69*(1), 30-42.

Bruner, B., Chad, K., & Chizen, D. (2006). Effects of exercise and nutritional counseling in women with polycystic ovary syndrome. *Appl Physiol Nutr Metab, 31*, 384-91.

Building Demand/International Year of Pulses. (2016a). Retrieved from http://saskpulse.com/building-demand/international-year-of-the-pulses/

Burger, H. G., Dudley, E. C., Cui, J., Dennerstein, L., & Hopper, J. L. (2000). A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-

binding globulin levels through the menopause transition. *J Clin Endocrinol Metab, 85*(8), 2832-8.

Buttram, V. C., Jr., & Vaquero, C. (1975). Post-ovarian wedge resection adhesive disease. *Fertil Steril, 26*(9), 874-6.

Bützow, T. L., Lehtovirta, M., Siegberg, R., Hovatta, O., Koistinen, R., Seppälä, M., & Apter, D. (2000). The decrease in luteinizing hormone secretion in response to weight reduction is inversely related to the severity of insulin resistance in overweight women. *J Clin Endocrinol Metab, 85*(9), 3271-5.

Cameron, A. J., Magliano, D. J., Zimmet, P. Z., Welborn, T. A., Colagiuri, S., Tonkin, A. M., & Shaw, J. E. (2008). The metabolic syndrome as a tool for predicting future diabetes: the AusDiab study. *J Intern Med, 264*(2), 177-86.

Carmina, E. (2006). Endothelial dysfunction in PCOS: role of obesity and adipose hormones. *Am J Med, 119*, 356.e1-.e6.

Carmina, E., & Lobo, R. A. (1999). Polycystic ovary syndrome (PCOS): arguably the most common endocrinopathy is associated with significant morbidity in women. *J Clin Endocrinol Metab, 84*(6), 1897-9.

Carmina, E., Napoli, N., Longo, R. A., Rini, G. B., & Lobo, R. A. (2006). Metabolic syndrome in polycystic ovary syndrome (PCOS): lower prevalence in southern Italy than in the USA and the influence of criteria for the diagnosis of PCOS. *Eur J Endocrinol, 154*(1), 141-5.

Carr, M. C. (2003). The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab, 88*(6), 2404-11.

Chavez-Ross, A., Franks, S., Mason, H. D., Hardy, K., & Stark, J. (1997). Modelling the control of ovulation and polycystic ovary syndrome. *J Math Biol, 36*(1), 95-118.

Chen, Y.-F., Naftilan, A. J., & Oparil, S. (1992). Androgen-dependent angiotensinogen and renin messenger RNA expression in hypertensive rats. *Hypertension, 19*(5), 456-63.

Chibbar, R. N., Ambigaipalan, P., & Hoover, R. (2010). Review: molecular diversity in pulse seed starch and complex carbohydrates and its role in human nutrition and health. *Cereal Chem, 87*(4), 342-52.

Chiu, K. C., Chu, A., Go, V. L. W., & Saad, M. F. (2004). Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *Am J Clin Nutr, 79*(5), 820-5.

Chiu, W.-L., Boyle, J., Vincent, A., Teede, H., & Moran, L. J. (2017). Cardiometabolic risks in polycystic ovary syndrome: non-traditional risk factors and the impact of obesity. *Neuroendocrinol, 104*(4), 412-24.

Cholesterol Treatment Trialists' (CTT) Collaborators, Mihaylova, B., Emberson, J., Blackwell, L., Keech, A., Simes, J., Barnes, E. H., Voysey, M., Gray, A., Collins, R., & Baigent, C. (2012). The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet, 380*(9841), 581-90.

Clark, A. F., Marcellus, S., deLory, B., & Bird, C. E. (1975). Plasma testosterone free index: a better indicator of plasma androgen activity? *Fertil Steril, 26*(10), 1001-5.

Clark, N. M., Podolski, A. J., Brooks, E. D., Chizen, D. R., Pierson, R. A., Lehotay, D. C., & Lujan, M. E. (2014). Prevalence of polycystic ovary syndrome phenotypes using updated criteria for polycystic ovarian morphology. *Reprod Sci, 21*(8), 1034-43.

Coffey, S., Bano, G., & Mason, H. D. (2006). Health-related quality of life in women with polycystic ovary syndrome: a comparison with the general population using the Polycystic Ovary Syndrome Questionnaire (PCOSQ) and the Short Form-36 (SF-36). *Gynecol Endocrinol, 22*(2), 80-6.

Coffey, S., & Mason, H. (2003). The effect of polycystic ovary syndrome on health-related quality of life. *Gynecol Endocrinol, 17*(5), 379-86.

Colwell, K., Lujan, M. E., Lawson, K. L., Pierson, R. A., & Chizen, D. R. (2010). Women's perceptions of polycystic ovary syndrome following participation in a clinical research study: implications for knowledge, feelings, and daily health practices. *J Obstet Gynaecol Can, 32*(5), 453-9.

Conte, F., Banting, L., Teede, H. J., & Stepto, N. K. (2015). Mental health and physical activity in women with polycystic ovary syndrome: a brief review. *Sports Med, 45*(4), 497-504.

Corbould, A., Kim, Y., Youngren, J., Pender, C., Kahn, B., Lee, A., & Dunaif, A. (2005). Insulin resistance in the skeletal muscle of women with PCOS involves intrinsic and acquired defects in insulin signaling. *Am J Obstet Gynecol, 288*(5), E1047-E54.

Corbould, A., Zhao, H., Mirzoeva, S., Aird, F., & Dunaif, A. (2006). Enhanced mitogenic signaling in skeletal muscle of women with polycystic ovary syndrome. *Diabetes, 55*(3), 751-9.

Cronin, L., Guyatt, G., Griffith, L., Wong, E., Azziz, R., Futterweit, W., Cook, D., & Dunaif, A. (1998). Development of a health-related quality-of-life questionnaire (PCOSQ) for women with polycystic ovary syndrome (PCOS). *J Clin Endocrinol Metab, 83*(6), 1976-87.

Crosby, R. D., Kolotkin, R. L., & Williams, G. R. (2003). Defining clinically meaningful change in health-related quality of life. *J Clin Epidemiol, 56*(5), 395-407.

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

Croston, G. E., Milan, L. B., Marschke, K. B., Reichman, M., & Briggs, M. R. (1997). Androgen receptor-mediated antagonism of estrogen-dependent low density lipoprotein receptor transcription in cultured hepatocytes. *Endocrinol, 138*(9), 3779-86.

Deeks, A. A., Gibson-Helm, M. E., Paul, E., & Teede, H. J. (2011). Is having polycystic ovary syndrome a predictor of poor psychological function including anxiety and depression? *Hum Reprod, 26*(6), 1399-407.

Deeks, A. A., Gibson-Helm, M. E., & Teede, H. J. (2010a). Anxiety and depression in polycystic ovary syndrome: a comprehensive investigation. *Fertil Steril, 93*(7), 2421-3.

Deeks, A. A., Gibson-Helm, M. E., & Teede, H. J. (2010b). Anxiety and depression in polycystic ovary syndrome: a comprehensive investigation. *Fertil Steril, 93*(7), 2421-3.

DeFronzo, R. A. (1988). The triumvirate: β-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes, 37*(6), 667-87.

Després, J.-P., Lemieux, I., & Prud'Homme, D. (2001). Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ, 322*(7288), 716-20.

DeUgarte, C. M., Bartolucci, A. A., & Azziz, R. (2005). Prevalence of insulin resistance in the polycystic ovary syndrome using the homeostasis model assessment. *Fertil Steril, 83*(5), 1454-60.

DeVane, G. W., Czekala, N. M., Judd, H. L., & Yen, S. S. (1975). Circulating gonadotropins, estrogens, and androgens in polycystic ovarian disease. *Am J Obstet Gynecol, 121*(4), 496-500.

Dewailly, D., Catteau-Jonard, S., Reyss, A.-C., Leroy, M., & Pigny, P. (2006). Oligoanovulation with polycystic ovaries but not overt hyperandrogenism. *J Clin Endocrinol Metab, 91*(10), 3922-7.

Dewailly, D., Lujan, M. E., Carmina, E., Cedars, M. I., Laven, J., Norman, R. J., & Escobar-Morreale, H. F. (2014). Definition and significance of polycystic ovarian morphology: a task force report from the Androgen Excess and Polycystic Ovary Syndrome Society. *Hum Reprod Update, 20*(3), 334-52.

Diamanti-Kandarakis, E., Argyrakopoulou, G., Economou, F., Kandaraki, E., & Koutsilieris, M. (2008). Defects in insulin signaling pathways in ovarian steroidogenesis and other tissues in polycystic ovary syndrome (PCOS). *J Steroid Biochem Mol Biol, 109*(3), 242-6.

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., Zoeller, R. T., & Gore, A. C. (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev, 30*(4), 293-342.

Diamanti-Kandarakis, E., Christakou, C. D., Kandaraki, E., & Alexandraki, K. I. (2007). Early onset adiposity: a pathway to polycystic ovary syndrome in adolescents? *Hormones (Athens), 6*(3), 210-7.

Diamanti-Kandarakis, E., Christakou, C. D., Kandaraki, E., & Economou, F. N. (2010). Metformin: an old medication of new fashion: evolving new molecular mechanisms and clinical implications in polycystic ovary syndrome. *Eur J Endocrinol, 162*, 193-212.

Diamanti-Kandarakis, E., & Dunaif, A. (2012). Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications. *Endocr Rev, 33*(6), 981-1030.

Diamanti-Kandarakis, E., Kandarakis, H., & Legro, R. S. (2006). The role of genes and environment in the etiology of PCOS. *Endocrine, 30*(1), 19-26.

Diamanti-Kandarakis, E., Alexandraki, K., Piperi, C., Protogerou, A., Katsikis, I., Paterakis, T., Lekakis, J., & Panidis, D. (2006). Inflammatory and endothelial markers in women with polycystic ovary syndrome. *Eur J Clin Invest, 36*(10), 691-7.

Dietary reference intakes: the essential guide to nutrient requirements. (2006). Washington, DC: The National Academies Press.

Ding, E. L., Song, Y., Malik, V. S., & Liu, S. (2006). Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis. *JAMA, 295*(11), 1288-99.

Dinicola, S., Chiu, T. T., Unfer, V., Carlomagno, G., & Bizzarri, M. (2014). The rationale of the myo-inositol and D-chiro-inositol combined treatment for polycystic ovary syndrome. *J Clin Pharmacol, 54*(10), 1079-92.

Domecq, J. P., Prutsky, G., Mullan, R. J., Hazem, A., Sundaresh, V., Elamin, M. B., Phung, O. J., Wang, A., Hoeger, K., Pasquali, R., Erwin, P., Bodde, A., Montori, V. M., & Murad, M. H. (2013). Lifestyle modification programs in polycystic ovary syndrome: systematic review and meta-analysis. *J Clin Endocrinol Metab, 98*(12), 4655-63.

Douglas, C. C., Gower, B. A., Darnell, B. E., Ovalle, F., Oster, R. A., & Azziz, R. (2006). Role of diet in the treatment of polycystic ovary syndrome. *Fertil Steril, 85*(3), 679-88.

Douglas, C. C., Norris, L. E., Oster, R. A., Darnell, B. E., Azziz, R., & Gower, B. A. (2006). Difference in dietary intake between women with polycystic ovary syndrome and healthy controls. *Fertil Steril, 86*(2), 411-7.

Duffield-Lillico, A., Dalkin, B., Reid, M., Turnbull, B., Slate, E., Jacobs, E., Marshall, J., & Clark, L. (2003). Selenium supplementation, baseline plasma selenium status and incidence of prostate cancer: an analysis of the complete treatment period of the Nutritional Prevention of Cancer Trial. *BJU Int, 91*(7), 608-12.

Duleba, A. J., & Dokras, A. (2012). Is PCOS an inflammatory process? *Fertil Steril, 97*(1), 7-12.

Dunaif, A. (1997). Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev, 18*(6), 774-800.

Dunaif, A. (2008). Drug Insight: insulin-sensitizing drugs in the treatment of polycystic ovary syndrome-a reappraisal. *Nat Rev Endocrinol, 4*(5), 272-83.

Dunaif, A., & Fauser, B. C. (2013). Renaming PCOS—a two-state solution. *J Clin Endocrinol Metab, 98*(11), 4325-8.

Dunaif, A., & Finegood, D. T. (1996). Beta-cell dysfunction independent of obesity and glucose intolerance in the polycystic ovary syndrome. *J Clin Endocrinol Metab, 81*(3), 942-7.

Dunaif, A., Graf, M., Mandeli, J., Laumas, V., & Dobrjansky, A. (1987). Characterization of groups of hyperandrogenic women with acanthosis nigricans, impaired glucose tolerance, and/or hyperinsulinemia. *J Clin Endocrinol Metab, 65*(3), 499-507.

Dunaif, A., Segal, K. R., Futterweit, W., & Dobrjansky, A. (1989). Profound peripheral insulin resistance, independent of obesity, in polycystic ovary syndrome. *Diabetes, 38*(9), 1165-74.

Dunn, J. F., Nisula, B. C., & Rodbard, D. (1981). Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J Clin Endocrinol Metab, 53*(1), 58-68.

Egan, N., Read, A., Riley, P., & Atiomo, W. (2011). Evaluating compliance to a low glycaemic index (GI) diet in women with polycystic ovary syndrome (PCOS). *BMC Research Notes, 4*(1), 53.

Ehrmann, D. A. (2005). Polycystic ovary syndrome. *N Eng J Med, 352*(12), 1223-36.

Ehrmann, D. A. (2006). Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 91*, 48-53.

Ehrmann, D. A., Barnes, R. B., Rosenfield, R. L., Cavaghan, M. K., & Imperial, J. (1999). Prevalence of impaired glucose tolerance and diabetes in women with polycystic ovary syndrome. *Diabetes Care, 22*, 141-6.

Ehrmann, D. A., Liljenquist, D. R., Kasza, K., Azziz, R., Legro, R. S., & Ghazzi, M. N. (2006). Prevalence and predictors of the metabolic syndrome in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 91*(1), 48-53.

Eli, C., & Edelson, S. K. (1984). The use of human skin fibroblasts to obtain potency estimates of drug binding to androgen receptors. *J Clin Endocrinol Metab, 59*(1), 51-5.

Elsenbruch, S., Hahn, S., Kowalsky, D., Offner, A. H., Schedlowski, M., Mann, K., & Janssen, O. E. (2003). Quality of life, psychosocial well-being, and sexual satisfaction in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 88*(12), 5801-7.

Enerbäck, S., & Gimble, J. M. (1993). Lipoprotein lipase gene expression: physiological regulators at the transcriptional and post-transcriptional level. *Biochim Biophys Acta 1169*(2), 107-25.

Environmental impacts on reproductive health and fertility. (2010). (T. J. Woodruff, S. J. Janssen, L. J. Guillette Jr, & L. C. Giudice Eds.). New York: Cambridge University Press.

Erickson, G. F., Magoffin, D. A., Garzo, V. G., Cheung, A. P., & Chang, R. J. (1992). Granulosa cells of polycystic ovaries: are they normal or abnormal? *Hum Reprod, 7*(3), 293-9.

Escobar-Morreale, H. F., Luque-Ramírez, M., & San Millán, J. L. (2005). The molecular-genetic basis of functional hyperandrogenism and the polycystic ovary syndrome. *Endocr Rev, 26*(2), 251-82.

Eslamian, G., Baghestani, A. R., Eghtesad, S., & Hekmatdoost, A. (2016). Dietary carbohydrate composition is associated with polycystic ovary syndrome: a case–control study. *J Hum Nutr Diet*. Retrieved from doi:doi: 10.1111/jhn.12388

FAO. (2014). *FAO Statistical Yearbook 2014: Europe and Central Asia Food and Agriculture* Retrieved from http://www.fao.org/3/a-i3621e.pdf

FAO. (2016). About the International Year of Pulses. Retrieved from http://www.fao.org/pulses-2016/about/en/

Farm and Food Care. (2016). *The Real Dirt on Farming. Saskatchewan Digest Edition* Retrieved from https://farmfoodcaresk.org/real-dirt-on-farming/#p=5

Fauser, B. C. J. M., Tarlatzis, B. C., Rebar, R. W., Legro, R. S., Balen, A. H., Lobo, R., Carmina, E., Chang, J., Yildiz, B. O., Laven, J. S. E., Boivin, J., Petraglia, F., Wijeyeratne, C. N., Norman, R. J., Dunaif, A., Franks, S., Wild, R. A., Dumesic, D., & Barnhart, K. (2012). Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. *Fertil Steril, 97*(1), 28-38.

Fenkci, V., Fenkci, S., Yilmazer, M., & Serteser, M. (2003). Decreased total antioxidant status and increased oxidative stress in women with polycystic ovary syndrome may contribute to the risk of cardiovascular disease. *Fertil Steril, 80*(1), 123-7.

Ferriman, D., & Gallwey, J. (1961). Clinical assessment of body hair growth in women. *J Clin Endocrinol Metab, 21*(11), 1440-7.

Fleming, S., O'Donnell, A., & Perman, J. (1985). Influence of frequent and long-term bean consumption on colonic function and fermentation. *AJCN, 41*(5), 909-18.

Foroozanfard, F., Jamilian, M., Jafari, Z., Khassaf, A., Hosseini, A., Khorammian, H., & Asemi, Z. (2015). Effects of zinc supplementation on markers of insulin resistance and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Exp Clin Endocrinol Diabetes, 123*(04), 215-20.

Franik, S., Kremer, J. A., Nelen, W. L., Farquhar, C., & Marjoribanks, J. (2015). Aromatase inhibitors for subfertile women with polycystic ovary syndrome: summary of a Cochrane review. *Fertil Steril, 103*(2), 353-5.

Franks, S. (1991). The ubiquitous polycystic ovary. *J Endocrinol, 129*(3), 317-9.

Franks, S. (2008). Polycystic ovary syndrome in adolescents. *Int J Obes 32*(7), 1035-41.

Franks, S., Mason, H., White, D., & Willis, D. (1998). Etiology of anovulation in polycystic ovary syndrome. *Steroids, 63*(5), 306-7.

Franks, S., McCarthy, M. I., & Hardy, K. (2006). Development of polycystic ovary syndrome: involvement of genetic and environmental factors. *Int J Androl, 29*(1), 278-85.

Franks, S., Stark, J., & Hardy, K. (2008). Follicle dynamics and anovulation in polycystic ovary syndrome. *Hum Reprod Update, 14*(4), 367-78.

Franks, S., Webber, L. J., Goh, M., Valentine, A., White, D. M., Conway, G. S., Wiltshire, S., & McCarthy, M. I. (2008). Ovarian morphology is a marker of heritable biochemical traits in sisters with polycystic ovaries. *J Clin Endocrinol Metab, 93*(9), 3396-402.

Frost, G., & Dornhorst, A. (2012). Glycemic Index. In B. Caballero (Ed.), *Encyclopedia of Human Nutrition* (3 ed., pp. 393-8). Waltham: Academic Press.

Functional foods fact sheet: Plant stanols and sterols. (2003). Retrieved from http://www.foodinsight.org/Functional_Foods_Fact_Sheet_Plant_Stanols_and_Sterols

Galisteo, M., Duarte, J., & Zarzuelo, A. (2008). Effects of dietary fibers on disturbances clustered in the metabolic syndrome. *J Nutr Biochem, 19*(2), 71-84.

Gambineri, A., Pelusi, C., Vicennati, V., Pagotto, U., & Pasquali, R. (2002). Obesity and the polycystic ovary syndrome. *Int J Obes Relat Metab Disord 26*(7), 883-97.

Ganie, M. A., Chakraborty, S., & Rehman, H. (2015). Treatment of polycystic ovary syndrome: recent trial results. *J Clin Invest, 5*(3), 337-50.

Garg, A. (1998). High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am J Clin Nutr, 67*(3), 577S-82S.

Garriguet, D. (2009). Diet quality in Canada. *Health Reports, 20*(3), 41.

Gedik, O., & Zileli, M. (1977). Effects of hypocalcemia and theophylline on glucose tolerance and insulin release in human beings. *Diabetes, 26*(9), 813-9.

Gilling-Smith, C., Willis, D. S., Beard, R. W., & Franks, S. (1994). Hypersecretion of androstenedione by isolated thecal cells from polycystic ovaries. *J Clin Endocrinol Metab, 79*(4), 1158-65.

Ginsberg, H. N., Kris-Etherton, P., Dennis, B., Elmer, P. J., Ershow, A., Lefevre, M., Pearson, T., Roheim, P., Ramakrishnan, R., Reed, R., Stewart, K., Stewart, P., Phillips, K., Anderson, N., & Group, f. t. D. R. (1998). Effects of reducing dietary saturated fatty acids on plasma lipids and ipoproteins in healthy subjects: the Delta Study protocol 1. *Arterioscler Thromb Vasc Biol, 18*(3), 441-9.

Givens, J. R., Andersen, R. N., Wiser, W. L., & Fish, S. A. (1974). Dynamics of suppression and recovery of plasma FSH, LH, androstenedione and testosterone in polycystic ovarian disease using an oral contraceptive. *J Clin Endocrinol Metab, 38*(5), 727-35.

Glueck, C. J., Papanna, R., Wang, P., Goldenberg, N., & Sieve-Smith, L. (2003). Incidence and treatment of metabolic syndrome in newly referred women with confirmed polycystic ovarian syndrome. *Metabolism, 52*(7), 908-15.

Godin, G., & Shephard, R. (1985). A simple method to assess exercise behaviour in the community. *Can J Appl Sport Sci*(10), 141-6.

Goldenberg, R., & Punthakee, Z. (2013). Definition, classification and diagnosis of diabetes, prediabetes and metabolic syndrome. *Can J Diabetes, 37*, S8-S11.

González, F. (2015). Nutrient-induced inflammation in polycystic ovary syndrome: role in the development of metabolic aberration and ovarian dysfunction. *Semin Reprod Med 33*(4), 276-86.

González, F., Kirwan, J. P., Rote, N. S., & Minium, J. (2013). Glucose ingestion stimulates atherothrombotic inflammation in polycystic ovary syndrome. *Am J Physiol Endocrinol Metab, 304*(4), E375-E83.

González, F., Rote, N. S., Minium, J., & Kirwan, J. P. (2006). Reactive oxygen species-induced oxidative stress in the development of insulin resistance and hyperandrogenism in polycystic ovary syndrome. *J Clin Endocrinol Metab, 91*(1), 336-40.

González, F., Sia, C. L., Shepard, M. K., Rote, N. S., & Minium, J. (2012). Hyperglycemia-induced oxidative stress is independent of excess abdominal adiposity in normal-weight women with polycystic ovary syndrome. *Hum Reprod, 27*(12), 3560-8.

Goodarzi, M. O., Dumesic, D. A., Chazenbalk, G., & Azziz, R. (2011). Polycystic ovary syndrome: etiology, pathogenesis and diagnosis. *Nat Rev Endocrinol, 7*(4), 219-31.

Goss, A. M., Chandler-Laney, P. C., Ovalle, F., Goree, L. L., Azziz, R., Desmond, R. A., Bates, G. W., & Gower, B. A. (2014). Effects of a eucaloric reduced-carbohydrate diet on body composition and fat distribution in women with PCOS. *Metabolism, 63*(10), 1257-64.

Gower, B. A., Chandler-Laney, P. C., Ovalle, F., Goree, L. L., Azziz, R., Desmond, R. A., Granger, W. M., Goss, A. M., & Bates, G. W. (2013). Favourable metabolic effects of a eucaloric lower-carbohydrate diet in women with PCOS. *Clin Endocrinol (Oxf), 79*(4), 550-7.

Graff, S. K., Mário, F. M., Alves, B. C., & Spritzer, P. M. (2013). Dietary glycemic index is associated with less favorable anthropometric and metabolic profiles in polycystic ovary syndrome women with different phenotypes. *Fertil Steril, 100*(4), 1081-8.

Gronbaek, H., Lange, A., Birkebaek, N. H., Holland-Fischer, P., Solvig, J., Horlyck, A., Kristensen, K., Rittig, S., & Vilstrup, H. (2012). Effect of a 10-week weight loss camp on fatty liver disease and insulin sensitivity in obese Danish children. *J Pediatr Gastroenterol Nutr, 54*(2), 223-8.

Grundy, S. M. (2008). Metabolic syndrome pandemic. *Arterioscler Thromb Vasc Biol, 28*(4), 629-36.

Guenther, P. M., Dodd, K. W., Reedy, J., & Krebs-Smith, S. M. (2006). Most Americans eat much less than recommended amounts of fruits and vegetables. *J Am Diet Assoc, 106*(9), 1371-9.

Guzick, D. S., Wing, R., Smith, D., Berga, S. L., & Winters, S. J. (1994). Endocrine consequences of weight loss in obese, hyperandrogenic, anovulatory women. *Fertil Steril, 61*, 598-604.

Ha, V., Sievenpiper, J. L., de Souza, R. J., Jayalath, V. H., Mirrahimi, A., Agarwal, A., Chiavaroli, L., Mejia, S. B., Sacks, F. M., & Di Buono, M. (2014). Effect of dietary pulse intake on established therapeutic lipid targets for cardiovascular risk reduction: a systematic review and meta-analysis of randomized controlled trials. *CMAJ, 186*(8), E252-E62.

Hahn, S., Benson, S., Elsenbruch, S., Pleger, K., Tan, S., Mann, K., Schedlowski, M., van Halteren, W. B., Kimmig, R., & Janssen, O. E. (2006). Metformin treatment of polycystic ovary syndrome improves health-related quality-of-life, emotional distress and sexuality. *Hum Reprod, 21*(7), 1925-34.

Hahn, S., Haselhorst, U., Tan, S., Quadbeck, B., Schmidt, M., Roesler, S., Kimmig, R., Mann, K., & Janssen, O. (2006). Low serum 25-hydroxyvitamin D concentrations are associated with insulin resistance and obesity in women with polycystic ovary syndrome. *Exp Clin Endocrinol Diabetes, 114*(10), 577-83.

Hahn, S., Janssen, O. E., Tan, S., Pleger, K., Mann, K., Schedlowski, M., Kimmig, R., Benson, S., Balamitsa, E., & Elsenbruch, S. (2005). Clinical and psychological correlates of quality-of-life in polycystic ovary syndrome. *Eur J Endocrinol, 153*(6), 853-60.

Hanefeld, M., Cagatay, M., Petrowitsch, T., Neuser, D., Petzinna, D., & Rupp, M. (2004). Acarbose reduces the risk for myocardial infarction in type 2 diabetic patients: meta-analysis of seven long-term studies. *Eur Heart J, 25*(1), 10-6.

Harbis, A., Defoort, C., Narbonne, H., Juhel, C., Senft, M., Latgé, C., Delenne, B., Portugal, H., Atlan-Gepner, C., Vialettes, B., & Lairon, D. (2001). Acute hyperinsulinism modulates plasma apolipoprotein B-48 triglyceride—rich lipoproteins in healthy subjects during the postprandial period. *Diabetes, 50*(2), 462-9.

Hardiman, P., Pillay, O. S., & Atiomo, W. (2003a). Polycystic ovary syndrome and endometrial carcinoma. *Lancet, 361*(9371), 1810-2.

Hardiman, P., Pillay, O. S., & Atiomo, W. (2003b). Polycystic ovary syndrome and endometrial carcinoma. *The lancet, 361*(9371), 1810-2.

Harlow, S. D., & Campbell, O. M. (2000). Menstrual dysfunction: a missed opportunity for improving reproductive health in developing countries. *Reprod Health Matters, 8*(15), 142-7.

Harris-Glocker, M., Davidson, K., Kochman, L., Guzick, D., & Hoeger, K. (2010). Improvement in quality-of-life questionnaire measures in obese adolescent females with polycystic ovary syndrome treated with lifestyle changes and oral contraceptives, with or without metformin. *Fertil Steril, 93*.

Harrison, C. L., Lombard, C. B., Moran, L. J., & Teede, H. J. (2011). Exercise therapy in polycystic ovary syndrome: a systematic review. *Hum Reprod Update, 17*(2), 171-83.

Hartman, T. J., Albert, P. S., Zhang, Z., Bagshaw, D., Kris-Etherton, P. M., Ulbrecht, J., Miller, C. K., Bobe, G., Colburn, N. H., & Lanza, E. (2010). Consumption of a legume-enriched, low-glycemic index diet is associated with biomarkers of insulin resistance and inflammation among men at risk for colorectal cancer. *J Nutr, 140*(1), 60-7.

Hawley, J. A. (2004). Exercise as a Therapeutic Intervention for the Prevention and Treatment of Insulin Resistance. *Diabtes Metab Res Rev, 20*(5), 383-93.

Hawley, J. A. (2004). Exercise as a therapeutic intervention for the prevention and treatment of insulin resistance. *Diabetes Metab Res Rev, 20*(5), 383-93.

He, C., Lin, Z., Robb, S. W., & Ezeamama, A. E. (2015). Serum vitamin D levels and polycystic ovary syndrome: a systematic review and meta-analysis. *Nutrients, 7*(6), 4555-77.

Health Canada. (2007). Eating Well with Canada's Food Guide. Retrieved from http://www.hc-sc.gc.ca/fn-an/alt_formats/hpfb-dgpsa/pdf/food-guide-aliment/print_eatwell_bienmang-eng.pdf

Health Canada. Nutrient Value of Some Common Foods. (2008). Retrieved from www.healthcanada.gc.ca/cnf

Hellendoorn, E. W. (1979). Beneficial physiological activity of leguminous seeds. *Qual Plant, 29*(1-2), 227-44.

Hermsdorff, H. H. M., Zulet, M. Á., Abete, I., & Martínez, J. A. (2011). A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects. *EJCN, 50*(1), 61-9.

Himelein, M. J., & Thatcher, S. S. (2006). Polycystic ovary syndrome and mental health: a review. *Obstet Gynecol Surv, 61*(11), 723-32.

Hoeger, K., Davidson, K., Kochman, L., Cherry, T., Kopin, L., & Guzick, D. S. (2008). The impact of metformin, oral contraceptives, and lifestyle modification on polycystic ovary syndrome in obese adolescent women in two randomized, placebo-controlled clinical trials. *J Clin Endocrinol Metab, 93*(11), 4299-306.

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

Hoeger, K. M., Kochman, L., Wixom, N., Craig, K., Miller, R. K., & Guzick, D. S. (2004). A randomized, 48-week, placebo-controlled trial of intensive lifestyle modification and/or metformin therapy in overweight women with polycystic ovary syndrome: a pilot study. *Fertil Steril, 82*(2), 421-9.

Holick, M. F. (2007). Vitamin D deficiency. *N Eng J Med, 357*(3), 266-81.

Homburg, R. (2006). Pregnancy complications in PCOS. *Best Pract Res Clin Endocrinol Metab, 20*(2), 281-92.

Hovatta, O., Silye, R., Abir, R., Krausz, T., & Winston, R. (1997). Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. *Hum Reprod, 12*(5), 1032-6.

Huang, P. L. (2009). A comprehensive definition for metabolic syndrome. *Dis Model Mech, 2*(5-6), 231-7.

Huber-Buchholz, M.-M., Carey, D., & Norman, R. (1999). Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. *J Clin Endocrinol Metab, 84*(4), 1470-4.

Huber-Buchholz, M. M., Carey, D. G., & Norman, R. J. (1999). Restoration of reproductive potential by lifestyle modification in obese polycystic ovary syndrome: role of insulin sensitivity and luteinizing hormone. *J Clin Endocrinol Metab, 84*, 1470-4.

Ikramuddin, S., Korner, J., Lee, W.-J., Connett, J. E., Inabnet, W. B., Billington, C. J., Thomas, A. J., Leslie, D. B., Chong, K., & Jeffery, R. W. (2013). Roux-en-Y gastric bypass vs intensive

medical management for the control of type 2 diabetes, hypertension, and hyperlipidemia: the Diabetes Surgery Study randomized clinical trial. *JAMA, 309*(21), 2240-9.

Indran, I. R., Lee, B. H., & Yong, E.-L. (2016). Cellular and animal studies: insights into pathophysiology and therapy of PCOS. *Best Pract Res Clin Obstet Gynaecol, 1*(37), 12-24.

Iqbal, A., Khalil, I. A., Ateeq, N., & Sayyar Khan, M. (2006). Nutritional quality of important food legumes. *Food Chem, 97*(2), 331-5.

Irani, M., Seifer, D. B., Grazi, R. V., Julka, N., Bhatt, D., Kalgi, B., Irani, S., Tal, O., Lambert-Messerlian, G., & Tal, R. (2015). Vitamin D supplementation decreases TGF-beta1 bioavailability in PCOS: a randomized placebo-controlled trial. *J Clin Endocrinol Metab, 100*(11), 4307-14.

Jacobs, D., Meyer, K. A., Kushi, L. H., & Folsom, A. R. (1998). Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. *Am J Clin Nutr, 68*(2), 248-57.

Jamilian, M., Razavi, M., Fakhrie Kashan, Z., Ghandi, Y., Bagherian, T., & Asemi, Z. (2015). Metabolic response to selenium supplementation in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. *Clin Endocrinol (Oxf), 82*(6), 885-91.

Jang, Y., Lee, J. H., Kim, O. Y., Park, H. Y., & Lee, S. Y. (2001). Consumption of whole grain and legume powder reduces insulin demand, lipid peroxidation, and plasma homocysteine concentrations in patients with coronary artery disease randomized controlled clinical trial. *Arterioscler Thromb Vasc Biol, 21*(12), 2065-71.

Jarrett, B. Y., & Lujan, M. E. (2017). Impact of hypocaloric dietary intervention on ovulation in obese women with PCOS. *Reproduction, 153*(1), R15-R27.

Jayalath, V. H., de Souza, R. J., Sievenpiper, J. L., Ha, V., Chiavaroli, L., Mirrahimi, A., Di Buono, M., Bernstein, A. M., Leiter, L. A., Kris-Etherton, P. M., Vuksan, V., Beyene, J., Kendall, C. W. C., & Jenkins, D. J. A. (2014). Effect of dietary pulses on blood pressure: a systematic review and meta-analysis of controlled feeding trials. *Am J Hypertens, 27*(1), 56-64.

Jayasena, C. N., & Franks, S. (2014). The management of patients with polycystic ovary syndrome. *Nat Rev Endocrinol, 10*, 624.

Jee, S. H., Miller, E. R., Guallar, E., Singh, V. K., Appel, L. J., & Klag, M. J. (2002). The effect of magnesium supplementation on blood pressure: a meta-analysis of randomized clinical trials. *Am J Hypertens, 15*(8), 691-6.

Jenkins, D., Wolever, T., Rao, A. V., Hegele, R. A., Mitchell, S. J., Ransom, T., Boctor, D. L., Spadafora, P. J., Jenkins, A. L., & Mehling, C. (1993). Effect on blood lipids of very high intakes of fiber in diets low in saturated fat and cholesterol. *N Eng J Med, 329*(1), 21-6.

Jenkins, D., Wolever, T., Taylor, R. H., Barker, H., Fielden, H., Baldwin, J. M., Bowling, A. C., Newman, H. C., Jenkins, A. L., & Goff, D. V. (1981). Glycemic index of foods: a physiological basis for carbohydrate exchange. *AJCN, 34*(3), 362-6.

Jenkins, D. J., Kendall, C. W., Augustin, L. S., Franceschi, S., Hamidi, M., Marchie, A., Jenkins, A. L., & Axelsen, M. (2002). Glycemic index: overview of implications in health and disease. *Am J Clin Nutr, 76*(1), 266S-73S.

Jenkins, D. J. A., Kendall, C. W. C., Marchie, A., Faulkner, D., Vidgen, E., Lapsley, K. G., Trautwein, E. A., Parker, T. L., Josse, R. G., Leiter, L. A., & Connelly, P. W. (2003). The effect of combining plant sterols, soy protein, viscous fibers, and almonds in treating hypercholesterolemia. *Metabolism, 52*(11), 1478-83.

Johnson-Down, L., Ritter, H., Starkey, L. J., & Gray-Donald, K. (2006). Primary food sources of nutrients in the diet of Canadian adults. *Can J Diet Pract Res, 67*(1), 7-13.

Jonard, S., & Dewailly, D. (2004). The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Hum Reprod Update, 10*(2), 107-17.

Kahn, S. E., Hull, R. L., & Utzschneider, K. M. (2006). Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature, 444*(7121), 840-6.

Kalupahana, N., & Moustaid-Moussa, N. (2012). The renin-angiotensin system: a link between obesity, inflammation and insulin resistance. *Obes Rev, 13*(2), 136-49.

Kandaraki, E., Chatzigeorgiou, A., Livadas, S., Palioura, E., Economou, F., Koutsilieris, M., Palimeri, S., Panidis, D., & Diamanti-Kandarakis, E. (2010). Endocrine disruptors and polycystic ovary syndrome (PCOS): elevated serum levels of bisphenol A in women with PCOS. *J Clin Endocrinol Metab, 96*(3), E480-E4.

Kasim-Karakas, S. E., Almario, R. U., Gregory, L., Wong, R., Todd, H., & Lasley, B. L. (2004). Metabolic and endocrine effects of a polyunsaturated fatty acid-rich diet in polycystic ovary syndrome. *J Clin Endocrinol Metab, 89*(2), 615-20.

Kaufman, M. L., & Homa, S. T. (1993). Defining a role for calcium in the resumption and progression of meiosis in the pig oocyte. *Exp Zool, 265*(1), 69-76.

Kern, P. A., Di Gregorio, G. B., Lu, T., Rassouli, N., & Ranganathan, G. (2003). Adiponectin expression from human adipose tissue. *Diabetes, 52*(7), 1779-85.

Kiddy, D., Sharp, P., White, D., Scanlon, M., Mason, H., Bray, C., Polson, D., Reed, M., & Franks, S. (1990). Differences in clinical and endocrine features between obese and non-obese subjects with polycystic ovary syndrome: an analysis of 263 consecutive cases. *Clin Endocrinol (Oxf), 32*(2), 213-20.

Kirchengast, S., & Huber, J. (2001). Body composition characteristics and body fat distribution in lean women with polycystic ovary syndrome. *Hum Reprod, 16*(6), 1255-60.

Kirk, A., Mutrie, N., MacIntyre, P., & Fisher, M. (2003). Increasing physical activity in people with type 2 diabetes. *Diabetes Care, 26*(4), 1186-92.

Kishimoto, Y., Wakabayashi, S., & Takeda, H. (1995). Hypocholesterolemic effect of dietary fiber: relation to intestinal fermentation and bile acid excretion. *J Nutr Sci Vitaminol (Tokyo), 41*(1), 151-61.

Kjerulff, L. E., Sanchez-Ramos, L., & Duffy, D. (2011). Pregnancy outcomes in women with polycystic ovary syndrome: a metaanalysis. *Am J Obstet Gynecol, 204*(6), 558.e1-6.

Knochenhauer, E. S. (1998). Prevalence of the polycystic ovary syndrome in unselected black and white women of the southeastern United States: a prospective study. *J Clin Endocrinol Metab, 83*, 3078-82.

Knowler, W. C., Barrett-Connor, E., Fowler, S. E., Hamman, R. F., Lachin, J. M., Walker, E. A., & Nathan, D. M. (2002). Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *New Eng J Med, 2002*(346), 393-403.

Kogure, G. S., Miranda-Furtado, C. L., Silva, R. C., Melo, A. S., Ferriani, R. A., De Sa, M. F., & Dos Reis, R. M. (2016). Resistance exercise impacts lean muscle mass in women with polycystic ovary syndrome. *Med Sci Sports Exerc, 48*(4), 589-98.

Koh, A., De Vadder, F., Kovatcheva-Datchary, P., & Bäckhed, F. (2016). From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell, 165*(6), 1332-45.

Kozarsky, K. F., Donahee, M. H., Glick, J. M., Krieger, M., & Rader, D. J. (2000). Gene transfer and hepatic overexpression of the HDL receptor SR-BI reduces atherosclerosis in the cholesterol-fed LDL receptor–deficient mouse. *Arterioscler Thromb Vasc Biol, 20*(3), 721-7.

Kravariti, M., Naka, K. K., Kalantaridou, S. N., Kazakos, N., Katsouras, C. S., Makrigiannakis, A., Paraskevaidis, E. A., Chrousos, G. P., Tsatsoulis, A., & Michalis, L. K. (2005). Predictors of endothelial dysfunction in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 90*(9), 5088-95.

Kuchenbecker, W. K. H., Groen, H., van Asselt, S. J., Bolster, J. H. T., Zwerver, J., Slart, R. H. J., vd Jagt, E. J., Muller Kobold, A. C., Wolffenbuttel, B. H. R., Land, J. A., & Hoek, A. (2011). In women with polycystic ovary syndrome and obesity, loss of intra-abdominal fat is associated with resumption of ovulation. *Hum Reprod, 26*(9), 2505-12.

Lago, R. M., Singh, P. P., & Nesto, R. W. (2007). Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. *Lancet, 370*(9593), 1129-36.

Lara, L. A., Ramos, F. K., Kogure, G. S., Costa, R. S., Silva de Sa, M. F., Ferriani, R. A., & dos Reis, R. M. (2015). Impact of physical resistance training on the sexual function of women with polycystic ovary syndrome *J Sex Med, 12*(7), 1584-90.

Lasley, B. L., Santoro, N., Randolf, J. F., Gold, E. B., Crawford, S., Weiss, G., McConnell, D. S., & Sowers, M. F. (2002). The relationship of circulating dehydroepiandrosterone, testosterone, and estradiol to stages of the menopausal transition and ethnicity. *J Clin Endocrinol Metab, 87*(8), 3760-7.

Lawlor, D. A., Ebrahim, S., & Smith, G. D. (2002). Socioeconomic position in childhood and adulthood and insulin resistance: cross sectional survey using data from British women's heart and health study. *BMJ, 325*(7368), 805.

Lefevre, M., Lovejoy, J. C., Smith, S. R., DeLany, J. P., Champagne, C., Most, M. M., Denkins, Y., de Jonge, L., Rood, J., & Bray, G. A. (2005). Comparison of the acute response to meals enriched with cis-or trans-fatty acids on glucose and lipids in overweight individuals with differing FABP2 genotypes. *Metabolism, 54*(12), 1652-8.

Legro, R. S. (2017). Effects of obesity treatment on female reproduction: results do not match expectations. *Fertil Steril, 107*(4), 860-7.

Legro, R. S., Arslanian, S. A., Ehrmann, D. A., Hoeger, K. M., Murad, M. H., Pasquali, R., & Welt, C. K. (2013). Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab, 98*(12), 4565-92.

Legro, R. S., Castracane, V. D., & Kauffman, R. P. (2004). Detecting insulin resistance in polycystic ovary syndrome: purposes and pitfalls. *Obstet Gynecol Surv, 59*(2), 141-54.

Legro, R. S., Driscoll, D., Strauss, J. F., Fox, J., & Dunaif, A. (1998). Evidence for a genetic basis for hyperandrogenemia in polycystic ovary syndrome. *Proc Natl Acad Sci USA, 95*, 14956-60.

Legro, R. S., Kunselman, A. R., Dodson, W. C., & Dunaif, A. (1999). Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab, 84*(1), 165-9.

Legro, R. S., Kunselman, A. R., & Dunaif, A. (2001). Prevalence and predictors of dyslipidemia in women with polycystic ovary syndrome. *Am J Med, 111*, 607-13.

Lewis, J. D., Ferrara, A., Peng, T., Hedderson, M., Bilker, W. B., Quesenberry, C. P., Vaughn, D. J., Nessel, L., Selby, J., & Strom, B. L. (2011). Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. *Diabetes Care, 34*(4), 916-22.

Li, H. W. R., Brereton, R. E., Anderson, R. A., Wallace, A. M., & Ho, C. K. (2011). Vitamin D deficiency is common and associated with metabolic risk factors in patients with polycystic ovary syndrome. *Metabolism, 60*(10), 1475-81.

Li, S. S., Kendall, C. W., Souza, R. J., Jayalath, V. H., Cozma, A. I., Ha, V., Mirrahimi, A., Chiavaroli, L., Augustin, L. S., & Blanco Mejia, S. (2014). Dietary pulses, satiety and food intake: a systematic review and meta-analysis of acute feeding trials. *Obesity, 22*(8), 1773-80.

Li, Y., Li, Y., Yu Ng, E. H., Stener-Victorin, E., Hou, L., Wu, T., Han, F., & Wu, X. (2011). Polycystic ovary syndrome is associated with negatively variable impacts on domains of health-related quality of life: evidence from a meta-analysis. *Fertil Steril, 96*(2), 452-8.

Liao, L. M., Nesic, J., Chadwick, P. M., Brooke-Wavell, K., & Prelevic, G. M. (2008). Exercise and body image distress in overweight and obese women with polycystic ovary syndrome: a pilot investigation. *Gynecol Endocrinol, 24*(10), 555-61.

Lim, S., Norman, R., Davies, M., & Moran, L. (2013). The effect of obesity on polycystic ovary syndrome: a systematic review and meta-analysis. *Obes Rev, 14*(2), 95-109.

Lin, A. W., & Lujan, M. E. (2014). Comparison of dietary intake and physical activity between women with and without polycystic ovary syndrome: a review. *Adv Nutr, 5*(5), 486-96.

Lindmark, S., Wiklund, U., Bjerle, P., & Eriksson, J. W. (2003). Does the autonomic nervous system play a role in the development of insulin resistance? A study on heart rate variability in first-degree relatives of type 2 diabetes patients and control subjects. *Diabet Med, 20*(5), 399-405.

Lord, J., Thomas, R., Fox, B., Acharya, U., & Wilkin, T. (2006). The central issue? visceral fat mass is a good marker of insulin resistance and metabolic disturbance in women with polycystic ovary syndrome. *BJOG, 113*(10), 1203-9.

Lujan, M. E., Brooks, E. D., Kepley, A. L., Chizen, D. R., Pierson, R. A., & Peppin, A. K. (2010). Grid analysis improves reliability in follicle counts made by ultrasonography in women with polycystic ovary syndrome. *Ultrasound Med Biol, 36*(5), 712-8.

Lujan, M. E., Chizen, D. R., & Pierson, R. A. (2008). Diagnostic criteria for polycystic ovary syndrome: pitfalls and controversies. *J Obstet Gynaecol Can, 30*(8), 671-9.

Lujan, M. E., Jarrett, B. Y., Brooks, E. D., Reines, J. K., Peppin, A. K., Muhn, N., Haider, E., Pierson, R. A., & Chizen, D. R. (2013). Updated ultrasound criteria for polycystic ovary syndrome: reliable thresholds for elevated follicle population and ovarian volume. *Hum Reprod, 28*(5), 1361-8.

Luque-Ramírez, M., Mendieta-Azcona, C., Álvarez-Blasco, F., & Escobar-Morreale, H. F. (2007). Androgen excess is associated with the increased carotid intima-media thickness observed in young women with polycystic ovary syndrome. *Hum Reprod, 22*(12), 3197-203.

MacDonald, P. C., Rombaut, R. P., & Siiteri, P. K. (1967). Plasma precursors of estrogen. I. Extent of conversion of plasma Δ4-androstenedione to estrone in normal males and nonpregnant normal, castrate and adrenalectomized females. *J Clin Endocrinol Metab, 27*(8), 1103-11.

Maciel, G. A., Baracat, E. C., Benda, J. A., Markham, S. M., Hensinger, K., Chang, R. J., & Erickson, G. F. (2004). Stockpiling of transitional and classic primary follicles in ovaries of women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 89*(11), 5321-7.

Mannerås-Holm, L., Baghaei, F., Holm, G., Janson, P. O., Ohlsson, C., Lönn, M., & Stener-Victorin, E. (2011). Coagulation and fibrinolytic disturbances in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 96*(4), 1068-76.

Manson , J. E., Tosteson , H., Ridker , P. M., Satterfield , S., Hebert , P., O'Connor , G. T., Buring , J. E., & Hennekens , C. H. (1992). The primary prevention of myocardial infarction. *New Eng J Med, 326*(21), 1406-16.

March, W. A., Moore, V. M., Willson, K. J., Phillips, D. I. W., Norman, R. J., & Davies, M. J. (2010). The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod, 25*(2), 544-51.

Marina Commodities. Pulse Facts. (2016). Retrieved from http://marinacommodities.com/pulse-facts/

Marinangeli, C. P. F., & Jones, P. J. H. (2012). Pulse grain consumption and obesity: Effects on energy expenditure, substrate oxidation, body composition, fat deposition and satiety. *Br J Nutr, 108*(S1), S46-S51.

Marsh, K., & Brand-Miller, J. (2005). The optimal diet for women with polycystic ovary syndrome? *Br J Nutr, 94*(02), 154-65.

Marsh, K. A., Steinbeck, K. S., Atkinson, F. S., Petocz, P., & Brand-Miller, J. C. (2010). Effect of a low glycemic index compared with a conventional healthy diet on polycystic ovary syndrome. *Am J Clin Nutr, 92*(1), 83-92.

Mathers, J. C. (2002). Pulses and carcinogenesis: potential for the prevention of colon, breast and other cancers. *Br J Nutr, 88*(Suppl3), 273-9.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia, 28*(7), 412-9.

McBreairty, L. E., Chilibeck, P. D., Chizen, D. R., Pierson, R. A., Tumback, L., Sherar, L. B., & Zello, G. A. (2017). The role of a pulse-based diet on infertility measures and metabolic syndrome risk: protocol of a randomized clinical trial in women with polycystic ovary syndrome. *BMC Nutr, 3*(1), 23.

McCarney, R., Warner, J., Iliffe, S., van Haselen, R., Griffin, M., & Fisher, P. (2007). The Hawthorne Effect: a randomised, controlled trial. *BMC Med Res Methodol 7*(1), 30.

McCook, J. G., Reame, N. E., & Thatcher, S. S. (2005). Health-related quality of life issues in women with polycystic ovary syndrome. *J Obstet Gynecol Neonatal Nurs, 34*(1), 12-20.

McCrory, M. A., Hamaker, B. R., Lovejoy, J. C., & Eichelsdoerfer, P. E. (2010). Pulse consumption, satiety, and weight management. *Adv Nutr, 1*(1), 17-30.

Mensink, R. P., & Katan, M. B. (1992). Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler Thromb, 12*(8), 911-9.

Meyer, C., McGrath, B., Cameron, J., Kotsopoulos, D., & Teede, H. (2005). Vascular dysfunction and metabolic parameters in polycystic ovary syndrome. *J Clin Endocrinol Metab, 90*(8), 4630-5.

Meyer, C., McGrath, B. P., & Teede, H. J. (2005). Overweight women with polycystic ovary syndrome have evidence of subclinical cardiovascular disease. *J Clin Endocrinol Metab, 90*, 5711-6.

Michelmore, K. F., Balen, A. H., Dunger, D. B., & Vessey, M. P. (1999). Polycystic ovaries and associated clinical and biochemical features in young women. *Clin Endocrinol (Oxf), 51*(6), 779-86.

Minorsky, P. V. (2003). The hot and the classic. *Plant Physiol, 131*(3), 1159-60.

Mitchell, D. C., Lawrence, F. R., Hartman, T. J., & Curran, J. M. (2009). Consumption of dry beans, peas, and lentils could improve diet quality in the US population. *J Acad Nutr Diet, 109*(5), 909-13.

Mollard, R., Zykus, A., Luhovyy, B., Nunez, M., Wong, C., & Anderson, G. (2012). The acute effects of a pulse-containing meal on glycaemic responses and measures of satiety and satiation within and at a later meal. *Br J Nutr, 108*(03), 509-17.

Moran, C. (2010). Prevalence of polycystic ovary syndrome and related disorders in Mexican women. *Gynecol Obstet Invest, 69*, 274-80.

Moran, L. J., Hutchison, S. K., Norman, R. J., & Teede, H. J. (2011). Lifestyle changes in women with polycystic ovary syndrome. *Cochrane Database Syst Rev*(7), Cd007506.

Moran, L. J., Ko, H., Misso, M., Marsh, K., Noakes, M., Talbot, M., Frearson, M., Thondan, M., Stepto, N., & Teede, H. J. (2013). Dietary composition in the treatment of polycystic ovary syndrome: a systematic review to inform evidence-based guidelines. *J Acad Nutr Diet, 113*(4), 520-45.

Moran, L. J., Misso, M. L., Wild, R. A., & Norman, R. J. (2010). Impaired glucose tolerance, type 2 diabetes and metabolic syndrome in polycystic ovary syndrome: a systematic review and meta-analysis. *Hum Reprod Update, 16*(4), 347-63.

Moran, L. J., Noakes, M., Clifton, P. M., Wittert, G. A., Le Roux, C. W., Ghatei, M. A., Bloom, S. R., & Norman, R. J. (2007). Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. *Am J Clin Nutr, 86*(6), 1603-10.

Moran, L. J., Pasquali, R., Teede, H. J., Hoeger, K. M., & Norman, R. J. (2009). Treatment of obesity in polycystic ovary syndrome: a position statement of the Androgen Excess and Polycystic Ovary Syndrome Society. *Fertil Steril, 92*(6), 1966-82.

Moran, L. J., Ranasinha, S., Zoungas, S., McNaughton, S. A., Brown, W. J., & Teede, H. J. (2013). The contribution of diet, physical activity and sedentary behavior to body mass index in women with and without polycystic ovary syndrome. *Hum Reprod, 28*(8), 2276-83.

Moran, L. J., & Teede, H. (2009). Metabolic features of the reproductive phenotypes of polycystic ovary syndrome. *Hum Reprod Update, 15*, 477-88.

Motta, A. B. (2012). The role of obesity in the development of polycystic ovary syndrome. *Curr Pharm Des, 18*(17), 2482-91.

Mozaffarian, D., Pischon, T., Hankinson, S. E., Rifai, N., Joshipura, K., Willett, W. C., & Rimm, E. B. (2004). Dietary intake of trans fatty acids and systemic inflammation in women. *Am J Clin Nutr, 79*(4), 606-12.

Mudryj, A. N., Yu, N., & Aukema, H. M. (2014). Nutritional and health benefits of pulses. *Appl Physiol Nutr Metab, 39*(11), 1197-204.

Mudryj, A. N., Yu, N., Hartman, T. J., Mitchell, D. C., Lawrence, F. R., & Aukema, H. M. (2012). Pulse consumption in Canadian adults influences nutrient intakes. *Br J Nutr, 108*(S1), S27-S36.

Murri, M., Luque-Ramírez, M., Insenser, M., Ojeda-Ojeda, M., & Escobar-Morreale, H. F. (2013). Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis. *Hum Reprod Update*. Retrieved from doi:10.1093/humupd/dms059

Musrap, N., & Diamandis, E. P. (2016). Prostate-specific antigen as a marker of hyperandrogenism in women and its implications for antidoping. *Clinical chemistry, 62*(8), 1066-74.

National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and

Treatment of High Blood Cholesterol in adults (Adult Treatment Panel III) final report. (2002). *Circulation, 106*(25), 3143-421.

Nelson, V. L., Legro, R. S., Strauss, J. F., 3rd, & McAllister, J. M. (1999). Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. *Mol Endocrinol, 13*(6), 946-57.

Norman, R. J., Davies, M. J., Lord, J., & Moran, L. J. (2002). The role of lifestyle modification in polycystic ovary syndrome. *Trends Endocrinol Metab, 13*(6), 251-7.

Norman, R. J., Dewailly, D., Legro, R. S., & Hickey, T. E. (2007). Polycystic ovary syndrome. *Lancet, 370*(9588), 685-97.

Nutrient Recommendations: Dietary Reference Intakes (DRI). (2011). Retrieved from https://ods.od.nih.gov/Health_Information/Dietary_Reference_Intakes.aspx

O'Donnell, A., & Fleming, S. (1984). Influence of frequent and long-term consumption of legume seeds on excretion of intestinal gases. *AJCN, 40*(1), 48-57.

O'Driscoll, J., Mamtora, H., Higginson, J., Pollock, A., Kane, J., & Anderson, D. (1994). A prospective study of the prevalence of clear-cut endocrine disorders and polycystic ovaries in 350 patients presenting with hirsutism or androgenic alopecia. *Clin Endocrinol (Oxf), 41*(2), 231-6.

O'Meara, N., Blackman, J., Ehrmann, D., Barnes, R., Jaspan, J., Rosenfield, R., & Polonsky, K. (1993). Defects in beta-cell function in functional ovarian hyperandrogenism. *J Clin Endocrinol Metab, 76*(5), 1241-7.

Orchard, T. J., Temprosa, M., Goldberg, R., Haffner, S., Ratner, R., Marcovina, S., & Fowler, S. (2005). The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann Int Med, 142*(8), 611-9.

Orio, F., Muscogiuri, G., Giallauria, F., Savastano, S., Bottiglieri, P., Tafuri, D., Predotti, P., Colarieti, G., Colao, A., & Palomba, S. (2016). Oral contraceptives versus physical exercise on cardiovascular and metabolic risk factors in women with polycystic ovary syndrome: a randomized controlled trial. *Clin Endocrinol (Oxf), 85*(5), 764-71.

Ortega, I., Villanueva, J. A., Wong, D. H., Cress, A. B., Sokalska, A., Stanley, S. D., & Duleba, A. J. (2014). Resveratrol potentiates effects of simvastatin on inhibition of rat ovarian theca-interstitial cells steroidogenesis. *J Ovarian Res, 7*(1), 1.

Ovesen, P., Moller, J., Ingerslev, H. J., Jorgensen, J. O., Mengel, A., Schmitz, O., Alberti, K. G., & Moller, N. (1993). Normal basal and insulin-stimulated fuel metabolism in lean women with the polycystic ovary syndrome. *J Clin Endocrinol Metab, 77*(6), 1636-40.

Palomba, S. (2015). Aromatase inhibitors for ovulation induction. *J Clin Endocrinol Metab, 100*(5), 1742-7.

Palomba, S., Falbo, A., Zullo, F., & Orio, F. (2009). Evidence-based and potential benefits of metformin in the polycystic ovary syndrome: a comprehensive review. *Endocr Rev, 30*, 1-50.

Palomba, S., Giallauria, F., Falbo, A., Russo, T., Oppedisano, R., Tolino, A., Colao, A., Vigorito, C., Zullo, F., & Orio, F. (2008). Structured exercise training programme versus hypocaloric hyperproteic diet in obese polycystic ovary syndrome patients with anovulatory infertility: a 24-week pilot study. *Hum Reprod, 23*(3), 642-50.

Palomba, S., Santagni, S., Falbo, A., & La Sala, G. B. (2015). Complications and challenges associated with polycystic ovary syndrome: current perspectives. *Int J Womens Health, 7*, 745-63.

Panidis, D., Skiadopoulos, S., Rousso, D., Ioannides, D., & Panidou, E. (1995). Association of acanthosis nigricans with insulin resistance in patients with polycystic ovary syndrome. *Br J Dermatol, 132*(6), 936-41.

Panidis, D., Tziomalos, K., Papadakis, E., Chatzis, P., Kandaraki, E. A., Tsourdi, E. A., & Katsikis, I. (2014). The role of orlistat combined with lifestyle changes in the management of overweight and obese patients with polycystic ovary syndrome. *Clin Endocrinol (Oxf), 80*(3), 432-8.

Pardridge, W. (1981). Transport of protein-bound hormones into tissues in vivo. *Endocrin Rev, 2*(1), 103.

Phillips, T., Zello, G. A., Chilibeck, P. D., & Vandenberg, A. (2014). Perceived benefits and barriers surrounding lentil consumption in families with young children. *Can J Diet Pract Res, 76*(1), 3-8.

Piotrowski, P., Rzepczynska, I., Kwintkiewicz, J., & Duleba, A. (2005). Oxidative stress induces expression of CYP11A, CYP17, STAR, and 3βHSD in rat theca-interstitial cells. *J Soc Gynecol Investig, 12*(Suppl 2), 319A.

Pittas, A. G., Lau, J., Hu, F. B., & Dawson-Hughes, B. (2007). The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab, 92*(6), 2017-29.

Plat, J., & Mensink, R. P. (2005). Plant stanol and sterol esters in the control of blood cholesterol levels: mechanism and safety aspects. *Am J Cardiol, 96*(1), 15-22.

Plymate, S. R., Matej, L. A., Jones, R. E., & Friedl, K. E. (1988). Inhibition of sex hormone-binding globulin production in the human hepatoma (Hep G2) cell line by insulin and prolactin. *J Clin Endocrinol Metab, 67*(3), 460-4.

Practice Committee of American Society for Reproductive Medicine, Society for Reproductive Endocrinology and Infertility. Optimizing natural fertility: a committee opinion. (2013). *Fertil Steril, 100*(3), 631-7.

Rafraf, M., Mohammadi, E., Asghari-Jafarabadi, M., & Farzadi, L. (2012). Omega-3 fatty acids improve glucose metabolism without effects on obesity values and serum visfatin levels in women with polycystic ovary syndrome. *J Am Coll Nutr, 31*(5), 361-8.

Ramos, F. K., Lara, L. A., Kogure, G. S., Silva, R. C., Ferriani, R. A., Silva de Sa, M. F., & Reis, R. M. (2016). Quality of life in women with polycystic ovary syndrome after a program of resistance exercise training. *Rev Bras Ginecol Obstet, 38*(7), 340-7.

Rasgon, N. L., Rao, R. C., Hwang, S., Altshuler, L. L., Elman, S., Zuckerbrow-Miller, J., & Korenman, S. G. (2003). Depression in women with polycystic ovary syndrome: clinical and biochemical correlates. *J Affect Disord, 74*(3), 299-304.

Rashti, F., Gupta, E., Shope, T. R., & Koch, T. R. (2015). Overview of Nutritional Deficiencies After Bariatric Surgery. In R. Rajendram, V. R. Preedy, & V. B. Patel (Eds.), *Diet and Nutrition in Critical Care* (pp. 1079-92). New York, NY: Springer New York.

Rebar, R., Judd, H., Yen, S., Rakoff, J., Vandenberg, G., & Naftolin, F. (1976). Characterization of the inappropriate gonadotropin secretion in polycystic ovary syndrome. *J Clin Invest, 57*(5), 1320.

Roberfroid, M., & Slavin, J. (2000). Nondigestible oligosaccharides. *Crit Rev Food Sci Nutr 40*(6), 461-80.

Roque, M., Tostes, A. C., Valle, M., Sampaio, M., & Geber, S. (2015). Letrozole versus clomiphene citrate in polycystic ovary syndrome: systematic review and meta-analysis. *Gynecol Endocrinol, 31*(12), 917-21.

Rosner, W., Auchus, R. J., Azziz, R., Sluss, P. M., & Raff, H. (2007). Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab, 92*, 405-13.

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 (PCOS). (2004). *Hum Reprod, 19*(1), 41-7.

Rubio, M. A., Gargallo, M., Isabel Millan, A., & Moreno, B. (2007). Drugs in the treatment of obesity: sibutramine, orlistat and rimonabant. *Public Health Nutr, 10*(10a), 1200-5.

Saad, M. F., Lillioja, S., Nyomba, B. L., Castillo, C., Ferraro, R., De Gregorio, M., Ravussin, E., Knowler, W. C., Bennett, P. H., & Howard, B. V. (1991). Racial differences in the relation between blood pressure and insulin resistance. *N Eng J Med, 324*(11), 733-9.

Sacks, F. M., Svetkey, L. P., Vollmer, W. M., Appel, L. J., Bray, G. A., Harsha, D., Obarzanek, E., Conlin, P. R., Miller, E. R., & Simons-Morton, D. G. (2001a). Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *N Eng J Med, 344*(1), 3-10.

Sacks, F. M., Svetkey, L. P., Vollmer, W. M., Appel, L. J., Bray, G. A., Harsha, D., Obarzanek, E., Conlin, P. R., Miller, E. R., & Simons-Morton, D. G. (2001b). Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. *New Eng J Med, 344*(1), 3-10.

Salley, K. E. S., Wickham, E. P., Cheang, K. I., Essah, P. A., Karjane, N. W., & Nestler, J. E. (2007). Position statement: glucose intolerance in polycystic ovary syndrome-a position statement of the Androgen Excess Society. *J Clin Endocrinol Metab, 92*(12), 4546-56.

Salmerón, J., Manson, J. E., Stampfer, M. J., Colditz, G. A., Wing, A. L., & Willett, W. C. (1997). Dietary fiber, glycemic load, and risk of non-insulin-dependent diabetes mellitus in women. *JAMA, 277*(6), 472-7.

Saltiel, A. R., & Kahn, C. R. (2001). Insulin signalling and the regulation of glucose and lipid metabolism. *Nature, 414*(6865), 799-806.

Saskatchewan Pulse Growers. Market Access. (2016b). Retrieved from http://saskpulse.com/markets/market-access/

Sasktachewan Pulse Growers. Strategic Plan. (2016c). Retrieved from http://saskpulse.com/about/spg-strategic-plan/

Schäfer, G., Schenk, U., Ritzel, U., Ramadori, G., & Leonhardt, U. (2003). Comparison of the effects of dried peas with those of potatoes in mixed meals on postprandial glucose and insulin concentrations in patients with type 2 diabetes. *Am J Clin Nutr, 78*(1), 99-103.

Schulz, K. F., Altman, D. G., Moher, D., & and the CONSORT Group. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Ann Int Med, 152*, 726-32.

Shah, M., Simha, V., & Garg, A. (2006). Long-term impact of bariatric surgery on body weight, comorbidities, and nutritional status. *J Clin Endocrinol Metab, 91*(11), 4223-31.

Shaw, L. J., Bairey Merz, C. N., Azziz, R., Stanczyk, F. Z., Sopko, G., Braunstein, G. D., Kelsey, S. F., Kip, K. E., Cooper-Dehoff, R. M., Johnson, B. D., Vaccarino, V., Reis, S. E., Bittner, V., Hodgson, T. K., Rogers, W., & Pepine, C. J. (2008). Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health-National Heart, Lung, and Blood Institute sponsored Women's Ischemia Syndrome Evaluation. *J Clin Endocrinol Metab, 93*, 1276-84.

Shishehgar, F., Tehrani, F. R., Mirmiran, P., Hajian, S., Baghestani, A. R., & Moslehi, N. (2016a). Comparison of dietary intake between polycystic ovary syndrome women and controls. *Glob J Health Sci, 8*(9), 302-11.

Shutler, S. M., Bircher, G. M., Tredger, J. A., Morgan, L. M., Walker, A. F., & LOW, A. G. (1989). The effect of daily baked bean (Phaseolus vulgaris) consumption on the plasma lipid levels of young, normo-cholesterolaemic men. *Br J Nutr, 61*(02), 257-65.

Sievenpiper, J. L., Kendall, C. W. C., Esfahani, A., Wong, J. M. W., Carleton, A. J., Jiang, H. Y., Bazinet, R. P., Vidgen, E., & Jenkins, D. J. A. (2009). Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. *Diabetologia, 52*(8), 1479-95.

Sjöström, C. D., Lissner, L., Wedel, H., & Sjöström, L. (1999). Reduction in incidence of diabetes, hypertension and lipid disturbances after intentional weight loss induced by bariatric surgery: the SOS Intervention Study. *Obesity, 7*(5), 477-84.

Skov, V., Glintborg, D., Knudsen, S., Jensen, T., Kruse, T., Tan, Q., Brusgaard, K., Beck-Nielsen, H., & Højlund, K. (2007). Reduced expression of nuclear-encoded genes involved in mitochondrial oxidative metabolism in skeletal muscle of insulin-resistant women with polycystic ovary syndrome. *Diabetes, 56*(9), 2349-55.

Smith, V., Osianlis, T., & Vollenhoven, B. (2015). Prevention of ovarian hyperstimulation syndrome: a review. *Obstet Gynecol Int, 2015*, 10.

Soares, E. M. M., Azevedo, G. D., Gadelha, R. G. N., Lemos, T. M. A. M., & Maranhão, T. M. O. (2008). Prevalence of the metabolic syndrome and its components in Brazilian women with polycystic ovary syndrome. *Fertil Steril, 89*(3), 649-55.

Stamets, K., Taylor, D. S., Kunselman, A., Demers, L. M., Pelkman, C. L., & Legro, R. S. (2004). 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, 81*(3), 630-7.

Stein, I., & Leventhal, M. (1935). Amenorrhea associated with bilateral polycystic ovaries. *Am J Obstet Gynecol, 35*(29), 181-5.

Steinhardt, R. A., Epel, D., Carroll, E. J., & Yanagimachi, R. (1974). Is calcium ionophore a universal activator for unfertilised eggs? *Nature, 252*(41-43).

Stener-Victorin, E., Jedel, E., Janson, P. O., & Sverrisdottir, Y. B. (2009). Low-frequency electroacupuncture and physical exercise decrease high muscle sympathetic nerve activity in polycystic ovary syndrome. *Am J Physiol Regul Integr Comp Physiol, 297*(2), R387-R95.

Stone, N. J., & Van Horn, L. (2002). Therapeutic lifestyle change and adult treatment panel III: evidence then and now. *Curr Atheroscler Rep, 4*(6), 433-43.

Sweatt, K., Ovalle, F., Azziz, R., & Gower, B. (2015). The effect of diet and exercise in women with polycystic ovary syndrome. *Faseb J, 29*(596), 12.

Swennen, K., Courtin, C. M., & Delcour, J. A. (2006). Non-digestible oligosaccharides with prebiotic properties. *Crit Rev Food Sci Nutr, 46*(6), 459-71.

Taghibiglou, C., Carpentier, A., Van Iderstine, S. C., Chen, B., Rudy, D., Aiton, A., Lewis, G. F., & Adeli, K. (2000). Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance *J Biol Chem, 275*(12), 8416-25.

Tai, M. M. (1994). A mathematical model for the determination of total area under glucose tolerance and other metabolic curves. *Diabetes Care, 17*(2), 152-4.

Takamiya, T., Zaky, W. R., Edmundowics, D., Kadowaki, T., Ueshima, H., Kuller, L. H., & Sekikawa, A. (2004). World Health Organization-defined metabolic syndrome is a better predictor of coronary calcium than the Adult Treatment Panel III criteria in American men aged 40–49 years. *Diabetes Care, 27*(12), 2977-9.

Talbott, E., Clerici, A., Berga, S. L., Kuller, L., Guzick, D., Detre, K., Daniels, T., & Engberg, R. A. (1998). Adverse lipid and coronary heart disease risk profiles in young women with polycystic ovary syndrome: results of a case-control study. *J Clin Epidemiol, 51*(5), 415-22.

Tarkun, I., Arslan, B. n. Ç., Canturk, Z., Turemen, E., Şahin, T., & Duman, C. (2004). Endothelial dysfunction in young women with polycystic ovary syndrome: relationship with insulin resistance and low-grade chronic inflammation. *J Clin Endocrinol Metab, 89*(11), 5592-6.

Teede, H., Deeks, A., & Moran, L. (2010). Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. *BMC Med, 8*(1), 41.

Teede, H. J., Hutchison, S., Zoungas, S., & Meyer, C. (2006). Insulin resistance, the metabolic syndrome, diabetes, and cardiovascular disease risk in women with PCOS. *Endocrine, 30*(1), 45-53.

Testa, M. A., & Simonson, D. C. (1996). Assessment of quality-of-life outcomes. *New Eng J Med, 334*(13), 835-40.

Thessaloniki ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group. Consensus on infertility treatment related to polycystic ovary syndrome. (2008). *Hum Reprod, 23*(3), 462-77.

Thompson, F. E., Kirkpatrick, S. I., Subar, A. F., Reedy, J., Schap, T. E., Wilson, M. M., & Krebs-Smith, S. M. (2015). The national cancer institute's dietary assessment primer: a resource for diet research. *J Acad Nutr Diet, 115*(12), 1986-95.

Thomson, R. L., Buckley, J. D., Lim, S. S., Noakes, M., Clifton, P. M., Norman, R. J., & Brinkworth, G. D. (2010). Lifestyle management improves quality of life and depression in overweight and obese women with polycystic ovary syndrome. *Fertil Steril, 94*(5), 1812-6.

Thomson, R. L., Buckley, J. D., Moran, L. J., Noakes, M., Clifton, P. M., Norman, R. J., & Brinkworth, G. D. (2009). Comparison of aerobic exercise capacity and muscle strength in overweight women with and without polycystic ovary syndrome. *BJOG, 116*(9), 1242-50.

Thomson, R. L., Buckley, J. D., Noakes, M., Clifton, P. M., Norman, R. J., & Brinkworth, G. D. (2008). The effect of a hypocaloric diet with and without exercise training on body composition, cardiometabolic risk profile, and reproductive function in overweight and obese women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 93*(9), 3373-80.

Thorne, M. J., Thompson, L., & Jenkins, D. (1983). Factors affecting starch digestibility and the glycemic response with special reference to legumes. *Am J Clin Nutr, 38*(3), 481-8.

Tielemans, S. M., Altorf-van Der Kuil, W., Engberink, M. F., Brink, E. J., Van Baak, M. A., Bakker, S. J., & Geleijnse, J. M. (2013). Intake of total protein, plant protein and animal protein in relation to blood pressure: a meta-analysis of observational and intervention studies. *J Hum Hypertens 27*(9), 564.

Tiitinen, A., Halttunen, M., Härkki, P., Vuoristo, P., & Hyden-Granskog, C. (2001). Elective single embryo transfer: the value of cryopreservation. *Hum Reprod, 16*(6), 1140-4.

Toscani, M. K., Mario, F. M., Radavelli-Bagatini, S., & Spritzer, P. M. (2011). Insulin resistance is not strictly associated with energy intake or dietary macronutrient composition in women with polycystic ovary syndrome. *Nutr Res, 31*(2), 97-103.

Toulis, K. A., Goulis, D. G., Mintziori, G., Kintiraki, E., Eukarpidis, E., Mouratoglou, S. A., Pavlaki, A., Stergianos, S., Poulasouchidou, M., Tzellos, T. G., Makedos, A., Chourdakis, M., & Tarlatzis, B. C. (2011). Meta-analysis of cardiovascular disease risk markers in women with polycystic ovary syndrome. *Hum Reprod Update, 17*(6), 741-60.

Tremblay, M. S., Warburton, D. E., Janssen, I., Paterson, D. H., Latimer, A. E., Rhodes, R. E., Kho, M. E., Hicks, A., LeBlanc, A. G., & Zehr, L. (2011). New Canadian physical activity guidelines. *Appl Physiol Nutr Metab, 36*(1), 36-46.

Trueb, R. M. (2002). Causes and management of hypertrichosis. *Am J Clin Dermatol, 3*(9), 617-27.

Tsai, Y.-H., Wang, T.-W., Wei, H.-J., Hsu, C.-Y., Ho, H.-J., Chen, W.-H., Young, R., Liaw, C.-M., & Chao, J. C.-J. (2013). Dietary intake, glucose metabolism and sex hormones in women with polycystic ovary syndrome (PCOS) compared with women with non-PCOS-related infertility. *Br J Nutr, 109*(12), 2190-8.

Turley, M., Skeaff, C., Mann, J., & Cox, B. (1998). The effect of a low-fat, high-carbohydrate diet on serum high density lipoprotein cholesterol and triglyceride. *Eur J Clin Nutr, 52*(10), 728-32.

Turner-McGrievy, G., Davidson, C. R., & Billings, D. L. (2015). Dietary intake, eating behaviors, and quality of life in women with polycystic ovary syndrome who are trying to conceive. *Hum Fertil (Camb), 18*(1), 16-21.

Turner-McGrievy, G. M., Davidson, C. R., Wingard, E. E., & Billings, D. L. (2014a). Low glycemic index vegan or low-calorie weight loss diets for women with polycystic ovary syndrome: a randomized controlled feasibility study. *Nutr Res, 34*(6), 552-8.

Turner-McGrievy, G. M., Davidson, C. R., Wingard, E. E., & Billings, D. L. (2014b). Low glycemic index vegan or low-calorie weight loss diets for women with polycystic ovary syndrome: A randomized controlled feasibility study. *Nutr Res, 34*.

Twells, L. K., Gregory, D. M., Reddigan, J., & Midodzi, W. K. (2014). Current and predicted prevalence of obesity in Canada: A trend analysis. *CMAJ open, 2*(1), E18-E26.

Unfer, V., Nestler, J. E., Kamenov, Z. A., Prapas, N., & Facchinetti, F. (2016). Effects of inositol(s) in women with PCOS: a systematic review of randomized controlled trials. *Int J Endocrinol, 2016*, 12.

US Department of Health and Human Services (HSS), National Center for Chronic Disease Prevention and Health Promotion, The Presidents' Council on Physical Fitness and Sports. Physical Activity and Health: A Report of the Surgeon General. (2008).

USDA Center for Nutrition and Policy Promotion. (2016). Inside the Pyramide: How many vegetables are needed daily or weekly? Retrieved from http://fit4maui.com/pages/popUps/usda_veg.html

van Bennekum, A. M., Nguyen, D. V., Schulthess, G., Hauser, H., & Phillips, M. C. (2005). Mechanisms of cholesterol-lowering effects of dietary insoluble fibres: relationships with intestinal and hepatic cholesterol parameters. *Br J Nutr, 94*(03), 331-7.

Vassilatou, E., Lafoyianni, S., Vryonidou, A., Ioannidis, D., Kosma, L., Katsoulis, K., Papavassiliou, E., & Tzavara, I. (2010). Increased androgen bioavailability is associated with non-alcoholic fatty liver disease in women with polycystic ovary syndrome. *Hum Reprod, 25*(1), 212-20.

Vause, T. D., Cheung, A. P., Sierra, S., Claman, P., Graham, J., Guillemin, J. A., Lapensee, L., Stewart, S., & Wong, B. C. (2010). Ovulation induction in polycystic ovary syndrome. *J Obstet Gynaecol Can, 32*(5), 495-502.

Vedovato, M., Lepore, G., Coracina, A., Dodesini, A., Jori, E., Tiengo, A., Del Prato, S., & Trevisan, R. (2004). Effect of sodium intake on blood pressure and albuminuria in Type 2 diabetic patients: the role of insulin resistance. *Diabetologia, 47*(2), 300-3.

Velazquez, E., Bellabarba, G. A., Mendoza, S., & Sánchez, L. (2000). Postprandial triglyceride response in patients with polycystic ovary syndrome: relationship with waist-to-hip ratio and insulin. *Fertil Steril, 74*(6), 1159-63.

Vendrell, J., Broch, M., Vilarrasa, N., Molina, A., Gómez, J. M., Gutiérrez, C., Simón, I., Soler, J., & Richart, C. (2004). Resistin, adiponectin, ghrelin, leptin, and proinflammatory cytokines: relationships in obesity. *Obesity Res, 12*(6), 962-71.

Venn, B., & Mann, J. (2004). Cereal grains, legumes and diabetes. *Eur J Clin Nutr, 58*(11), 1443-61.

Victor, M. V., Rocha, M., Bañuls, C., Sanchez-Serrano, M., Sola, S., Gomez, M., & Hernandez-Mijares, A. (2009). Mitochondrial complex I impairment in leukocytes from polycystic ovary syndrome patients with insulin resistance. *J Clin Endrocinol Metab, 94*(9), 3505-12.

Vigorito, C., Giallauria, F., Palomba, S., Cascella, T., Manguso, F., Lucci, R., De Lorenzo, A., Tafuri, D., Lombardi, G., Colao, A., & Orio, F. (2007). Beneficial effects of a three-month structured exercise training program on cardiopulmonary functional capacity in young women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 92*(4), 1379-84.

Vincent, H. K., & Taylor, A. G. (2005). Biomarkers and potential mechanisms of obesity-induced oxidant stress in humans. *Int J Obes, 30*(3), 400-18.

von Eckardstein, A. (1998). Androgens, cardiovascular risk factors, and atherosclerosis. In E. Nieschlag & H. Behre (Eds.), *Testosterone: action, deficiency, substiution* (2 ed., pp. 229-57): Springer.

Vuksan, V., Sievenpiper, J. L., Owen, R., Swilley, J. A., Spadafora, P., Jenkins, D., Vidgen, E., Brighenti, F., Josse, R. G., & Leiter, L. (2000). Beneficial effects of viscous dietary fiber from Konjac-Mannan in subjects with the insulin resistance syndrome: results of a controlled metabolic trial. *Diabetes care, 23*(1), 9-14.

Wang, T. J., Pencina, M. J., Booth, S. L., Jacques, P. F., Ingelsson, E., Lanier, K., Benjamin, E. J., D'Agostino, R. B., Wolf, M., & Vasan, R. S. (2008). Vitamin D deficiency and risk of cardiovascular disease. *Circulation, 117*(4), 503-11.

Wang, Y., & Beydoun, M. A. (2007). The obesity epidemic in the United States—gender, age, socioeconomic, racial/ethnic, and geographic characteristics: a systematic review and meta-regression analysis. *Epidemiol Rev, 29*(1), 6-28.

Webber, L., Stubbs, S., Stark, J., Trew, G., Margara, R., Hardy, K., & Franks, S. (2003). Formation and early development of follicles in the polycystic ovary. *Lancet, 362*(9389), 1017-21.

Webber, L. J., Stubbs, S. A., Stark, J., Margara, R. A., Trew, G. H., Lavery, S. A., Hardy, K., & Franks, S. (2007). Prolonged survival in culture of preantral follicles from polycystic ovaries. *J Clin Endocrinol Metab, 92*(5), 1975-8.

Wehr E, Pieber TR, & Obermayer-Pietsch B. (2011). Effect of vitamin D3 treatment on glucose metabolism and menstrual frequency in PCOS women: a pilot study. *J Endocrinol Invest, 34*(10), 757-63.

Weiner, C. L., Primeau, M., & Ehrmann, D. A. (2004). Androgens and mood dysfunction in women: comparison of women with polycystic ovarian syndrome to healthy controls. *Psychosom Med, 66*(3), 356-62.

Welt, C. K. (2006). Characterizing discrete subsets of polycystic ovary syndrome as defined by the Rotterdam Criteria: the impact of weight on phenotype and metabolic features. *J Clin Endocrinol Metab, 91*, 4842-8.

Whitsel, E. A., Boyko, E. J., Matsumoto, A. M., Anawalt, B. D., & Siscovick, D. S. (2001). Intramuscular testosterone esters and plasma lipids in hypogonadal men: a meta-analysis. *Am J Med, 111*(4), 261-9.

Wild, R. A., & Bartholomew, M. J. (1988). The influence of body weight on lipoprotein lipids in patients with polycystic ovary syndrome. *Am J Obstet Gynecol, 159*(2), 423-7.

Wild, R. A., Carmina, E., Diamanti-Kandarakis, E., Dokras, A., Escobar-Morreale, H. F., Futterweit, W., Lobo, R., Norman, R. J., Talbott, E., & Dumesic, D. A. (2010). Assessment of cardiovascular risk and prevention of cardiovascular disease in women with the polycystic ovary syndrome: a consensus statement by the Androgen Excess and Polycystic Ovary Syndrome (AE-PCOS) Society. *J Clin Endocrinol Metab, 95*(5), 2038-49.

Wild, R. A., Painter, P., Coulson, P. B., Carruth, K. B., & Ranney, G. (1985). Lipoprotein lipid concentrations and cardiovascular risk in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 61*(5), 946-51.

Wild, R. A., Umstot, E. S., Andersen, R. N., & Givens, J. R. (1982). Adrenal function in hirsutism. II. Effect of an oral contraceptive. *J Clin Endocrinol Metab, 54*(4), 676-81.

Wild, R. A., Vesely, S., Beebe, L., Whitsett, T., & Owen, W. (2005). Ferriman Gallwey self-scoring I: performance assessment in women with polycystic ovary syndrome. *J Clin Endocrinol Metab, 90*(7), 4112-4.

Willett, W. C., Sacks, F., Trichopoulou, A., Drescher, G., Ferro-Luzzi, A., Helsing, E., & Trichopoulos, D. (1995). Mediterranean diet pyramid: a cultural model for healthy eating. *Am J Clin Nutr, 61*(6), 1402S-6S.

Wilson, I. B., & Cleary, P. D. (1995). Linking clinical variables with health-related quality of life. *JAMA, 273*(1), 59-65.

Wiseman, M. (2008). The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. *Proc Nutr Soc, 67*(03), 253-6.

World Health Organization. Preventing and managing the global epidemic. (1998). *WHO Technical Report Series 894 Geneva: World Health Organization*, 1-276.

World Health Organization. Waist circumference and waist-hip ratio: report of a WHO Expert Consultation, Geneva, 8–11 December 2008. Retrieved from http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.418.302&rep=rep1&type=pdf

Wright, C., Zborowski, J., Talbott, E., McHugh-Pemu, K., & Youk, A. (2004). Dietary intake, physical activity, and obesity in women with polycystic ovary syndrome. *Int J Obes Relat Metab Disord, 28*(8), 1026-32.

Wu, T., Gao, X., Chen, M., & Van Dam, R. M. (2009). Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: a meta-analysis. *Obes Rev, 10*(3), 313-23.

Yasuda, K., Hurukawa, Y., Okuyama, M., Kikuchi, M., & Yoshinaga, K. (1975). Glucose tolerance and insulin secretion in patients with parathyroid disorders: effect of serum calcium on insulin release. *New Engl J Med, 292*(10), 501-4.

Yildiz, B. O., Bolour, S., Woods, K., Moore, A., & Azziz, R. (2010). Visually scoring hirsutism. *Hum Reprod Update, 16*(1), 51-64.

Yildiz, B. O., Knochenhauer, E. S., & Azziz, R. (2008). Impact of obesity on the risk for polycystic ovary syndrome. *J Clin Endocrinol Metab, 93*, 162-8.

Zawadzki, J., & Dunaif, A. (1992). Diagnostic criteria for polycystic ovary syndrome: towards a rational approach. In A. Dunaif, J. R. Givens, F. P. Haseltine, & G. R. Merriam (Eds.), *Polycystic ovary syndrome* (pp. 377-84). Boston: Blackwell Scientific.

Zhao, S., Tian, Y., Gao, X., Zhang, X., Liu, H., You, L., Cao, Y., Su, S., Chan, W.-Y., Sun, Y., Zhao, H., & Chen, Z.-J. (2015). Family-based analysis of eight susceptibility loci in polycystic ovary syndrome. *Sci Rep, 5*, 12619. Retrieved from doi:10.1038/srep12619.

APPENDICES

Appendix A. Characteristics of the health-related quality of life survey and the mean scores of groups for each survey item (n=55).

Quantitative information section		
Domain 1: Health concerns (12-item scores) Before/after the lifestyle intervention, I was concerned about: Choices: 1. This is not an issue for me 2. Not concerned 3. Indifferent 4. Somewhat concerned 5. Very concerned		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)
1. Fertility, and my ability to become pregnant in the future	4.1±1.3	4.2±1.1
2. Irregular menstrual bleeding	4.2±0.8	3.8±1.1
3. Unwanted male pattern hair growth (hirsutism)	4.1±1.1	4.2±0.8
4. High male hormone levels (hyperandrogenemia)	3.9±1.1	4.3±0.8
5. Acne		
6. Scalp hair loss (alopecia)	3.4±1.4	4.0±1.2
7. A weight problem	3.3±1.5	3.7±1.3
8. High insulin levels and insulin resistance	4.6±0.9	4.6±0.8
9. My risk of developing cancer of the uterus or breast	4.1±1.2	4.5±0.8
10. My risk of developing diabetes	4.4±1.0	4.4±0.7
11. My risk of developing cardiovascular disease		
12. My risk of developing the metabolic syndrome (a group of symptoms, including elevated levels of blood triglyceride, glucose, waist circumference, blood pressure, and decreased HDL cholesterol levels)	4.6±0.8 4.3±0.9 4.4±0.9	4.6±0.6 4.4±0.6 4.7±0.6
Domain 2: Healthcare satisfaction (5-item scores) Before/after the lifestyle intervention: Choices: 1. I did not see a healthcare provider 2. Don't know		

3. No 4. Yes somewhat 5. Yes to a large extent		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)
13. I had discussed my symptoms with my primary healthcare providers	4.2±1.0	4.7±0.6
14. I felt satisfied with the explanation given to me about my symptoms by my healthcare providers	4.0±1.0	4.6±0.7
15. I felt satisfied with the treatment options offered to me by my healthcare providers	3.4±1.0	4.3±0.9
16. I felt satisfied with the treatment I was receiving (or had received) for my symptoms by my healthcare providers	3.3±1.0	4.3±0.9
17. I felt satisfied with the on-going care I was receiving for my symptoms by my healthcare providers	3.3±1.0	4.3±0.8
Domain 3: Knowledge about PCOS (23-item scores) Before/after the lifestyle intervention I understood [that]: Choices: 1. I do not understand this information 2. I am unsure 3. Yes, I somewhat understand 4. Yes, I understand		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)
18. A follicle is a small fluid-filled sac with a single egg inside and that follicles are often called cysts	3.5±0.7	3.8±0.05
19. How follicle growth and egg release occur	3.0±0.8	3.7±0.4
20. Polycystic ovaries contain more visible follicles than the average ovary	3.5±0.7	3.9±0.5
21. Polycystic ovaries tend to ovulate less frequently than the average ovary	3.5±0.7	3.9±0.2
22. Ovulating more frequently would improve my fertility	3.7±0.7 3.3±0.8	3.9±0.3 3.9±0.3
23. After egg release (ovulation), progesterone is released: progesterone would allow my uterine lining		

to shed and make me have a regular menstrual period (if I'm not pregnant)	3.1±0.9	3.8±0.6
24. Having monthly increases in progesterone and therefore, menstrual periods would decrease my risk of cancer of the uterus	3.3±0.9	4.0±0.2
25. Polycystic ovary tends to make more male hormone (like testosterone) than the average ovary	2.8±0.9	3.7±0.5
26. The amount of fat in the body affects the amount of free male hormone in my body	3.1±0.9	3.9±0.4
27. More free male hormone promotes unwanted hair growth, acne and scalp hair loss (alopecia)	3.0±0.9	3.7±0.6
28. Medications can stop the production of male hormone by the ovary (like hormonal contraception) or block the effects of male hormone, and would help to prevent unwanted hair growth, acne or alopecia (male pattern balding)	2.9±0.9	3.7±0.5
29. Insulin helps the ovary make more male hormone (like testosterone)	3.3±0.9	3.9±0.2
30. High insulin levels are common in women with PCOS	3.4±0.8	4.0±0.2
31. High insulin levels promote weight gain and make it harder to lose weight	3.5±0.8	4.0±0.2
32. High insulin levels place individuals at high risk for developing diabetes	3.3±0.8	3.9±0.3
33. Lowering insulin levels may help to decrease male hormone levels, help with weight loss and help trigger ovulation	3.5±0.8	4.0±0.2
34. Insulin levels can be lowered by exercise and by certain changes in diet	3.3±0.8	3.8±0.5
35. Insulin levels can be lowered by medications (like metformin) that make the body more sensitive to insulin	3.5±0.7	3.9±0.4
36. Increasing the amount of muscle in my body will increase my body's metabolic rate, that is my body's ability to burn calories	3.2±0.9	3.9±0.3
37. The metabolic syndrome increases my risk for developing diabetes and cardiovascular disease.	3.2±0.9	3.9±0.4
38. The metabolic syndrome is common in women with PCOS	3.4±0.9	4.0±0.2

39. That exercise, appropriate changes in diet, and weight loss can decrease the risk of developing the metabolic syndrome 40. Enough about PCOS to explain to another person what PCOS is, and how it affects health parameters	3.1±0.7	3.7±0.6
Domain 4: Active living (6-item scores) Before/after the lifestyle intervention:		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)
41. I led an active lifestyle Choices: 1. Strongly disagree 2. Disagree 3. Neutral 4. Agree 5. Strongly agree	3.0±1.2	4.1±1.0
42. Total number of times I exercised per week Choices: 1. No, I didn't really exercise 2. 1 to 2 days each week 3. 3 to 4 days each week 4. 5 to 6 days each week 5. Daily	2.2±1.2	3.2±1.1
43. The number of days per week of doing aerobic exercise (that is, exercise that increases heart rate and makes you warm and sweaty) was Choices: 1. Occasional 2. 1-2 times per week 3. 3-4 times per week 4. 5-6 times per week 5. Daily	2.4±1.4	3.7±1.2
44. The number of minutes per session of doing aerobic exercise was (examples of non-aerobic exercise include resistance bands, hand weights, Pilates or Yoga but not exercises such as jogging or walking) Choices: 1. 2-5 minutes	3.1±2.0	5.0±1.2

2. 6-10 minutes 3. 11-15 minutes 4. 16-30 minutes 5. 31-44 minutes 6. greater than 45 minutes		
45. The number of days per week of non-aerobic exercise was Choices: 1. Occasional 2. 1-2 times per week 3. 3-4 times per week 4. 5-6 times per week 5. Daily	2.1±1.1	2.7±1.2
46. The number of minutes per session of non-aerobic exercise was Choices: 1. 2-5 minutes 2. 6-10 minutes 3. 11-15 minutes 4. 16-30 minutes 5. 31-44 minutes 6. Greater than 45 minutes	2.7±0.2	3.7±1.5
Domain 5: Healthy Dieting (8-item scores) Before/after the lifestyle intervention: Choices: 1. Never 2. Hardly ever 3. Sometimes 4. Almost always 5. Always		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)

47. I thought about the types of food I was eating when planning every meal	3.0±0.8	4.0±0.8
48. I ate three meals per day	3.5±1.2	4.0±0.9
49. I ate protein with every meal	3.5±0.9	3.9±0.8
50. I ate a diet low in fat	2.8±0.8	3.8±0.8
51. I made healthy food choices for snacks	3.0±0.7	3.9±0.9
52. I ate a diet rich in fruits and vegetables	3.3±0.8	4.2±0.7
53. I knew which foods were high in protein	3.6±1.0	4.3±0.9
54. I knew the difference between simple and complex carbohydrate-containing foods	1.6±1.2	4.2±1.2
Domain 6: Feelings and experiences about PCOS diagnosis (7-item scores) • Choose the response that best describes your feelings: 1. Don't know 2. No 3. Yes		
Domain item	Pre-intervention scores* (n = 55)	Post-intervention scores* (n = 55)
55. I was first diagnosed with PCOS as a result of	2.6±0.5	-
56. I had an ultrasound in the last three years	2.4±0.5	-
57. The type of ultrasound was transvaginal [In case of positive response to the last question]	2.2±0.6	-
58. I suspect that other women in my family (like mother, sisters) or extended family (like aunts, cousins, grandmothers) have PCOS	2.5±0.8	-
59. Excess weight (being overweight or obese) runs in my family	2.7±0.5	-
60. I feel I need to lose weight for health reasons	2.7±0.5	-
61. I didn't know that I had hirsutism (male pattern hair growth) until I was told during the pre-study diagnosis	2.1±0.9	-
Domain 7: Feelings and experiences about participating in the lifestyle intervention (13-item scores) Before/after participating in the study, I felt:		

Choices: 1. Strongly disagree 2. Disagree 3. Neutral 4. Agree 5. Strongly agree		
62. Comfortable about asking questions [from the study researchers]	4.4±0.6	4.7±0.5
63. Comfortable about discussing personal or sensitive subjects [with the study researchers]	4.2±0.7	4.5±0.7
64. My information would remain confidential (unless I agreed to have my information sent to my family doctor)	4.5±0.6	4.7±0.5
65. The [study] researchers were knowledgeable about PCOS	4.7±0.6	4.7±0.6
66. The [study] researchers would gain helpful information about PCOS	4.6±0.6	4.6±0.6
67. The [study] researchers would be able to provide answers regarding my concerns about PCOS	4.6±0.6	4.76±0.5
68. It was helpful to look at the ultrasound images of my ovaries. Now it is now easier for me to understand what it means to have polycystic ovaries	4.4±0.8	4.67±0.5
Before/after participating in the study, my level of comfort with:		
69. The idea of having blood tests was Choices: 1. Painful 2. Very uncomfortable 3. Uncomfortable 4. Slightly uncomfortable 5. Comfortable 6. Very comfortable	4.1±0.2	4.2±0.9
70. The idea of having physical examinations, including having my height, weight, and waist circumference measured, hair growth assessed, and body composition scans done was Choices: 1. Painful	3.1±0.2	4.0±0.8

2. Very uncomfortable 3. Uncomfortable 4. Slightly uncomfortable 5. Comfortable 6. Very comfortable	2.7±1.2	2.9±1.1
71. The idea of having a transvaginal ultrasound was Choices: 1. Uncomfortable 2. Slightly uncomfortable 3. Neutral 4. Comfortable 5. Very comfortable		
72. The experience of doing blood tests was Choices: 1. Too painful 2. Painful and I wanted to quit but didn't say so 3. Painful but I didn't want to quit 4. Not overly painful 5. Not at all painful/no discomfort	3.5±1.2	3.6±1.4
73. The experience of doing physical examination tests was Choices: 1. Painful 2. Very uncomfortable 3. Uncomfortable 4. Slightly uncomfortable 5. Comfortable 6. Very comfortable	3.6±1.1	4.4±0.6
74. The experience of doing transvaginal ultrasound was Choices: 1. Too painful 2. Painful and I wanted to quit but didn't say so 3. Painful but I didn't want to quit 4. Not overly painful 5. Not at all painful/no discomfort	3.9±0.7	4.1±0.8
Qualitative information section Questions asked from participants after completing the lifestyle intervention study 75. If you have been advised to lose weight, how does this make you feel?		

76. We are interested in learning more about how women experience the physical examination. Please tell us anything about your experience that you think we should know
77. We are interested in learning more about how women experience the transvaginal ultrasound, which was part of the PCOS study. Please tell us anything about your experience that you think we should know
78. Is there any topic that you think we should have asked you about?
79. Is there any comment you would like to make about the topic of PCOS or the topic of being involved in a study?
80. Is there anything that you would like to add, ask or comment about?
81. If you learned something about PCOS during the study, what ONE thing did you learn that is most helpful to you?
82. If you learned something about PCOS over the course of the study, what ONE thing came as a surprise to you?

Values are mean±SD. n = 55 at pre- and post-intervention (pooled groups).

Appendix B. Pulse Consumption Survey

Subject ID:_____

Date: _____

Observation:_____

UNIVERSITY OF SASKATCHEWAN

PULSE CONSUMPTION SURVEY

Dear Study Participant,

We are interested in understanding more about why people do or do not eat pulses. This may be something you have or have not thought much about but please note, there are no right or wrong answers. Do your best to answer all the questions.
For your information, the term **pulses** refer to:

- **Beans** (i.e. Kidney beans, white beans, black beans, navy beans)
- **Chickpeas**
- **Peas** (i.e. Split peas)
- **Lentils (**i.e. Red, yellow and green)

There are **three parts** to the questionnaire, which should take approximately **10 minutes** to complete.

PART ONE

Please check (√) one response for each question in regard to **pulse consumption**.

	Strongly Disagree	Disagree	Not Sure	Agree	Strongly Agree
1. I need more information about how to cook pulses.					
2. Pulse-based meals or snacks are not available when I eat out.					
3. I'm too busy to prepare a pulse-based meal.					
4. I believe it would be too expensive to eat pulses.					
5. I believe I would have to go shopping too often if I ate pulses.					
6. I would buy a prepackaged pulse-based snack.					
7. I would get indigestion, bloating or gas eating pulses.					
8. I don't know how to prepare pulses.					
9. Pulses are not tasty enough.					
10. I believe it takes too long to prepare pulses.					
11. I never think of using pulses when I cook.					
12. I would try a pulse meal in a restaurant.					
13. I would eat pulses if they had a more attractive appearance.					
14. I would buy a prepackaged pulse-based meal.					
15. Pulses are expensive to add to meals.					
16. I am motivated to eat pulses.					
17. I know how to cook pulses.					
18. I believe that pulse-based meals can help					
19. Pulses can be a part of a tasty diet.					
20. Pulses are part of my traditional diet.					
21. I believe pulses are a healthy food.					

PART TWO

The following items are designed to record your **usual** food habits.

1. How often do you eat pulses?
   - □ Never or rarely **(Go to question 2)**
   - □ 1-3 times per month **(Go to PART THREE)**
   - □ 1-2 times per week **(Go to PART THREE)**
   - □ 3-4 times per week **(Go to PART THREE)**
   - □ 5-6 times per week **(Go to PART THREE)**
   - □ Once a day **(Go to PART THREE)**
   - □ Two or more times a day **(Go to PART THREE)**

2. People have given many reasons for not eating pulses or rarely eating pulses. Of the reasons listed at the bottom of the page, indicate which ones are the most important regarding why you never or rarely eat pulses? (**Please put letter in box**).
   - □ **Most important** reason why I do not or rarely eat pulses.
   - □ **Second most important** reason why I do not or rarely eat pulses.
   - □ **Third most important** reason why I do not or rarely eat pulses.

   These include:
   - **A.** I believe pulses do not taste good.
   - **B.** I believe pulses take a long time to cook.
   - **C.** I do not know where to find pulses.
   - **D.** I believe my family would not like pulses.
   - **E.** I believe pulses are expensive.
   - **F.** I do not know how to cook pulses.
   - **G.** I do not want to try new foods.
   - **H.** I believe pulses are not very healthy.
   - **I.** Other. Please explain:

PART THREE

The following section is regarding your knowledge of pulses and food. If you are unsure, do your best in picking what you believe to be the best answer.

1. According to Canada's Food Guide, pulses are an example of a food in the (please check one):
   - □ Vegetable and Fruit Group
   - □ Grain Products Group
   - □ Milk and Alternatives Group
   - □ Meat and Alternatives Group
2. Eating a proper diet will help to reduce your risk of certain types of diseases:
   - □ True

□ False

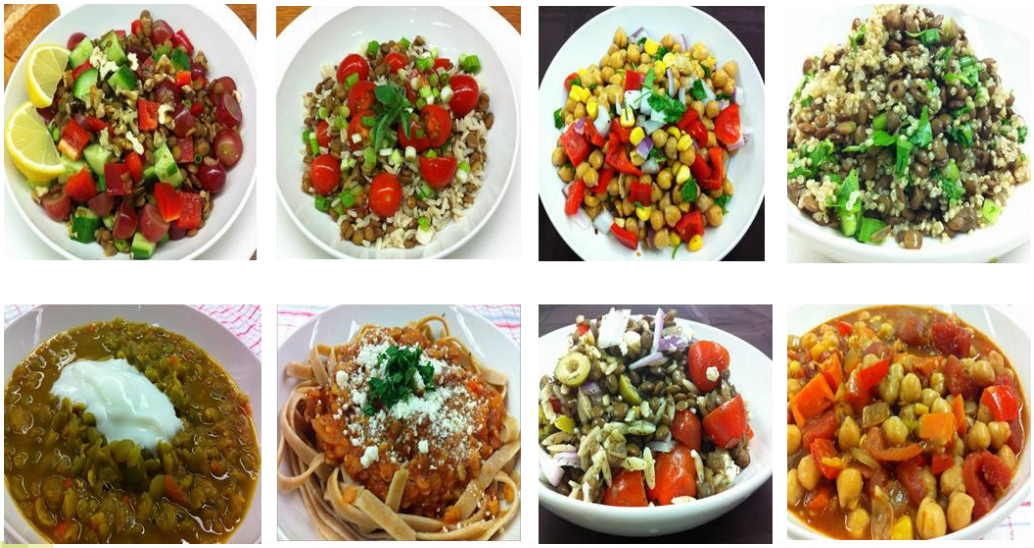
3. One serving of cooked pulses according to Canada's Food Guide equals (please check one):
   - □ ¼ cup (60 ml)
   - □ ½ cup (125 ml)
   - □ ¾ cup (175 ml)
   - □ 1 cup (250 ml)
4. Pulses are a good source of protein:
   - □ True
   - □ False
5. Pulses are a good source of fibre:
   - □ True
   - □ False
6. Pulses are a poor source of iron:
   - □ True
   - □ False
7. Pulses have too much saturated fat:
   - □ True
   - □ False
8. Which of the following does not belong in the Meat and Alternatives Group in Canada's Food Guide (please check one):
   - □ Eggs
   - □ Kidney Beans
   - □ Tofu
   - □ Peanut Butter
   - □ Cottage Cheese
9. Pulses are grown in Saskatchewan:
   - □ True
   - □ False
10. Where do you access information on healthy eating? (check all that apply)
    - □ Internet
    - □ Magazines
    - □ Cookbooks
    - □ Chefs
    - □ Television
    - □ Friends, family, colleagues
    - □ Grocery store
    - □ Radio

Thank you

Appendix C. Pulse-Based Diet Recipe Resource Guide.

To assist with the integration of pulses into the diet, a pulsed-based food recipe booklet was developed throughout the course of the study. The booklet has been developed to promote pulse consumption and provide general population with an improved insight about preparation and consumption of pulse-based meals. The booklet comprised of 59 healthy, easy, and affordable recipes in three main categories, entrees, soups, and salads, that featured whole pulses and pulse ingredients. The recipes were all tested, evaluated, and modified several times to ensure high quality, palatability, profitability, appearance, and nutritional value for Canadian consumers. The recipes were modified based on feedback from participants in order to make the meals both healthy and tasty. For general educational purposes, the standard Nutrition Facts Labels were provided for all recipes, based on the Food and Drug Administration Guidelines. The recipes analyses were performed using the Food Processor Nutrition Analysis Software (version 7, ESHA Research, Oregon).

Appendix C. Pulse-Based Diet Recipe Booklet



UNIVERSITY OF SASKATCHEWAN

Pulse-Based Diet Recipe Booklet

Submitted to the Saskatchewan Pulse Growers
in partial fulfillment of the requirements of the research project entitled:

A Lifestyle Intervention for Women with Polycystic Ovary Syndrome: The Role of a Pulse-Based Diet and Aerobic Exercise on Infertility Measures and Metabolic Syndrome Risk

Edited by:

Maryam Kazemi

CONTENTS

Acknowledgements

We appreciate everyone who has contributed to the development of this booklet. We thank Christianne Rooke, Lindsay Tumback, Jennifer Wright, Katia Hucl, Alexandra Babcock, Kiley Zdunich, Kavitha Ramachandran, Michele Sheikh, and Amanda Kilduff for their contribution to the development of an earlier version of the recipe book. We are also immensely grateful to our study participants who provided insight and feedback that greatly assisted the improvement of recipes. Thanks to Maryam Kazemi for her review and editorial contribution.

The present work was supported by the Saskatchewan Pulse Growers, and Agriculture and Agri- Food Canada.

Introduction

Welcome to the Pulse-based Recipe Booklet! In this booklet, we will introduce you to 59 healthy, easy, and affordable recipes that feature whole pulses and pulse ingredients.

Pulses include peas, beans, chickpeas, and lentils. Pulses are super-healthy foods with a favorable nutritional composition. Pulses are a rich source of fiber, starch, and vegetable protein, and vitamins and minerals, such as iron, zinc, folate, and magnesium. Pulse consumption helps to control weight, and fight against diabetes, cardiovascular disease and cancer.

Pulses are surprisingly good when you have the right recipe! Try one of our delicious recipes for an easy, healthy meal today. With these recipes, we hope to fight the notion that healthy food has to be bland or boring, and to provide you with an improved insight about preparation and consumption of pulse-based meals. The recipes offered in the present booklet are full of life, colour, and flavour. All of the recipes have been tested, evaluated, and modified several times to ensure they are easy to make and taste fantastic. We hope you have fun exploring pulsed-based cooking style and share it with your friends and family.

The Pulse-based Diet Recipe Booklet is organized in two sections. In the first section, The Kitchen Companion, you will be introduced to the basic instructions of cooking pulses and commonly used grains in the recipes provided. In the second section, pulse-based recipes will be introduced in three main categories, entrees, soups, and salads. A note on the times listed to complete the recipes: cooking and preparation times are estimates. You can often speed up time spent in the kitchen with advanced preparation, or by preparing other ingredients while cooking a grain or simmering a soup.

We like you to enjoy the recipes we've provided. In case you have questions or suggestions about the recipes, please don't hesitate to mention it to us, and we will be happy to assist you. Please email us at pcos.pulsestudy@usask.ca.

Good luck with discovering the healthy pulse-meals, and bon appétit!

Kitchen Companion

Following are some cooking basics that will be needed for many of the recipes.

How to Cook Pasta

Steps

- Use a large pot as full of water as possible. For 450 grams dried pasta, place 6 liters water in an 8-litre pot.
- Cover pot, and bring water to a full rolling boil over high heat before adding pasta.
- Add pasta, and stir with a pasta fork or large tongs to separate strands.
- Start timing cooking when water returns to a boil. If you use fresh pasta, remember that it cooks more quickly than dried.
- Always cook pasta uncovered, or only partially covered, over high heat.
- Start testing for doneness a few minutes before indicated cooking time. Pasta that offers resistance to the bite, but has no trace of brittleness is al dente. This is how you want it. If an undercooked piece of pasta is cut in half, a white dot or line is clearly visible in the center. Al dente pasta has only a speck of white remaining, meaning the pasta has absorbed just enough water to hydrate it.
- Set a large colander in the sink so water drains quickly. Do not rinse the pasta.
- Return pasta to the warm cooking pot or add to the skillet with sauce, and toss immediately with large tongs or a pasta fork.

How to Cook Basic White

Rice Steps

- Select a saucepan, deep skillet or sauté pan with a snug-fitting lid.
- Heat water to boiling.

    - For soft, tender rice, use 2 cups water per 1 cup rice.
    - For dry separate grains, use 1¾ cups water per 1 cup rice.
- Add the rice and salt. Stir once.
- Return to boiling. Stir once.
- Cover and reduce heat to low.
- Cook for 15 minutes or until all the water is absorbed. Don't lift the lid or stir. Lifting the lid allows steam to escape, and stirring the rice releases more starch, causing the grains to stick together in lumps.
- Remove the lid carefully (try not to let the condensation on the lid drip onto the rice).

How to Cook Pearl Barley

Steps

- Bring 1 cup barley and 2 ½ cups water or low sodium broth to a boil (Should yield a generous 3 cups of cooked barley).
- Reduce heat to a simmer. Cook, covered, until tender and most of the liquid has been absorbed, 40 to 50 minutes.
- Let stand 5 minutes.

How to Cook Chickpeas and Beans

Steps

- Place the chickpeas/beans in a bowl and fill with water. Move the chickpeas/beans around a little with your hand or a spoon to allow any dirt to come loose in the water. Drain the water. Repeat until the water appears clear. Don't worry too much if you can never get the water to be perfectly clear. If any chickpeas/beans float to the top, pick them out and throw them away. These are bad chickpeas/beans. Pick out and throw out any other chickpeas/beans that look bad.

- Soak chickpeas/beans. You will use 3 cups of water for each cup of chickpea/bean. If you don't have a large bowl, use a large pot or any other large container that you have.

  * Do not soak chickpeas/beans in the fridge! Soak them at room temperature.

  * Do not add baking soda or salt to the beans. Some advice adding 1/8 teaspoon of baking soda if you have particularly hard water, but usually, it is not recommended.

- Drain the chickpeas/beans and Replace the Water. Drain the water from chickpeas/beans and move them to a large pot. Add 3 cups water for each cup of dried beans that you used.

- Boil the chickpeas/beans. They should take between 2 and 3 hours to cook (excluding the time it takes to bring the chickpeas/beans to a simmer). First, bring the water to a boil under medium-high heat. Scoop out any froth that forms with a spoon.

  Once the water starts to boil, set the heat to low and cover the pot. Set a timer to 3 hours. I keep them in for the full 3 hours to ensure that they are fully cooked, however, cooking times vary depending on the amount of beans, the pot, and time spent soaking the beans. For your first-time cooking chickpeas/beans, you may want to check after 2 hours and keep checking the beans until they are done. Whenever you take the lid off the pot and replace it, check to make sure the water is still at a simmer. If it isn't, temporarily bring the heat up to medium heat until the water is simmering again, and then set the heat back to low.

  * Should I stir the beans?

  Yes. You may want to stir the chickpeas/beans a few times throughout the cooking process to ensure that the beans get cooked evenly.

  * How do I know when the chickpeas/beans are done?

  Take a chickpea/bean and bite into it or squeeze it between your fingers. You should be able to squeeze it with your fingers, at least. It should have a very soft consistency. Note that you can decide how cooked you want the beans to be. If you find that they are cooked but you prefer them to be a little softer, go ahead and let them simmer for a little while longer.

- Drain the chickpeas/beans and add any apices or add-ins. Makes about 4 cups cooked lentils.

How to Cook Lentils

Steps

- Rinse the lentils under running water and pick through them to remove any bits of soil or rocks. You will use 2 cups of water for each cup of lentil. If you don't have a large bowl, use a large pot or any other large container that you have.
- Add lentils and water to a saucepan (with a lid) and bring to a boil. Turn heat down to low and cover to let the lentils simmer, but leave the lid ajar a bit so that they don't boil over. Check on them occasionally to make sure the water has not boiled down below the level of the lentils and add more as needed. When the lentils are tender and can easily be mashed with a fork, they are done. It usually takes about 30-45 minutes for them to cook (older lentils take longer to cook, so it's best to just test them to decide when they are ready), or 20 minutes if using the split red lentils.
- When they are finished cooking, take the saucepan off the heat and cover tightly with the lid. Optional: leave the lid to sit for 5-10 minutes. The lentils will absorb more of the water making them juicier and tenderer. Makes about 4 cups cooked lentils.

How to Cook Split Peas

Steps

- Pour the split peas into large colander or sieve. Pick out and discard any shriveled or broken beans, stones or debris, and rinse under cold water and pick through them to remove any bits of soil or rocks. You will use about 1.5 cups of water for each cup of split pea. If you don't have a large bowl, use a large pot or any other large container that you have.
- Add the peas and water to a saucepan (with a lid), and allow water to return to boiling, reduce heat, partially cover pan, and simmer for 30 to 45 minutes, depending on the variety. Check on them occasionally to make sure the water has not boiled down below

the level of the peas and add more as needed. When the peas are tender and can easily be mashed with a fork, they are done. Makes about 3 cups cooked peas.

Pulse Recipes

Entrees

CHICKPEA and CAULIFLOWER CURRY

Yield: 4 servings

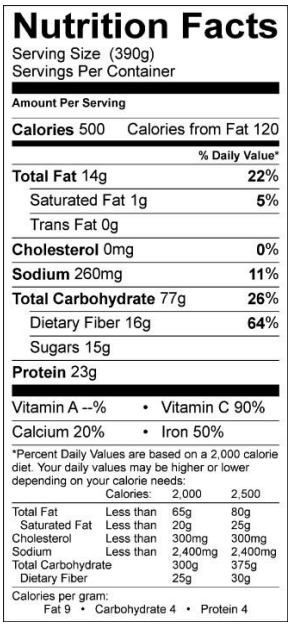
Preparation time: 30 minutes **Cook time:** 1 hour 10 minutes **Total time:** 1 hour and 40 minutes

Ingredients:

- 900 grams chickpeas, cooked and thawed
- ½ tablespoon madras curry powder
- 1 bay leaf
- 1 teaspoon cinnamon
- 1 teaspoon cardamom
- 2 teaspoons ground cloves
- 2 teaspoons black pepper
- ¼ red onion, quartered
- 2 tablespoons garlic, minced
- 2 teaspoons ginger, minced
- ¼ teaspoon salt
- 2 tablespoons canola oil
- ½ red onion, diced
- 1 tablespoon cumin
- 1 tablespoon coriander
- ½ teaspoon turmeric
- 1 plum tomato, diced
- 3 cups cauliflower florets, frozen
- 2 teaspoons cornstarch
- 2 teaspoons water
- 1 tablespoon lemon juice
- ¼ cup fresh cilantro, chopped

Directions:

1. Cover chickpeas with water. Add curry powder, bay leaf, cinnamon, cardamom, cloves, and black pepper. Bring to a boil. Reduce heat and simmer until chickpeas are tender.
2. Pulse quartered onion, garlic, ginger and salt in a food processor to form a paste.
3. Heat oil in a pot over medium-high heat. Add onion and cook until browned, about 6 to 7 minutes.
4. Add onion, garlic and ginger paste. Cook until dry, about 5 to 7 minutes more.
5. Add cumin, coriander and turmeric. Cook until fragrant, about 1 minute more.
6. Add chickpeas and cooking liquid, tomatoes, and cauliflower.
7. Combine cornstarch and water in a small bowl. Add to curry slowly, stirring constantly. Allow to sit for thirty minutes.
8. Add lemon juice and cilantro. Stir to combine.
9. Portion out curry. Remember to remove bay leaf.
10. Freeze.



Nutrition Facts

Serving Size (390g)
Servings Per Container

Amount Per Serving

Calories 500	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22**%
Saturated Fat 1g	**5**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 260mg	**11**%
Total Carbohydrate 77g	**26**%
Dietary Fiber 16g	**64**%
Sugars 15g	
Protein 23g	

Vitamin A --%	• Vitamin C 90%
Calcium 20%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

CURRIED COCONUT RICE

Yield: 5 servings

Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

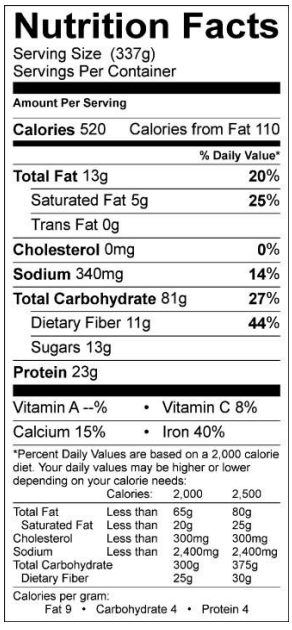
Ingredients:

- 1125 grams chickpeas, cooked and thawed
- 1 tablespoon olive oil
- 1 small onion, chopped
- 1 jalapeno pepper, minced
- 1 teaspoon madras curry powder
- 1 tablespoon garlic, minced
- 1 can light coconut milk
- 1 ¼ cup low-sodium vegetable broth
- ½ teaspoon salt
- 1 bay leaf
- ½ cup brown rice, dry
- 1 cup cold water

Directions:

1. Heat oil in a pot over medium-high heat. Add onion, jalapeno pepper and curry powder. Sauté until onion is translucent.
2. Add garlic and sauté for 1 minute more.
3. Add coconut milk, vegetable broth, chickpeas, bay leaves and salt. Stir to combine. Reduce heat and simmer.
4. Combine brown rice and water in another pot. Bring to a boil. Reduce heat and simmer until cooked.
5. Add rice to chickpea mixture. Stir to combine.

6. Portion out rice and chickpea mixture. Remember to remove bay leaf.
7. Freeze.



Nutrition Facts

Serving Size (337g)
Servings Per Container

Amount Per Serving

Calories 520	Calories from Fat 110
	% Daily Value*
Total Fat 13g	**20**%
Saturated Fat 5g	**25**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 340mg	**14**%
Total Carbohydrate 81g	**27**%
Dietary Fiber 11g	**44**%
Sugars 13g	
Protein 23g	

Vitamin A --%	• Vitamin C 8%
Calcium 15%	• Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

PASTA WITH GINGER AND CORIANDER SAUCE

Yield: 4 servings

Preparation time: 20 minutes **Cook time:** 40 minutes **Total time:** 1 hour

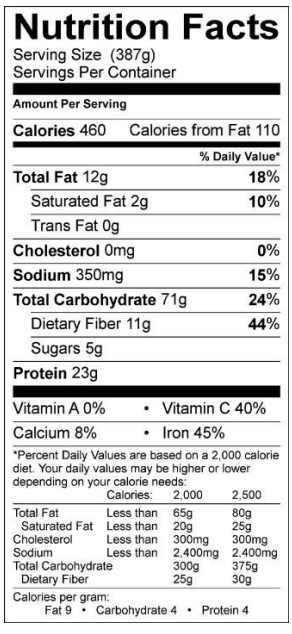
Ingredients:

- 300 grams red lentils, dry
- 3 tablespoons olive oil
- 2 small onions, finely chopped
- 1 tablespoon garlic, minced
- 3 tablespoons ginger, minced
- 1 tablespoon white wine vinegar
- 2 teaspoons coriander
- 2 teaspoons cumin
- 2 cups low-sodium vegetable broth
- ½ teaspoon salt
- 1 tablespoon no-salt seasoning (such as garlic herb)
- black pepper to taste
- 1 ½ cups whole wheat pasta (such as fusilli or macaroni)
- 4 cups cold water
- 1 cup fresh parsley, packed

Directions:

1. Cook red lentils according to pulse cooking instructions. Add a pinch of coriander and cumin to cooking water. Drain once cooked.
2. Heat oil in another pot over medium-high heat. Add onion and sauté until onions begin to wilt.
3. Add garlic and ginger and sauté for 30 seconds more.
4. Deglaze with vinegar and evaporate.

5. Add vegetable broth, lentils, coriander and cumin. Stir to combine. Bring to a boil. Reduce heat, cover and simmer until lentils are tender and soupy, about 10 to 15 minutes.
6. Add salt and no-salt seasoning. Season to taste with pepper.
7. Bring cold water to a boil in another pot. Cook pasta according to package instructions until al dente. Drain.
8. Portion out pasta. Portion out sauce on top of pasta. Garnish with parsley.
9. Freeze.



Nutrition Facts

Serving Size (387g)
Servings Per Container

Amount Per Serving

Calories 460	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 350mg	**15%**
Total Carbohydrate 71g	**24%**
Dietary Fiber 11g	**44%**
Sugars 5g	
Protein 23g	

Vitamin A 0% • Vitamin C 40%
Calcium 8% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LENTILS WITH BULGUR WHEAT AND CARAMELIZED ONIONS

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 1 hours **Total time:** 1 hour and 30 minutes

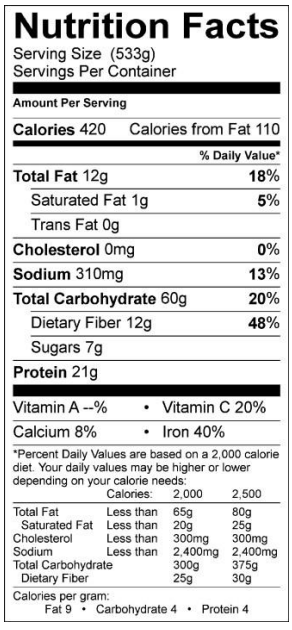
Ingredients:

- 450 grams lentils, dry
- 6 cups water
- 1 cup bulgur wheat, dry
- ½ teaspoon salt
- 2 tablespoons olive oil
- 1 large onion, finely chopped
- 1 tablespoon garlic, minced
- 2 teaspoons cumin
- 1 teaspoon cinnamon
- ½ teaspoon nutmeg
- ½ teaspoon allspice
- ½ teaspoon salt
- ½ teaspoon black pepper
- 1/3 cup walnuts, chopped
- 1 tablespoon olive oil
- 3 large onions, thinly sliced
- 1/3 cup parsley, chopped

Directions:

1. Cook lentils in water until almost done but still firm, about 15 to 18 minutes.
2. Add bulgur wheat and salt. Remove from heat and cover. Allow to sit until bulgur wheat is tender and water is absorbed, about 20 to 25 minutes.
   NOTE: If bulgur wheat seems dry, add more water.

3. Heat oil in another pot over medium-high heat. Add onions and sauté until very soft, about 5 minutes.
4. Add garlic, cumin, cinnamon, nutmeg, allspice, salt and black pepper. Cook for 1 minute.
5. Add walnuts. Stir to combine.
6. Add lentil and bulgur wheat mixture. Stir to combine. Remove from heat.
7. Heat oil in a large skillet over medium heat. Add sliced onions and cook until caramelized to very dark brown, about 20 to 25 minutes. Remove from heat.
8. Portion lentil and bulgur wheat mixture. Portion caramelized onions on top of lentils and bulgur wheat. Garnish with parsley.



Nutrition Facts

Serving Size (533g)
Servings Per Container

Amount Per Serving

Calories 420	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 310mg	**13%**
Total Carbohydrate 60g	**20%**
Dietary Fiber 12g	**48%**
Sugars 7g	
Protein 21g	

Vitamin A --%	• Vitamin C 20%
Calcium 8%	• Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

OAXACAN GREEN MOLE STEW

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 40 minutes **Total time:** 1 hour and 40 minutes

Ingredients:

- 900 grams chickpeas, cooked and thawed
- 3 tablespoons oil
- 2 large onions, chopped
- 1 tablespoon garlic, minced
- 1 tablespoon ground fennel
- 3 medium zucchinis, sliced
- 2 cups corn, frozen
- 1 cup water
- 2 cans salsa verde
- ½ cup fresh parsley, packed
- ½ cup fresh cilantro, packed
- 1 tablespoon garlic, minced
- 2 teaspoons cumin
- 1 teaspoon black pepper
- ½ teaspoon cinnamon
- ½ teaspoons cloves
- 2 whole wheat tortillas
- cinnamon and chili powder

Directions:

1. Heat oil in a pot over medium-high heat. Add onions and cook until translucent, about 8 minutes.
2. Add garlic and fennel. Cook for 2 minutes more.

3. Add zucchini, corn, and water. Cook until zucchini softens, about 15 minutes.
4. Combine salsa verde, parsley, cilantro, garlic, cumin, pepper, cinnamon and cloves in a food processor. Pulse until sauce consistency is achieved.
5. Add mole sauce to stew. Stir to combine and cook for 5 more minutes.
6. Portion out stew.
7. Freeze.

Serving Instructions:

1. Preheat oven to 350°F.
2. Cut tortillas into eighths. Place in a single layer on an ungreased baking sheet.
3. Sprinkle with cinnamon and chili powder as desired.
4. Bake until tortilla chips brown, about 5 to 10 minutes.

Serve with 4 tortilla chips each.

Nutrition Facts

Serving Size (626g)
Servings Per Container

Amount Per Serving

Calories 600 Calories from Fat 150

	% Daily Value*
Total Fat 17g	**26**%
Saturated Fat 1.5g	**8**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 110mg	**5**%
Total Carbohydrate 94g	**31**%
Dietary Fiber 17g	**68**%
Sugars 23g	
Protein 26g	

Vitamin A --% • Vitamin C 160%
Calcium 20% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

TOMATO BASIL BOWTIES

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

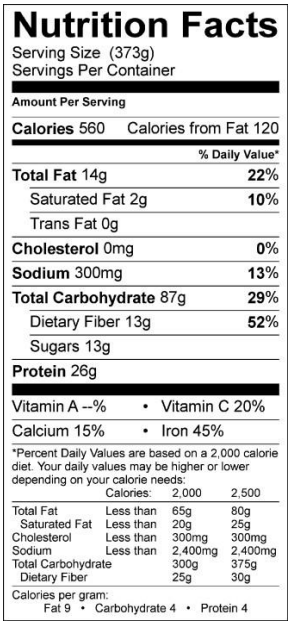
Ingredients:

- 1350 grams chickpeas, cooked and thawed
- 3 tablespoon olive oil
- 1 small onion, chopped
- 1 ½ cups cherry tomatoes, halved
- 1 369ml can tomato sauce
- 1 tablespoon garlic, minced
- 1 tablespoon lemon juice
- 1 tablespoon white wine vinegar
- 1 ½ cups low-sodium vegetable broth
- 1 teaspoon crushed red pepper flakes
- 1 teaspoon basil, dry
- 1 teaspoon salt
- 1 ½ cups whole wheat bowtie pasta, dry
- 4 ½ cups cold water
- ¼ cup fresh parsley, chopped

Directions:

1. Heat oil in a pot over medium-high heat. Add onion, garlic, tomatoes, lemon juice, and parsley. Sauté for 1 minute.
2. Deglaze with vinegar.
3. Add vegetable broth, chickpeas, red pepper flakes and basil. Bring to a boil. Reduce heat, cover and simmer until chickpeas are soft.
4. Put chickpea mixture in food processor. Add salt. Blend until smooth.

5. Bring cold water to a boil in another pot. Cook pasta according to package instructions until al dente. Drain.
6. Portion out pasta. Portion out chickpea mixture on top of pasta. Garnish with parsley.
7. Freeze.



Nutrition Facts

Serving Size (373g)
Servings Per Container

Amount Per Serving

Calories 560	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 300mg	**13%**
Total Carbohydrate 87g	**29%**
Dietary Fiber 13g	**52%**
Sugars 13g	
Protein 26g	

Vitamin A --%	• Vitamin C 20%
Calcium 15%	• Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

CHANNA MASALA

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 1 hour and 10 minutes **Total time:** 1 hour and 40 minutes

Ingredients:

- 1350 grams chickpeas, cooked and thawed
- 1 796ml can diced tomatoes
- 3 tablespoons tomato paste
- 3 tablespoons olive oil
- 2 cups red onion, chopped
- 1 tablespoon garlic, minced
- 2 teaspoons ginger, minced
- 2 tablespoons ground coriander
- 1 tablespoon garam masala
- 1 tablespoon turmeric
- 1 tablespoon paprika
- 2 teaspoons chili powder
- ½ teaspoon salt
- 5 tablespoons nonfat yogurt, plain, beaten until smooth
- 1 tablespoon lemon juice
- 1 cup water
- 3 naan breads, whole wheat

Directions:

1. Combine diced tomatoes and tomato paste in food processor. Process until smooth.
2. Heat oil over medium-high heat. Add onion and fry until golden-brown.

3. Add garlic and ginger and fry for at least 1 minute. Continue frying until onions are as brown as possible without burning.
4. Add spices and fry for 30 seconds.
5. Add tomatoes, yogurt and lemon juice and stir to combine. Simmer for 5 to 10 minutes, or until oil separates. Oil may not separate as little was used.
6. Add chickpeas. Stir to combine and simmer for five to ten minutes.
7. Add water. Stir to combine and simmer, covered, for 30 minutes. Stir occasionally throughout.

   NOTE: If water is not evaporating to desired consistency fast enough, boil vigorously until it does so. If water must be added to achieve desired consistency, bring to boil to combine it, then lower heat back to simmer.
8. Portion out Channa Masala.
9. Freeze

Serving Instructions:

Serve with ½ naan bread and garnish with cilantro if desired.

Nutrition Facts

Serving Size (460g)
Servings Per Container 6

Amount Per Serving	
Calories 600	Calories from Fat 130
	% Daily Values*
Total Fat 17g	**26%**
Saturated Fat 3g	**15%**
Trans Fat 0g	
Cholesterol 11mg	**4%**
Sodium 650mg	**27%**
Total Carbohydrate 104g	**35%**
Dietary Fiber 24g	**96%**
Sugars 24g	
Protein 28g	**56%**

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

CHICKPEA AND SPINACH CASSEROLE

Yield: 4 servings

Preparation time: 30 minutes **Cook Time: 1 hour** **Total Time:** 1 hour and 30 minutes

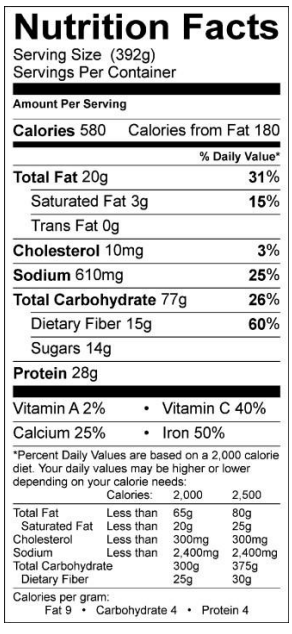
Ingredients:

- 900 grams chickpeas, cooked and thawed
- 5 slices back bacon or ½ package of regular bacon
- 2 tablespoons canola oil
- 1 small onion, thinly sliced
- 1 red bell pepper, chopped
- ¼ teaspoon salt
- 1 teaspoon red pepper flakes
- 2 bay leaves
- 2 tablespoons garlic, minced
- 330 grams spinach, frozen and chopped
- ½ cup dry white wine or cooking wine

Directions:

1. Preheat oven to 375°F.
2. Cook bacon until crispy in a pot over medium-high heat. Remove and drain on paper towels. Chop into bite sized pieces.
3. Drain bacon fat from pot. Add canola oil to pot and heat. Add onion, bell pepper, salt, red pepper flakes, and bay leaves. Cook until onion is soft, about 3 minutes.
4. Add garlic, bacon and chickpeas. Cook, stirring constantly, until chickpeas begin to turn golden, about 3 minutes.
5. Remove from heat.

6. Layer a third of spinach in a casserole dish. Pour half of chickpea mixture on top of spinach, and top with another third of spinach. Pour other half of the chickpea mixture on top, and top with last third of spinach.
7. Pour white or cooking wine over the dish.
8. Cover dish. Bake until chickpeas are tender and spinach is wilted, about 15 minutes.
9. Portion out casserole. Remember to remove bay leaves.
10. Freeze.



Nutrition Facts

Serving Size (392g)
Servings Per Container

Amount Per Serving

Calories 580	Calories from Fat 180
	% Daily Value*
Total Fat 20g	**31**%
Saturated Fat 3g	**15**%
Trans Fat 0g	
Cholesterol 10mg	**3**%
Sodium 610mg	**25**%
Total Carbohydrate 77g	**26**%
Dietary Fiber 15g	**60**%
Sugars 14g	
Protein 28g	

Vitamin A 2%	• Vitamin C 40%
Calcium 25%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

GINGERED VEGETABLE CURRY

Yield: 6 servings

Preparation time: 45 minutes **Cook time:** 45 minutes **Total time:** 1 hours and 30 minutes

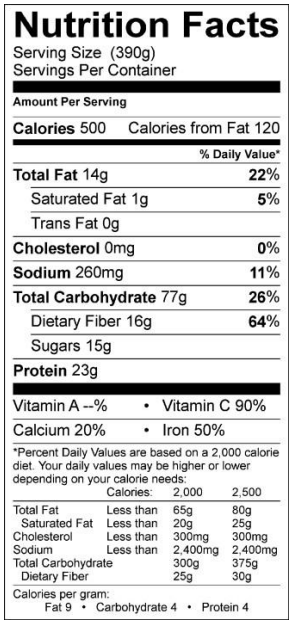
Ingredients:

- 540 grams split peas, dry
- 12 cups water
- 1 small potato, peeled and cubed
- 1 cup cauliflower florets, chopped
- 1 cup green beans, chopped
- 2 carrots, chopped
- 1 teaspoon turmeric
- ½ teaspoon salt
- 1 tablespoon garlic
- 2 teaspoons cumin
- 1 teaspoon curry powder
- 1 tablespoon cornstarch
- 3 tablespoons water
- 3 tablespoons ginger, minced
- 1 tablespoon lime juice
- 1 tablespoon margarine
- 1 cup brown rice, dry
- 2 cups cold water

Directions:

1. Cover potatoes in cold water and allow to soak.

2. Combine split peas and water in a large pot. Bring to a boil. Skim foam from surface.
3. Drain potatoes and add to split peas. Bring back to a boil. Reduce heat to medium, and simmer uncovered for 5 minutes.
4. Add cauliflower, green beans, carrots, turmeric and salt. Bring back to a boil again. Reduce heat to low, cover, and simmer gently for about 10 minutes more.
5. Combine brown rice and water in another pot. Bring to a boil. Reduce heat and simmer until cooked.
6. Heat oil in another pot over medium-high heat. Add garlic, cumin and curry powder. Cook, stirring constantly, until garlic is lightly browned, about 1 to 2 minutes.
7. Add garlic mixture, ginger, and cilantro to split pea mixture. Stir to combine.
8. Whisk cornstarch and water in a small bowl until smooth. Pour slowly into curry, stirring constantly.
9. Turn up heat to medium-high. Simmer uncovered, stirring occasionally, until the curry thickens, about 20 minutes.
10. Add cooked brown rice, lime juice, and margarine. Stir to combine.
11. Portion out curry and rice.
12. Freeze.



Nutrition Facts

Serving Size (390g)
Servings Per Container

Amount Per Serving

Calories 500	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 260mg	**11%**
Total Carbohydrate 77g	**26%**
Dietary Fiber 16g	**64%**
Sugars 15g	
Protein 23g	

Vitamin A --% • Vitamin C 90%
Calcium 20% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SPAGHETTI WITH MARINARA SAUCE

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 40 minute **Total time:** 1 hour and 10 minutes

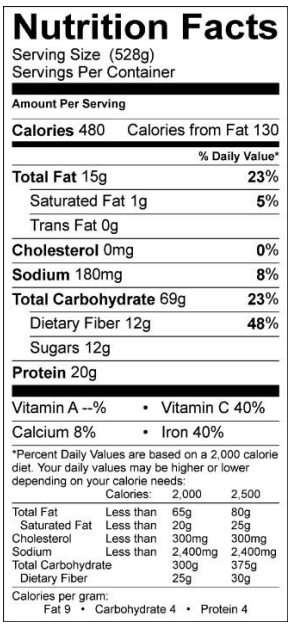
Ingredients:

- 215 grams red lentils, dry
- 150 grams whole wheat spaghetti, dry
- 1 tablespoon canola oil
- 2 tablespoons canola oil
- 1 tablespoon garlic, minced
- 1 cup onion, finely chopped
- 1 cup carrot, finely chopped
- 1 359 ml can tomato sauce
- 1 cup water
- ½ teaspoon tabasco sauce
- 2 teaspoons basil
- 2 teaspoons oregano
- 1 teaspoon thyme
- ½ teaspoon cinnamon
- 1 teaspoon sugar
- ¼ teaspoon salt

Directions:

1. Cook red lentils according to pulse cooking instructions. Drain.
2. Bring cold water to a boil in another pot. Cook spaghetti according to package instructions until al dente. Drain. Add oil and toss to coat.
3. Heat oil in another pot over medium-high heat. Add garlic, onions and carrots. Sauté until onions are tender.

4. Add tomato sauce, water, basil, oregano, thyme, cinnamon, sugar, salt and tabasco. Stir to combine. Simmer until vegetables are tender.
5. Add red lentils. Stir to combine.
6. Portion out pasta. Portion out lentil sauce on top of pasta.
7. Freeze.



Nutrition Facts

Serving Size (528g)
Servings Per Container

Amount Per Serving

Calories 480	Calories from Fat 130
	% Daily Value*
Total Fat 15g	**23%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 180mg	**8%**
Total Carbohydrate 69g	**23%**
Dietary Fiber 12g	**48%**
Sugars 12g	
Protein 20g	

Vitamin A --%	• Vitamin C 40%
Calcium 8%	• Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SWEET POTATO QUESADILLA

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

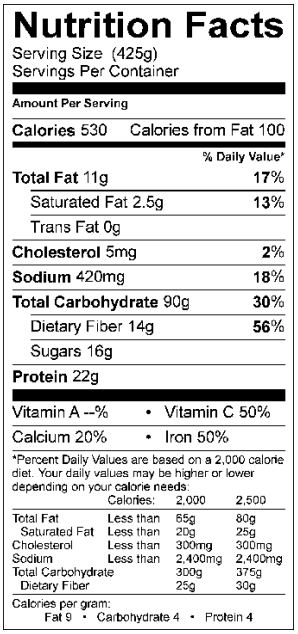
Ingredients:

- 300 grams lentils, dry
- 4 tablespoons canola oil
- 1 large sweet potato, peeled and diced
- 1 tablespoon garlic, minced
- 2 tablespoons cumin
- 1 tablespoon chili powder
- ¼ teaspoon salt
- 4 whole wheat tortillas
- 4 tablespoons shredded cheese
- 1 tablespoon canola oil

Directions:

1. Cook lentils according to pulse cooking instructions. Drain.
2. Heat oil in a pot over medium-high heat. Add sweet potatoes. Cook until they are tender and can be easily pierced with a fork.
3. Add lentils, garlic, cumin, chili powder, and salt. Stir to combine. Cook for 2 minutes more. Remove from heat.
4. Lay tortillas flat. Sprinkle each tortilla with ¼ of the shredded cheese. Top half of each tortilla with ¼ of the sweet potato mixture. Fold each tortilla in half into a quesadilla.
5. Heat oil in a pan over medium heat. Fry each quesadilla, pressing down with a spatula, until cheese melts and tortilla begins to brown. Flip quesadilla over and repeat.
6. Cut each quesadilla in half. Wrap the halves in waxed paper or aluminum foil.

7. Freeze.



Nutrition Facts

Serving Size (425g)
Servings Per Container

Amount Per Serving

Calories 530	Calories from Fat 100
	% Daily Value*
Total Fat 11g	**17%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 420mg	**18%**
Total Carbohydrate 90g	**30%**
Dietary Fiber 14g	**56%**
Sugars 16g	
Protein 22g	

Vitamin A --%	• Vitamin C 50%
Calcium 20%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

VEGETARIAN CHILI

Yield: 5 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

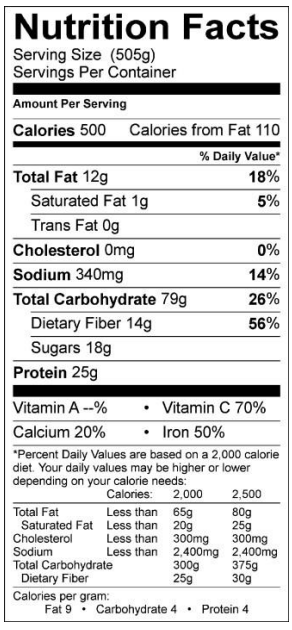
Ingredients:

- 1125 grams chickpeas, cooked and thawed
- 2 tablespoons canola oil
- 2 tablespoons garlic, minced
- 1 onion, chopped
- 2 celery stalks, chopped
- 1 red bell pepper, chopped
- 1 carrot, finely chopped
- 2 cans mushrooms
- ½ cup corn, frozen
- 1 796ml can diced tomatoes
- 1 tablespoon chili powder
- 2 teaspoons paprika
- 2 teaspoons cumin
- 2 bay leaves
- 1 teaspoon oregano
- ¼ teaspoon salt
- black pepper to taste

Directions:

1. Heat oil in a pot over medium heat. Add garlic, onion, celery, bell pepper, carrot, mushrooms and corn. Sauté until the vegetables are slightly tender.
2. Add chickpeas, diced tomatoes and spices. Bring to a boil. Reduce heat and simmer for 20 minutes or longer.

3. Portion out chili. Remember to remove bay leaves.
4. Freeze.



Nutrition Facts

Serving Size (505g)
Servings Per Container

Amount Per Serving

Calories 500	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 340mg	**14%**
Total Carbohydrate 79g	**26%**
Dietary Fiber 14g	**56%**
Sugars 18g	
Protein 25g	

Vitamin A --% • Vitamin C 70%
Calcium 20% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

ZUCCHINI AND SPLIT PEA SAUTÉE

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 20 minutes **Total time:** 50 minutes

Ingredients:

- 540 grams split peas, dry
- 3 tablespoons canola oil
- 1 onion, sliced into rings
- 1 tablespoon garlic, minced
- 3 green onions, chopped
- 2 medium zucchinis, sliced
- 1 796ml can diced tomatoes
- 1 cup shredded cheese
- ¼ teaspoon soy sauce, reduced sodium
- ½ teaspoon salt
- ¼ teaspoon black pepper
- 6 whole wheat buns
- 6 teaspoons margarine

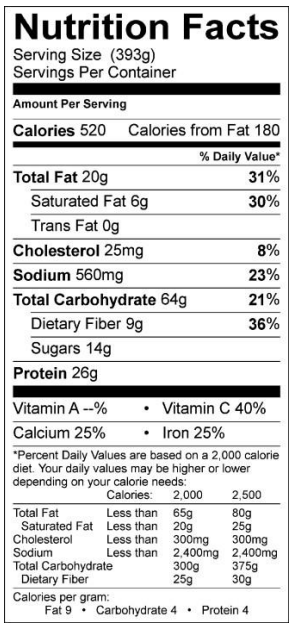
Directions:

1. Cook split peas according to pulse cooking instructions. Drain.
2. Heat oil in a pot over medium-low heat. Add onion rings. Sauté until browned. Remove from pot and set aside.
3. Add garlic. Sauté until fragrant.
4. Add green onions and zucchini. Sauté until slightly tender, about 5 minutes.
5. Pour diced tomatoes on top of zucchini mixture. Sprinkle two thirds of the cheese on top of the tomato layer.

6. Lay onion rings on top of cheese layer. Sprinkle the rest of the cheese on top of the onion layer.
7. Sprinkle soy sauce, salt and pepper on top of the sautée.
8. Reduce heat to low, cover, and cook for about 5 minutes, until cheese is melted and tomatoes are heated through.
9. Portion out split peas. Portion out sautée on top of split peas.
10. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (393g)
Servings Per Container

Amount Per Serving

Calories 520	Calories from Fat 180
	% Daily Value*
Total Fat 20g	**31%**
Saturated Fat 6g	**30%**
Trans Fat 0g	
Cholesterol 25mg	**8%**
Sodium 560mg	**23%**
Total Carbohydrate 64g	**21%**
Dietary Fiber 9g	**36%**
Sugars 14g	
Protein 26g	

Vitamin A --% • Vitamin C 40%
Calcium 25% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

APRICOT TAGINE

Yield: 5 servings

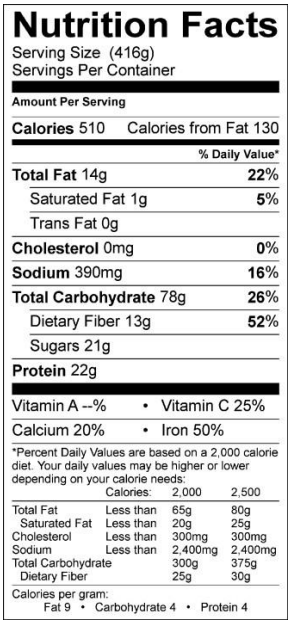
Preparation time: 30 minutes **Cook time:** 1 hour **Total time:** 1 hour and 30 minutes

Ingredients:

- 1125 grams chickpeas, cooked
- 3 tablespoons canola oil
- 1 large onion, chopped
- 2 tablespoons garlic, minced
- 2 796ml cans diced tomatoes
- ½ cup dried apricots, chopped
- 2 teaspoons oregano
- 1 teaspoon cumin
- 1 teaspoon coriander
- 1 teaspoon cinnamon
- 1 teaspoon black pepper
- ¼ teaspoon salt
- 1 cup water

Directions:

1. Heat oil in a pot. Add onion and garlic. Sauté until browned.
2. Add tomatoes, apricots and spices. Stir to combine. Sauté for five minutes.
3. Add chickpeas. Stir to combine.
4. Add water. Reduce heat to low, cover, and simmer until apricots are tender, about 30 to 45 minutes.
5. Add more water if necessary.
6. Portion out tagine.
7. Freeze.


Nutrition Facts
Serving Size (416g)
Servings Per Container
Amount Per Serving
Calories 510 Calories from Fat 130
% Daily Value*
Total Fat 14g 22%
Saturated Fat 1g 5%
Trans Fat 0g
Cholesterol 0mg 0%
Sodium 390mg 16%
Total Carbohydrate 78g 26%
Dietary Fiber 13g 52%
Sugars 21g
Protein 22g
Vitamin A --% • Vitamin C 25%
Calcium 20% • Iron 50%
*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:
Calories: 2,000 2,500
Total Fat Less than 65g 80g
Saturated Fat Less than 20g 25g
Cholesterol Less than 300mg 300mg
Sodium Less than 2,400mg 2,400mg
Total Carbohydrate 300g 375g
Dietary Fiber 25g 30g
Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

BAKED BURRITO

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 300 grams red lentils, dry
- 1 cup salsa
- 2 teaspoons chili powder
- 1 bunch green onions, sliced
- 4 whole wheat tortillas
- 90 grams light cheddar cheese, shredded

Directions:

1. Preheat oven to 350°F.
2. Cook red lentils according to pulse cooking instructions. Drain.
3. Heat a pan over low heat. Add lentils, salsa, and chili powder. Stir to combine. Allow to warm through.
4. Lay tortillas flat. Portion a quarter of the lentil mixture onto each tortilla. Sprinkle half of the green onions and cheddar cheese on top of the lentil mixture.
5. Roll tortillas into burritos. Sprinkle the remaining half of the green onions and cheddar cheese on top of the burritos.
6. Place burritos on a baking sheet. Bake until cheese is melted, about 10 minutes.
7. Wrap burritos in tinfoil.
8. Freeze.

Nutrition Facts

Serving Size (279g)
Servings Per Container

Amount Per Serving

Calories 440	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 6g	**30%**
Trans Fat 0g	
Cholesterol 25mg	**8%**
Sodium 830mg	**35%**
Total Carbohydrate 62g	**21%**
Dietary Fiber 9g	**36%**
Sugars 5g	
Protein 24g	

Vitamin A --% • Vitamin C 8%
Calcium 25% • Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

BROCCOLI PASTA ITALIANO

Yield: 4 servings

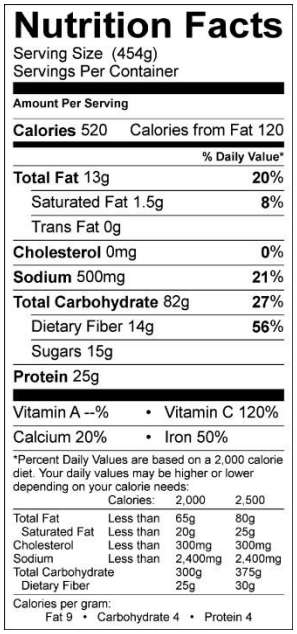
Preparation time: 20 minutes **Cook time:** 25 minutes **Total time:** 45 minutes

Ingredients:

- 900 grams chickpeas, cooked and thawed
- 1 ½ cups whole wheat pasta (such as fusilli or macaroni)
- 2 tablespoons olive oil
- ¼ cup red onion, minced
- 2 tablespoons garlic, minced
- 1 teaspoon oregano
- 1 teaspoon thyme
- 1 teaspoon basil
- 1 796ml can diced tomatoes
- 2 cups broccoli florets, frozen
- ½ teaspoon salt
- black pepper to taste

Directions:

1. Bring a pot of water to a boil. Cook pasta according to package directions until al dente. Drain.
2. Heat oil in another pot over medium heat. Add onion. Sauté until tender.
3. Add garlic and herbs. Sauté for another 2 minutes.
4. Add diced tomatoes and broccoli. Bring to a boil. Reduce heat to low and simmer until broccoli is tender, about 20 minutes.
5. Season with salt and pepper.
6. Add cooked pasta. Stir to combine. Allow to warm through.
7. Portion out pasta.
8. Freeze.



Nutrition Facts

Serving Size (454g)
Servings Per Container

Amount Per Serving

Calories 520	Calories from Fat 120
	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 500mg	**21%**
Total Carbohydrate 82g	**27%**
Dietary Fiber 14g	**56%**
Sugars 15g	
Protein 25g	

Vitamin A --% • Vitamin C 120%
Calcium 20% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LENTIL CHILI CON CARNE

Yield: 5 servings

Preparation time: 20 minutes **Cook time:** 1 hour 40 minutes **Total time:** 2 hours

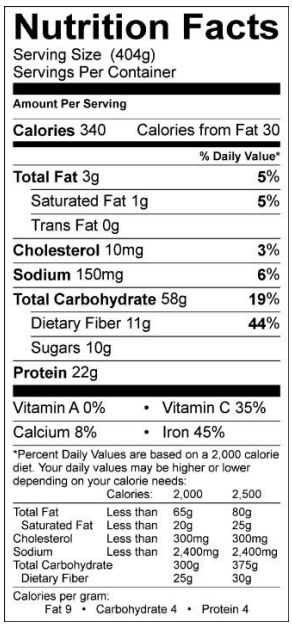
Ingredients:

- 450 grams red lentils, dry
- ¼ pound extra-lean ground beef
- 1 medium onion, chopped
- 1 large carrot, chopped
- 1 tablespoon garlic, minced
- 1 cup water
- ½ cup wild rice, dry
- 1 369ml can tomato sauce
- 2 284ml cans sliced mushrooms
- 2 tablespoon chili powder
- 1 teaspoon black pepper
- ¼ teaspoon salt
- 1 tablespoon white vinegar

Directions:

1. Cook red lentils according to pulse cooking instructions. Drain.
2. Heat a pot over medium-high heat. Add ground beef, onions, carrots and garlic. Sauté until beef has browned.
3. Lift beef mixture from pot with slotted spoon and drain fat. Return beef mixture to pot.
4. Add water, wild rice, tomato sauce, mushrooms and spices. Bring to a boil. Reduce heat to low, cover, and simmer until rice is cooked, about 1 hour.
5. Add vinegar. Simmer for another 15 minutes.
6. Portion out lentils. Portion out chili on top of lentils.

7. Freeze.



Nutrition Facts

Serving Size (404g)
Servings Per Container

Amount Per Serving

Calories 340	Calories from Fat 30
	% Daily Value*
Total Fat 3g	**5%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 150mg	**6%**
Total Carbohydrate 58g	**19%**
Dietary Fiber 11g	**44%**
Sugars 10g	
Protein 22g	

Vitamin A 0% • Vitamin C 35%

Calcium 8% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

EAST INDIAN CINNAMON PILAF

Yield: 6 servings

Preparation time: 10 minutes **Cook time:** 1 hour **Total time:** 1 hour 10 minutes

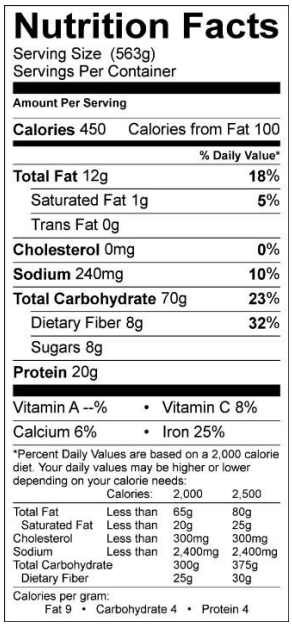
Ingredients:

- 540 grams split peas, dry
- 4 tablespoons canola oil
- 2 bay leaves
- 2 large onions, chopped
- 2 teaspoons cumin
- 1 teaspoon cumin seeds
- 1 teaspoon cinnamon
- 1 teaspoon madras curry powder
- ½ teaspoon turmeric
- ½ teaspoon hot pepper flakes
- black pepper to taste
- 1 cup brown rice, dry
- 2 ½ cups low-sodium vegetable broth

Directions:

1. Cook split peas according to pulse cooking instructions. Drain and keep warm.
2. Heat oil in a pan over medium-high heat. Add bay leaf. Cook until golden, about 3 minutes.
3. Add onion. Sauté until tender.
4. Add spices. Sauté for 30 seconds.
5. Add rice. Cook, stirring constantly, for 3 minutes.
6. Add broth. Bring to a boil. Reduce heat to low, cover, and simmer until liquid is mostly absorbed, about 20 to 25 minutes.

7. Add split peas. Stir to combine. Allow to warm through.
8. Portion out pilaf. Remember to remove bay leaf.
9. Freeze.



Nutrition Facts

Serving Size (563g)
Servings Per Container

Amount Per Serving

Calories 450	Calories from Fat 100
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 240mg	**10%**
Total Carbohydrate 70g	**23%**
Dietary Fiber 8g	**32%**
Sugars 8g	
Protein 20g	

Vitamin A --% • Vitamin C 8%
Calcium 6% • Iron 25%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

FALAFEL

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 10 minutes **Total time:** 40 minutes

Ingredients:

- 900 grams chickpeas, cooked and thawed
- 1 bunch green onions, sliced
- ¼ cup egg whites
- 3 tablespoons all-purpose flour
- 2 teaspoons oregano
- 2 teaspoons cumin
- ¼ teaspoon salt
- 1 tablespoon olive oil
- ¾ cup nonfat yogurt, plain
- 4 teaspoons tahini
- 1 ½ tablespoon lemon juice
- ½ cup fresh parsley, finely chopped
- 4 whole wheat pita breads, cut in half

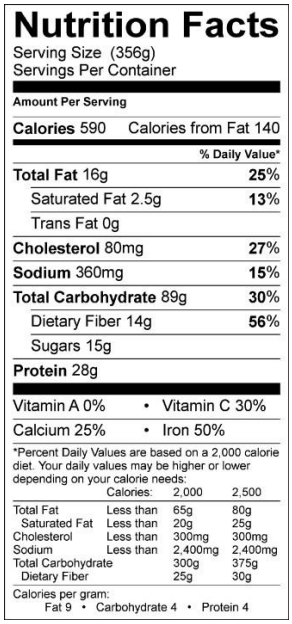
Directions:

1. Place chickpeas, egg whites, flour, green onions and spices in food processor. Pulse until a coarse mixture forms which holds together when pressed.
2. Form chickpea mixture into four patties.
3. Heat oil in a pan over medium-high heat. Add patties. Fry until golden and beginning to crisp, about 4 to 5 minutes.
4. Flip patties. Fry until other side is also golden and crisp, about 2 to 4 minutes.
5. Wrap patties in tinfoil.
6. Freeze.

Serving Instructions:

1. Combine yogurt, tahini, lemon juice and parsley in a bowl. Stir to combine.
2. Portion sauce into separate containers.

Serve with 1 container of sauce and 1 pita bread each.



Nutrition Facts

Serving Size (356g)
Servings Per Container

Amount Per Serving

Calories 590	Calories from Fat 140
	% Daily Value*
Total Fat 16g	**25%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 80mg	**27%**
Sodium 360mg	**15%**
Total Carbohydrate 89g	**30%**
Dietary Fiber 14g	**56%**
Sugars 15g	
Protein 28g	

Vitamin A 0% • Vitamin C 30%
Calcium 25% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SAUSAGE AND SWEET POTATO CHILI

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 1 hour **Total time:** 1 hour 30 minutes

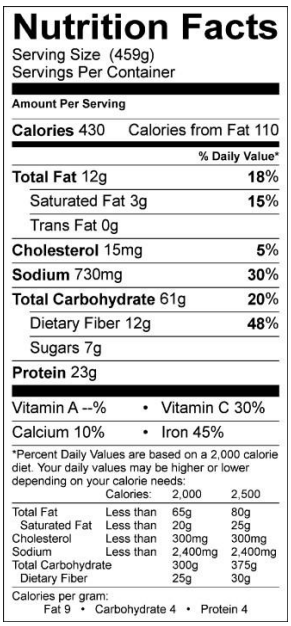
Ingredients:

- 300 grams red lentils, dry
- 1 teaspoon chili powder
- 1 teaspoon cumin
- 1 teaspoon no-salt seasoning (such as garlic herb)
- 1 teaspoon oregano
- ¼ pound hot italian sausage
- 1 tablespoon canola oil
- 1 small onion, chopped
- 2 tablespoons garlic, minced
- 1 ½ tablespoon. chili powder
- ¼ cup chipotle peppers
- 1 teaspoon cumin
- 1 teaspoon oregano
- black pepper to taste
- 1 796ml can diced tomatoes
- 1 large sweet potato, diced
- ½ cup water

Directions:

1. Cook red lentils according to pulse cooking instructions. Add chili powder, cumin, no- salt seasoning, and oregano to cooking water. Drain.
2. Heat a pan over medium heat. Add sausage. Cook until browned. Remove sausage from pan and slice thinly.

3. Heat oil in a pot over medium heat. Add onion, garlic and spices. Sauté for 2 minutes, stirring frequently.
4. Add diced tomatoes, sweet potato and water. Bring to a boil over medium heat. Reduce heat to low, cover, and simmer until sweet potato is tender, about 25 minutes.
5. Add water and adjust seasoning if necessary.
6. Portion out lentils. Portion out chili on top of lentils.
7. Freeze.



Nutrition Facts

Serving Size (459g)
Servings Per Container

Amount Per Serving

Calories 430	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 3g	**15%**
Trans Fat 0g	
Cholesterol 15mg	**5%**
Sodium 730mg	**30%**
Total Carbohydrate 61g	**20%**
Dietary Fiber 12g	**48%**
Sugars 7g	
Protein 23g	

Vitamin A --%	• Vitamin C 30%
Calcium 10%	• Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

APPLE CINNAMON OATMEAL

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 1 hour and 30 minutes **Total time:** 2 hours

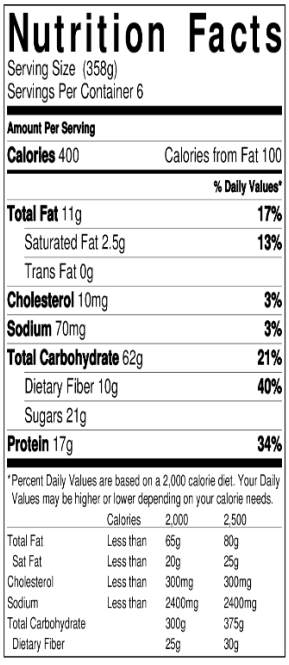
Ingredients:

- 450 grams red lentils, dry
- 2 cups water
- 1 ½ tablespoon vanilla extract
- 1 cup brown sugar
- 2 tablespoons cinnamon
- 1 tablespoon allspice
- 1 teaspoon ground cloves
- 1 teaspoon nutmeg
- 1/8 teaspoon salt
- 1 cup oats
- 2 tablespoons skim milk powder
- 2 cups water
- 1 ½ tablespoon margarine or unsalted butter
- 6 medium apples, peeled and chopped
- ½ cup almonds, slivered

Directions:

1. Combine red lentils, water, vanilla extract, half of brown sugar and half of spices in a pot. Bring to a boil. Reduce heat to low and simmer until lentils are almost done.
2. Add oats, skim milk powder and water. Cook until oatmeal consistency is achieved.
3. Heat margarine or butter in pan over medium-low heat. Add apples. Cook until softened.
4. Add almonds and remaining half of spices to apple mixture. Cook over medium heat until almonds are toasted and apples are lightly browned.

5. Add remaining half of brown sugar and lemon juice to apple mixture. Cook until sugar dissolves.
6. Portion out oatmeal mixture. Portion out apple mixture on top of oatmeal.
7. Freeze.



Nutrition Facts

Serving Size (358g)
Servings Per Container 6

Amount Per Serving	
Calories 400	Calories from Fat 100
	% Daily Values*
Total Fat 11g	**17%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 70mg	**3%**
Total Carbohydrate 62g	**21%**
Dietary Fiber 10g	**40%**
Sugars 21g	
Protein 17g	**34%**

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

CHEESY SPINACH PASTA

Yield: 5 servings

Preparation time: 25 minutes **Cook time:** 20 minutes **Total time:** 45 minutes

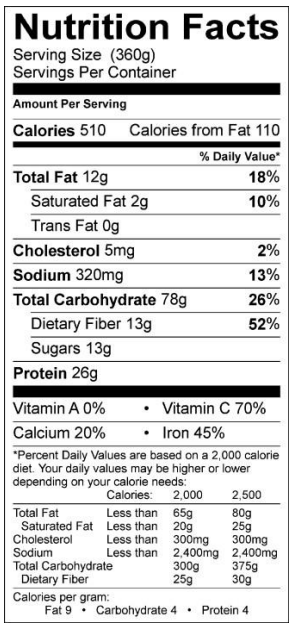
Ingredients:

- 1125 grams chickpeas, cooked and thawed
- 2 cups whole wheat pasta (such as fusilli or macaroni)
- 1 tablespoon olive oil
- 1 small onion, chopped
- 1 bell pepper, chopped
- 2 tablespoons garlic, minced
- 1 teaspoon red pepper flakes
- ½ teaspoon black pepper
- ½ teaspoon salt
- 1 cup low-sodium vegetable broth
- ½ 300 grams package frozen spinach, thawed
- 1 ½ tablespoon olive oil
- 2 tablespoons nonfat yogurt, plain
- 2 tablespoons part-skim mozzarella cheese, shredded
- 2 tablespoons parmesan cheese, grated

Directions:

1. Cook pasta according to package instructions until al dente. Drain.
2. Heat oil in a pot over medium-high heat. Add onions and bell pepper. Sauté until softened, about 4 minutes.
3. Add garlic, red pepper flakes, salt and pepper. Sauté for 1 minute more.
4. Add chickpeas and vegetable broth. Bring to a boil. Reduce heat to low and simmer until chickpeas are tender, about 5 minutes.

5. Crush chickpeas against the side of the pot using the back of a spoon.
6. Add pasta, spinach, olive oil, yogurt, mozzarella and parmesan. Stir to combine. Allow to heat through.
7. Portion out pasta.
8. Freeze.



Nutrition Facts

Serving Size (360g)
Servings Per Container

Amount Per Serving

Calories 510	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 320mg	**13%**
Total Carbohydrate 78g	**26%**
Dietary Fiber 13g	**52%**
Sugars 13g	
Protein 26g	

Vitamin A 0% • Vitamin C 70%
Calcium 20% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HERBED LENTILS

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 300 grams lentils, dry
- 2 tablespoons olive oil
- 1 cup broccoli florets, fresh or frozen and thawed
- 3 cups baby spinach, fresh or frozen and thawed
- ¼ cup green onions, finely chopped
- 1 cup cherry tomatoes, halved
- ¼ cup fresh basil, finely chopped
- ¼ cup fresh parsley, finely chopped
- ¼ cup fresh mint, finely chopped
- ¼ cup parmesan cheese, grated
- 2 tablespoons lemon juice
- ¼ teaspoon black pepper
- ¼ teaspoon salt
- 4 whole wheat buns
- 4 teaspoon margarine

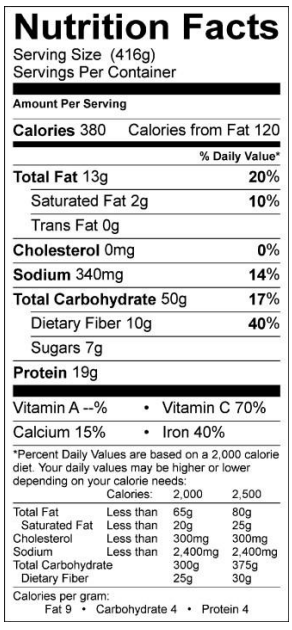
Directions:

1. Cook lentils according to pulse cooking instructions. Drain.
2. Heat oil in a pot over medium-high heat. Add broccoli, spinach and green onion. Cook until softened and wilted, about 2 minutes.
3. Add lentils, tomatoes, basil, parsley and mint. Stir to combine. Allow to heat through.
4. Add parmesan, lemon juice, salt and pepper. Stir to combine.
5. Portion out lentil mixture.

6. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (416g)
Servings Per Container

Amount Per Serving

Calories 380	Calories from Fat 120
	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 340mg	**14%**
Total Carbohydrate 50g	**17%**
Dietary Fiber 10g	**40%**
Sugars 7g	
Protein 19g	

Vitamin A --% • Vitamin C 70%

Calcium 15% • Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LENTIL SOFT TACOS

Yield: 3 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 450 grams lentils, cooked and thawed
- 1 teaspoon canola oil
- 1 small onion, chopped
- 1 tablespoon garlic, minced
- 2 teaspoon chili powder
- 1 teaspoon cumin
- 1 teaspoon oregano
- 1 ¼ cup low-sodium vegetable broth
- ¾ cup lettuce, shredded
- ¾ cup cherry tomatoes, quartered
- ½ cup salsa
- 3 tablespoon low-fat sour cream
- ¾ cup light cheddar cheese, shredded
- 6 small or 3 large whole-wheat tortillas

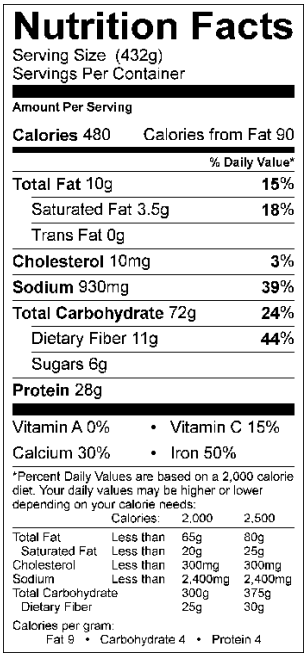
Directions:

1. Heat oil in a pot over medium-high heat. Add onions and garlic. Sauté until tender.
2. Add chili powder, cumin and oregano. Sauté for 1 minute more.
3. Add lentils and vegetable broth. Bring to a boil. Reduce heat to low, cover and simmer until lentils are soft, about 20 minutes.
4. Portion out lentil mixture.
5. Freeze.

Serving Instructions:

Serve with ¼ cup lettuce and ¼ cup tomatoes, 1 tablespoon salsa, 1 tablespoon sour cream, and ¼ cup cheddar cheese, each in separate containers.

Also serve with either 2 small or 1 large whole-wheat tortilla.



Nutrition Facts

Serving Size (432g)
Servings Per Container

Amount Per Serving

Calories 480 Calories from Fat 90

	% Daily Value*
Total Fat 10g	**15**%
Saturated Fat 3.5g	**18**%
Trans Fat 0g	
Cholesterol 10mg	**3**%
Sodium 930mg	**39**%
Total **Carbohydrate** 72g	**24**%
Dietary Fiber 11g	**44**%
Sugars 6g	
Protein 28g	

Vitamin A 0% • Vitamin C 15%
Calcium 30% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

MULTIGRAIN LENTIL PILAF

Yield: 5 servings

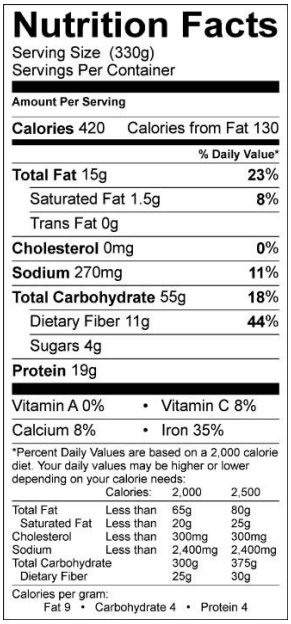
Preparation time: 25 minutes **Cook time:** 1 hour 15 minutes **Total time:** 1 hour 40 minutes

Ingredients:

- 375 grams lentils, dry
- 2 ½ tablespoons canola oil
- 1 medium onion, chopped
- ½ cup almonds, sliced
- ¼ cup brown rice, dry
- ¼ cup barley, dry
- ¼ cup wild rice, dry
- 2 ¼ cups low-sodium vegetable broth
- 1 tablespoon cooking sherry
- 1 tablespoon no-salt seasoning (such as garlic herb)
- ½ teaspoon salt

Directions:

1. Preheat oven to 350°F.
2. Cook lentils according to pulse cooking instructions. Drain.
3. Heat oil in a pot over medium-high heat. Add onions. Sauté until onions are translucent.
4. Add almonds, brown rice and barley. Sauté until rice is slightly browned.
5. Add lentils, wild rice, vegetable broth, sherry, seasoning and salt. Stir to combine.
6. Pour mixture into a casserole dish. Loosely cover with tinfoil. Bake for about 1 hour, stirring every 20 minutes.
7. Portion out pilaf.
8. Freeze.



Nutrition Facts

Serving Size (330g)
Servings Per Container

Amount Per Serving

Calories 420 Calories from Fat 130

	% Daily Value*
Total Fat 15g	**23**%
Saturated Fat 1.5g	**8**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 270mg	**11**%
Total Carbohydrate 55g	**18**%
Dietary Fiber 11g	**44**%
Sugars 4g	
Protein 19g	

Vitamin A 0% • Vitamin C 8%

Calcium 8% • Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LENTIL PIZZA WITH HERB PESTO

Yield: 4 servings

Preparation time: 20 minutes **Cook time:** 40 minutes **Total time:** 1 hour

Ingredients:

- 300 grams red lentils, dry
- 4 whole wheat thin buns
- ¼ cup basil pesto
- 1 cup artichoke hearts, chopped
- 1 cup roasted red peppers, chopped
- ½ cup part-skim mozzarella cheese, shredded
- fresh ground black pepper to taste

Directions:

1. Preheat oven to 350°F.
2. Cook red lentils according to pulse cooking instructions. Drain.
3. Split thin buns open. Place facing upward on baking sheet. Toast in oven until lightly golden.
4. Mix pesto into lentils.
5. Spread lentil mixture evenly onto each thin bun. Distribute artichoke hearts and roasted red peppers on top of lentil mixture. Sprinkle mozzarella cheese over pizzas.
6. Return pizzas to oven. Toast until cheese melts.
7. Wrap pizzas in tinfoil.
8. Freeze.

Nutrition Facts

Serving Size (187g)
Servings Per Container 4

Amount Per Serving	
Calories 435	Calories from Fat 72
	% Daily Values*
Total Fat 8g	**12%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 345mg	**14%**
Total Carbohydrate 58g	**19%**
Dietary Fiber 12g	**48%**
Sugars 2g	
Protein 27g	**54%**

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

ROSE
LENTILS

Yield: 6 servings

Preparation time: 15 minutes **Cook time:** 45 minutes **Total time:** 1 hour

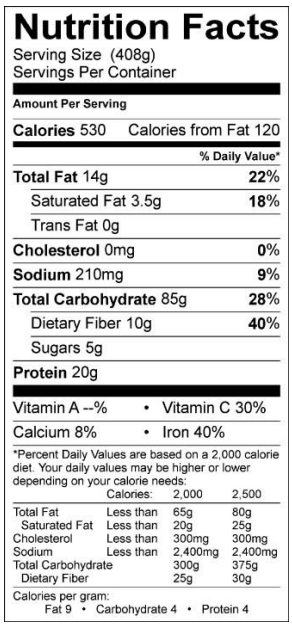
Ingredients:

- 450 grams red lentils, dry
- 2 tablespoons canola oil
- 1 small onion, chopped
- 2 tablespoons garlic powder
- 1 tablespoon madras curry powder
- 1 tablespoon ground ginger
- 1 tablespoon ground thyme
- 1 teaspoon cinnamon
- ½ teaspoon salt
- 1 398ml can light coconut milk
- 2 cups brown rice, dry
- 1 cup cauliflower florets, fresh or frozen and thawed, chopped
- 2 cups green beans, fresh or frozen and thawed, chopped

Directions:

1. Cook red lentils according to pulse cooking instructions. Drain.
2. Heat oil in a pot over medium-high heat. Add onions. Sauté until softened.
3. Add spices. Sauté for 1 minute more.
4. Add coconut milk, brown rice, cauliflower and green beans. Bring to a boil. Reduce heat to low, cover and simmer until rice is cooked, about 30 minutes.
5. Portion out lentils. Portion out rice mixture on top of lentils.

6. Freeze.



Nutrition Facts

Serving Size (408g)
Servings Per Container

Amount Per Serving

Calories 530	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 3.5g	**18%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 210mg	**9%**
Total Carbohydrate 85g	**28%**
Dietary Fiber 10g	**40%**
Sugars 5g	
Protein 20g	

Vitamin A --%	• Vitamin C 30%
Calcium 8%	• Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SHEPHERDLESS PIE

Yield: 5 servings

Preparation time: 30 minutes **Cook time:** 1 hour **Total time:** 1 hour 30 minutes

Ingredients:

- 375 grams lentils, dry
- 2 teaspoons vegetable stock powder
- 2 teaspoons cumin
- 2 teaspoons oregano
- 1 large sweet potato, peeled and diced
- 1 medium russet potato, peeled and diced
- 2 tablespoons nonfat yogurt, plain
- 2 tablespoons margarine
- ½ teaspoon salt
- black pepper to taste
- 1 ½ tablespoons olive oil
- 1 small onion, diced
- 1 small carrot, diced
- 1 celery stalk, diced
- 2 tablespoons garlic, minced
- 2 teaspoons cumin
- 2 teaspoons oregano
- ½ teaspoon black pepper
- 1 796ml can diced tomatoes
- 1 medium zucchini, diced
- ½ cup light cheddar cheese, shredded
- 2 green onions, thinly sliced

Nutrition Facts

Serving Size (442g)
Servings Per Container

Amount Per Serving

Calories 420	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 3.5g	**18%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 340mg	**14%**
Total Carbohydrate 63g	**21%**
Dietary Fiber 11g	**44%**
Sugars 7g	
Protein 21g	
Vitamin A --%	• Vitamin C 60%
Calcium 20%	• Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Directions:

1. Preheat oven to 375°F.

2. Cook lentils according to pulse cooking instructions. Add vegetable broth, cumin and oregano to cooking water. Drain and set aside.
3. Bring a pot of salted water to a boil. Add sweet potatoes and russet potatoes. Cover and cook until tender, about 20 minutes. Drain.
4. Return potatoes to pot. Add yogurt, margarine, salt, pepper and half of the cheese. Mash together and set aside.
5. Heat oil in a pot over medium heat. Add onions, carrot, celery and garlic. Sauté until softened, about 6 minutes.
6. Add cumin, oregano and pepper. Sauté for 2 minutes more.
7. Add tomatoes, zucchini and lentils. Bring to a boil. Reduce heat to low, cover and simmer until thickened, stirring often.
8. Pour lentil mixture into a casserole dish. Spread mashed potato mixture on top of lentils. Sprinkle remaining half of cheese and green onions over potatoes.
9. Bake in oven until cheese is bubbly, about 30 minutes.
10. Portion out pie.
11. Freeze.

Soups

SPLIT PEA VEGETABLE SOUP

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 40 minutes **Total time:** 1 hour 10 minutes

Ingredients:

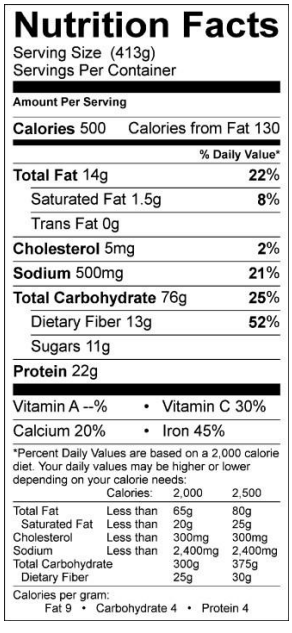
- 3 slices bacon
- 498 grams split peas, dry
- 4 tablespoons canola oil
- 1 onion, chopped (~150g)
- 6 celery stalks, chopped
- 2 teaspoons garlic ,minced
- 185 grams carrots, diced
- 2 bay leaves
- 1 teaspoon freshly ground black pepper
- 1 teaspoon turmeric
- 28 ounces diced tomatoes, no salt added
- 1 tablespoon fresh rosemary (or 1 tsp dry)
- 3 whole wheat pitas (½ per serving)
- 12 teaspoons sour cream, 2 per serving

Directions:

- Sort and rinse peas. Put in a large pot with 6 cups water, bring to a boil and reduce heat to maintain a simmer.
- While the peas cook, chop bacon into small pieces and brown in a skillet over medium heat, stirring occasionally. While the bacon cooks, chop onion, carrots and celery, including celery leaves, if available. Add the chopped veggies to bacon and cook, stirring occasionally, until onion softens, about 10 minutes. Add rosemary, turmeric, bay leaves, and tomatoes and minced or pressed garlic. Stir in well and cook for several minutes to develop flavors.

- Add bacon-veggie mix to peas and simmer until peas are soft, 10 to 30 minutes, depending on the age of the peas. Taste for seasoning and serve hot. Good with crusty wholegrain bread.

TIP: To reduce work time, add all ingredients to the pot with the peas and skip the browning steps. The finished soup won't have as rich a flavor, but will still be very good.



Nutrition Facts

Serving Size (413g)
Servings Per Container

Amount Per Serving

Calories 500	Calories from Fat 130
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 500mg	**21%**
Total Carbohydrate 76g	**25%**
Dietary Fiber 13g	**52%**
Sugars 11g	
Protein 22g	

Vitamin A --% • Vitamin C 30%

Calcium 20% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

BARLEY AND LENTIL SOUP

Yield: 6 servings

Preparation time: 20 minutes **Cook time:** 50 minutes **Total time:** 1 hour 10 minutes

Ingredients:

- 450 grams lentils, dry
- 4 cups low-sodium vegetable broth
- 4 cups cold water
- ½ cup pearl barley, dry
- 1 cup cold water
- 3 tablespoons olive oil
- 5 large carrots, finely chopped
- 1 medium red onion, diced
- ¾ teaspoon salt
- black pepper to taste
- 2 tablespoons garlic, minced
- 2 teaspoons cumin
- 300 grams spinach, frozen and chopped
- 3 tablespoons lemon juice
- 6 whole wheat buns

  6 teaspoons margarine

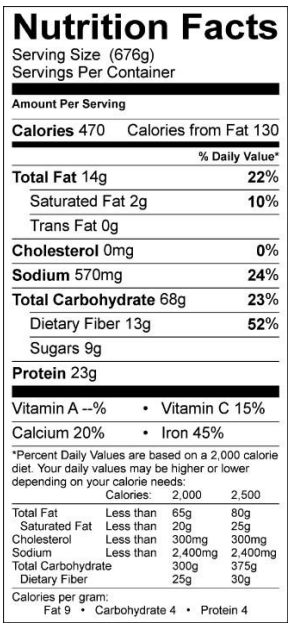
Directions:

1. Heat oil in a pot over medium heat. Add onions, carrots, salt and pepper. Cook until vegetables begin to brown, about 15 minutes.
2. Add garlic and cumin. Cook for 30 seconds more, stirring constantly.
3. Add lentils, vegetable broth and water. Bring to a boil. Reduce heat, cover and simmer until lentils are tender but not mushy, about 20 to 25 minutes.

4. Combine pearl barley and water in another pot. Bring to a boil. Reduce heat and simmer until cooked, about 20 minutes.
5. Add spinach and pearl barley to soup. Stir to combine. Cover and simmer until spinach is cooked, about 5 to 10 minutes.
6. Add lemon juice. Stir to combine.
7. Portion out soup.
8. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (676g)
Servings Per Container

Amount Per Serving

Calories 470	Calories from Fat 130
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 570mg	**24%**
Total Carbohydrate 68g	**23%**
Dietary Fiber 13g	**52%**
Sugars 9g	
Protein 23g	

Vitamin A --% • Vitamin C 15%
Calcium 20% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

GREEK SOUPA

Yield: 6 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 450 grams lentils, dry
- 4 tablespoons olive oil
- 1 cup carrot, chopped
- 1 cup celery, chopped
- 2 tablespoons garlic
- 2 tablespoons tomato paste
- 8 cups water
- 1 796ml can diced tomatoes
- 2 tablespoons lemon juice
- ¼ cup fresh parsley, chopped
- 1 tablespoon oregano
- 1 teaspoon rosemary
- 1 teaspoon black pepper
- ½ teaspoon sugar
- ½ teaspoon salt
- 6 whole wheat buns

  6 teaspoons margarine

Directions:

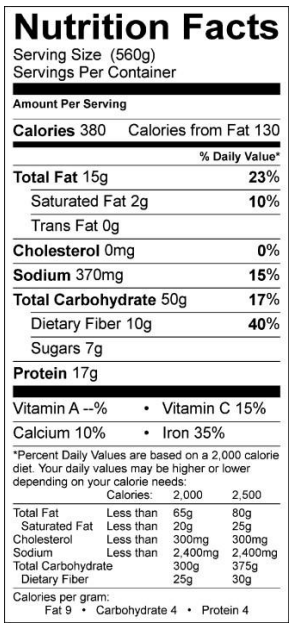
1. Heat oil in a pot over medium-high heat. Add carrot and celery. Cook until vegetables begin to brown.
2. Add garlic and tomato paste. Cook for 1 minute more, stirring constantly.

3. Add lentils, water, diced tomatoes, lemon juice, parsley, oregano, rosemary, pepper, sugar and salt. Bring to a boil. Reduce heat, cover and simmer until vegetables are soft.

   NOTE: Add more water as necessary to maintain soup consistency.
4. Portion out soup.
5. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (560g)
Servings Per Container

Amount Per Serving

Calories 380	Calories from Fat 130
	% Daily Value*
Total Fat 15g	**23%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 370mg	**15%**
Total Carbohydrate 50g	**17%**
Dietary Fiber 10g	**40%**
Sugars 7g	
Protein 17g	

Vitamin A --%	• Vitamin C 15%
Calcium 10%	• Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HONEY CARAMELIZED CARROT SOUP

Yield: 6 servings

Preparation time: 45 minutes **Cook time:** 45 minutes **Total time:** 1 hours and 30 minutes

Ingredients:

- 540 grams split peas, dry
- 1 tablespoon canola oil
- 1 small onion, chopped
- 1 medium bell pepper, chopped
- 1 tablespoon garlic, minced
- 3 cups low-sodium vegetable broth
- 1 tablespoon no-salt garlic and herb seasoning
- ¼ teaspoon salt
- 1 tablespoon canola oil
- 2 medium carrots, peeled and sliced into coins
- 1 tablespoon honey
- 6 whole wheat buns
- 6 teaspoons margarine

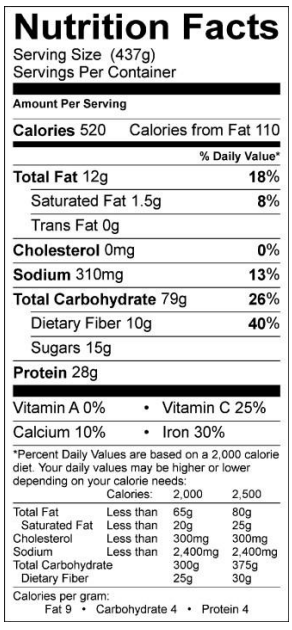
Directions:

1. Heat canola oil in a pot over medium heat. Add onion and bell pepper. Cook until onions are soft, about 5 minutes.
2. Add garlic. Cook for 4 minutes more.
3. Add vegetable broth, split peas, garlic and herb seasoning and salt. Bring to a boil. Reduce heat and simmer until split peas are cooked.
   NOTE: Add water if necessary.

4. Heat canola oil in a pan over medium heat. Add carrots and cook until tender enough to pierce with a fork.
5. Add honey. Stir or toss to combine. Turn heat to high and caramelize for 5 minutes.
6. Portion out soup. Portion out caramelized carrots on top of soup.
7. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (437g)
Servings Per Container

Amount Per Serving

Calories 520	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 310mg	**13%**
Total Carbohydrate 79g	**26%**
Dietary Fiber 10g	**40%**
Sugars 15g	
Protein 28g	

Vitamin A 0% • Vitamin C 25%
Calcium 10% • Iron 30%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

ROSEMARY INFUSED RED LENTIL SOUP

Yield: 5 servings

Preparation time: 20 minutes **Cook time:** 40 minutes **Total time:** 1 hour

Ingredients:

- 375 grams red lentils, dry
- 2 ½ tablespoons canola oil
- 1 teaspoon dried rosemary
- ½ teaspoon dried thyme
- ½ teaspoon dried basil
- 1 small onion, chopped
- 1 tablespoon garlic, minced
- 1 bay leaf
- 5 cups water
- ¼ cup pearl barley
- ½ teaspoon salt
- 1 large carrot, chopped
- 2 celery stalks, chopped
- 1 small potato, peeled and diced
- ¼ cup fresh parsley, chopped
- ½ teaspoon black pepper
- 5 whole wheat buns
- 5 teaspoons margarine

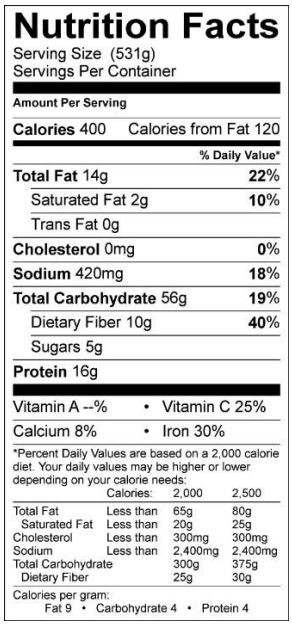
Directions:

1. Heat oil in a pot over medium heat. Add rosemary, thyme and basil. Temper for 1 minute until fragrant.

2. Add onion, garlic and bay leaf. Turn heat to medium-high. Sauté until onions are translucent, about 5 minutes.
3. Add water, red lentils, barley and salt. Bring to a boil. Reduce heat and simmer for 20 minutes, stirring occasionally.
4. Add carrots, celery, potato, parsley and black pepper. Simmer until vegetables are tender and lentils and barley are cooked through, about 20 minutes more.
5. Portion out soup. Remember to remove bay leaf.
6. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (531g)
Servings Per Container

Amount Per Serving

Calories 400	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 420mg	**18%**
Total Carbohydrate 56g	**19%**
Dietary Fiber 10g	**40%**
Sugars 5g	
Protein 16g	

Vitamin A --% • Vitamin C 25%
Calcium 8% • Iron 30%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SPLIT PEA AND BARLEY SOUP

Yield: 7 servings

Preparation time: 30 minutes **Cook time:** 1 hour 30 minutes **Total time:** 2 hours

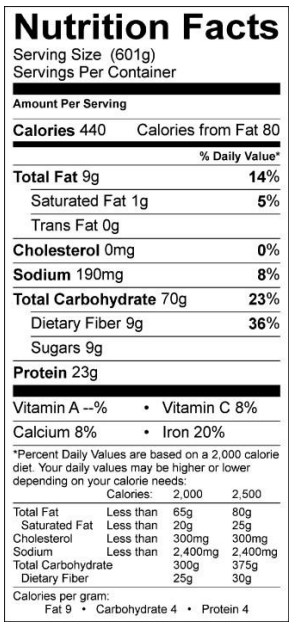
Ingredients:

- 630 grams split peas, dry
- 5 tablespoons canola oil
- 1 large onion, coarsely chopped
- 1 large carrot, peeled and coarsely chopped
- 3 celery stalks, coarsely chopped
- 1 tablespoon garlic
- 6 cups low-sodium vegetable broth
- 1 cup pearl barley, dry
- 3 cups cold water
- ¼ teaspoon salt
- white pepper to taste

Directions:

1. Heat oil in a pot over medium-high heat. Add onion, carrot, celery and garlic. Sauté until onions are translucent.
2. Add vegetable broth and split peas. Bring to a boil. Reduce heat and simmer uncovered for 1 hour.
3. Combine pearl barley and water in another pot. Bring to a boil. Reduce heat and simmer until cooked, about 30 to 40 minutes.
4. Blend soup with immersion blender until smooth.
5. Add pearl barley to blended soup. Stir to combine.
6. Season with salt and white pepper.
7. Portion out soup.

8. Freeze.



Nutrition Facts

Serving Size (601g)
Servings Per Container

Amount Per Serving

Calories 440	Calories from Fat 80
	% Daily Value*
Total Fat 9g	**14%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 190mg	**8%**
Total Carbohydrate 70g	**23%**
Dietary Fiber 9g	**36%**
Sugars 9g	
Protein 23g	

Vitamin A --% • Vitamin C 8%

Calcium 8% • Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

CURRIED APPLE SOUP

Yield: 4 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 300 grams red lentils, dry
- 1 teaspoon minced garlic
- 1 teaspoon curry powder
- 1 tablespoon margarine
- ½ cup chopped onion
- 2 tablespoons chopped celery leaves and/or celery
- 1 tablespoon minced garlic
- 1 teaspoon curry powder
- 4 cups low-sodium vegetable broth
- 3 apples, grated
- ½ cup carrot, grated
- 4 tablespoons nonfat yogurt, plain
- 4 whole wheat buns
- 4 teaspoons margarine

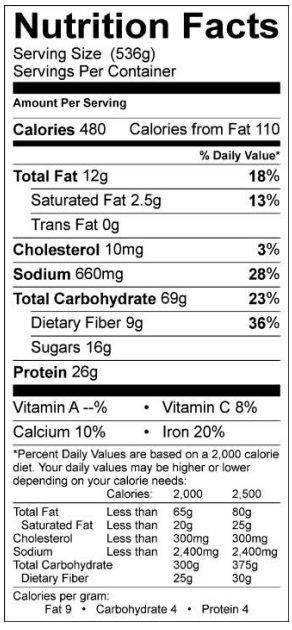
Directions:

1. Cook red lentils according to pulse cooking instructions. Add garlic and curry powder to cooking water. Drain.
2. Melt margarine in a pot over medium heat. Add garlic, onion and celery. Sauté until onion is soft, about 3 minutes.
3. Add curry powder. Sauté for 1 minute more, stirring constantly.
4. Add vegetable broth, carrot and apple. Bring to a boil. Reduce heat to low and simmer for 10 minutes.

5. Add lentils to soup and stir to combine.
6. Portion out soup.
7. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (536g)
Servings Per Container

Amount Per Serving

Calories 480	Calories from Fat 110
	% Daily Value*
Total Fat 12g	**18%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 660mg	**28%**
Total Carbohydrate 69g	**23%**
Dietary Fiber 9g	**36%**
Sugars 16g	
Protein 26g	

Vitamin A --%	• Vitamin C 8%
Calcium 10%	• Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

MINESTRONE SOUP

Yield: 5 servings

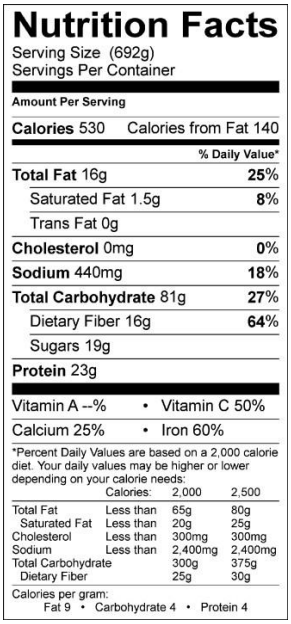
Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 1125 grams chickpeas, cooked and thawed
- 2 tablespoons canola oil
- 1 small onion, chopped
- 2 tablespoons garlic, minced
- ¼ cup dried oregano
- 1 teaspoon salt
- 2 teaspoons black pepper
- 1 medium carrot, chopped
- 2 celery stalks, chopped
- 5 cups water
- 1 796ml can diced tomatoes
- 1 156ml can tomato paste

Directions:

1. Heat oil in a pot over medium-high heat. Add onions. Sauté until softened.
2. Add garlic, oregano, salt and pepper. Temper for 30 seconds to 1 minute.
3. Add carrots and celery. Sauté until lightly browned.
4. Add water, diced tomatoes, tomato paste and chickpeas. Bring to a boil. Reduce heat to low and simmer until vegetables are tender.
5. Portion out soup.
6. Freeze.



Nutrition Facts

Serving Size (692g)
Servings Per Container

Amount Per Serving

Calories 530 Calories from Fat 140

	% Daily Value*
Total Fat 16g	**25**%
Saturated Fat 1.5g	**8**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 440mg	**18**%
Total Carbohydrate 81g	**27**%
Dietary Fiber 16g	**64**%
Sugars 19g	
Protein 23g	

Vitamin A --% • Vitamin C 50%

Calcium 25% • Iron 60%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SOUTHWESTERN CORN SOUP

Yield: 4 servings

Preparation time: 15 minutes **Cook time:** 45 minutes **Total time:** 1 hours

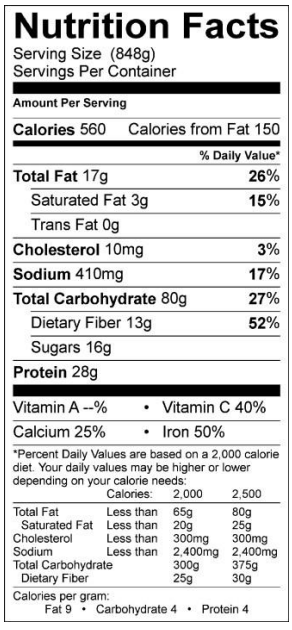
Ingredients:

- 900 grams chickpeas, cooked and thawed
- 2 tablespoons canola oil
- 1 tablespoon garlic, minced
- 1 small onion, diced
- 2 celery stalks, diced
- 1 teaspoon dried thyme
- 2 teaspoons cumin
- 1 teaspoon chili powder
- 1 teaspoon cinnamon
- 4 cups low-sodium vegetable broth
- 1 796ml can diced tomatoes
- 2 cups corn, frozen
- ¼ teaspoon salt
- black pepper to taste
- 4 tablespoons light cheddar cheese, shredded

Directions:

1. Heat oil in a pot over medium-high heat. Add garlic, onion and celery. Sauté until onion is translucent.
2. Add thyme, cumin, chili powder and cinnamon. Sauté for 1 minute more.
3. Add vegetable broth, diced tomatoes, corn, salt, and pepper. Bring to a boil. Reduce heat to low, cover, and simmer until celery is tender, about 30 minutes.
4. Add chickpeas. Stir to combine. Allow to warm through.

5. Portion out soup. Sprinkle 1 tablespoon cheddar cheese on top of each portion.
6. Freeze.



Nutrition Facts

Serving Size (848g)
Servings Per Container

Amount Per Serving

Calories 560	Calories from Fat 150
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 3g	**15%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 410mg	**17%**
Total Carbohydrate 80g	**27%**
Dietary Fiber 13g	**52%**
Sugars 16g	
Protein 28g	

Vitamin A --%	• Vitamin C 40%
Calcium 25%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

BABA'S BORSCHT

Yield: 5 servings

Preparation time: 30 minutes **Cook time:** 20 minutes **Total time:** 50 minutes

Ingredients:

- 1125 grams chickpeas, cooked and thawed
- 2 tablespoons canola oil
- 1 medium onion, chopped
- 3 medium beets, peeled and chopped
- 1 cup red cabbage, shredded
- 1 cup celery, chopped
- 1 cup green beans, chopped
- 2 ½ cups water
- 1 796ml can diced tomatoes
- 1 teaspoon vegetable stock powder
- 1 teaspoon thyme
- 1 teaspoon dill seeds
- 1 teaspoon no-salt seasoning (such as garlic herb)
- ¼ teaspoon salt
- ¼ teaspoon black pepper
- 1 bay leaf
- 1 tablespoon white vinegar
- 5 whole wheat buns
- 5 teaspoons margarine

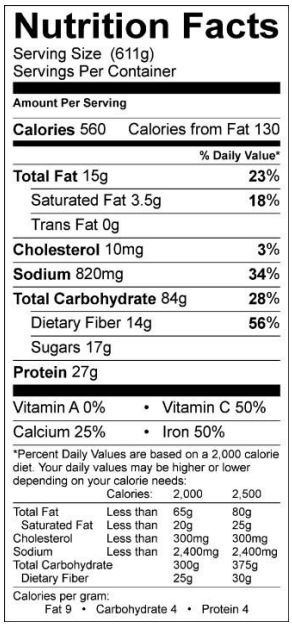
Directions:

1. Heat oil in a pot over medium-high heat. Add onion, beets, cabbage, celery and green beans. Sauté until onion is translucent.

2. Add chickpeas, water, diced tomatoes, vegetable stock, and spices. Bring to a boil. Reduce heat to low, cover and simmer until chickpeas and vegetables are tender.
3. Add vinegar. Stir to combine.
4. Portion out soup. Remember to remove bay leaf.
5. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (611g)
Servings Per Container

Amount Per Serving

Calories 560	Calories from Fat 130
	% Daily Value*
Total Fat 15g	**23**%
Saturated Fat 3.5g	**18**%
Trans Fat 0g	
Cholesterol 10mg	**3**%
Sodium 820mg	**34**%
Total Carbohydrate 84g	**28**%
Dietary Fiber 14g	**56**%
Sugars 17g	
Protein 27g	

Vitamin A 0%	• Vitamin C 50%
Calcium 25%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

FRENCH CANADIAN SPLIT PEA SOUP

Yield: 6 servings

Preparation time: 45 minutes **Cook time:** 2 hours **Total time:** 4 hours 45 minutes

Ingredients:

- 540 grams split peas, dry
- 12 cups water
- 1 bay leaf
- 5 strips bacon
- 3 tablespoons canola oil
- 2 medium onions, chopped
- 2 medium carrots, chopped
- 3 celery stalks, chopped
- 2 tablespoons garlic, minced
- ½ cup fresh parsley, packed
- 1 tablespoon dried sage
- ½ teaspoon salt
- black pepper to taste
- 6 whole wheat buns
- 6 teaspoons margarine

Directions:

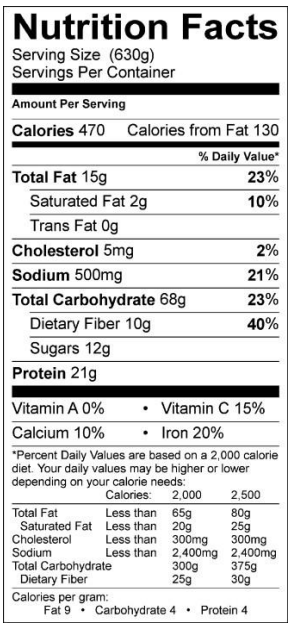
1. Combine split peas, water and bay leaf. Bring to a boil. Reduce heat and simmer until split peas puree, about 2 ½ to 3 hours. Add more water as necessary.
2. Heat pan over medium heat. Add bacon strips and cook bacon strips. Remove from pan and chop into pieces.
3. Heat oil in another pan over medium heat. Add onions, carrots, celery and garlic. Sauté

until onions are transparent.

4. Add bacon, vegetable mixture, parsley, sage, salt and pepper to split peas. Stir to combine. Simmer for another ½ hour.
5. Remove soup from heat. Allow to cool slightly. Puree with immersion blender until smooth.
6. Portion out soup.
7. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (630g)
Servings Per Container

Amount Per Serving

Calories 470	Calories from Fat 130
	% Daily Value*
Total Fat 15g	**23%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 500mg	**21%**
Total Carbohydrate 68g	**23%**
Dietary Fiber 10g	**40%**
Sugars 12g	
Protein 21g	

Vitamin A 0% • Vitamin C 15%
Calcium 10% • Iron 20%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HEARTY LENTIL SOUP

Yield: 5 servings

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 375 grams lentils, dry
- 120 grams back bacon, chopped
- 1 tablespoon canola oil
- ½ cup onions, finely chopped
- ½ cup carrots, finely chopped
- 1 tablespoon garlic, minced
- 3 cups low-sodium vegetable broth
- 1 ½ cups water
- 1 796ml can diced tomatoes
- ½ cup fresh parsley, chopped
- 1 teaspoon oregano
- fresh ground black pepper to taste
- ¼ teaspoon salt
- 5 whole wheat buns
- 5 teaspoons margarine

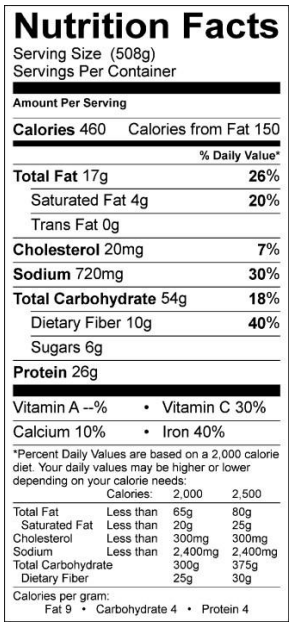
Directions:

1. Cook lentils according to pulse cooking instructions. Drain.
2. Heat a pot over medium-high heat. Add bacon. Sauté for about 3 minutes, stirring often.
3. Add oil, onions, carrots and garlic. Sauté until vegetables are tender but not browned, about 5 to 8 minutes.
4. Add lentils, vegetable broth, water, tomatoes, parsley, oregano, salt and pepper. Bring to a boil. Reduce heat to low and simmer until heated through.

5. Portion out soup.
6. Freeze.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (508g)
Servings Per Container

Amount Per Serving

Calories 460	Calories from Fat 150
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 4g	**20%**
Trans Fat 0g	
Cholesterol 20mg	**7%**
Sodium 720mg	**30%**
Total Carbohydrate 54g	**18%**
Dietary Fiber 10g	**40%**
Sugars 6g	
Protein 26g	

Vitamin A --% • Vitamin C 30%

Calcium 10% • Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

WINTER VEGETABLE SOUP

Yield: 5 servings

Preparation time: 30 minutes **Cook time:** 40 minutes **Total time:** 1 hour and 10 minutes

Ingredients:

- 375 grams lentils, dry
- 2 tablespoons canola oil
- 1 large onion, sliced thinly
- 2 large carrots, grated
- 2 celery stalks, sliced
- ½ head of cabbage, shredded
- 2 tablespoons garlic, minced
- 2 tablespoons tomato paste
- 2 bay leaves
- 1 container dill seed
- black pepper to taste
- 5 cups low-sodium vegetable broth
- 3 cups water
- ¼ cup nonfat yogurt, plain
- ¼ teaspoon salt
- 5 whole wheat buns
- 10 teaspoons margarine

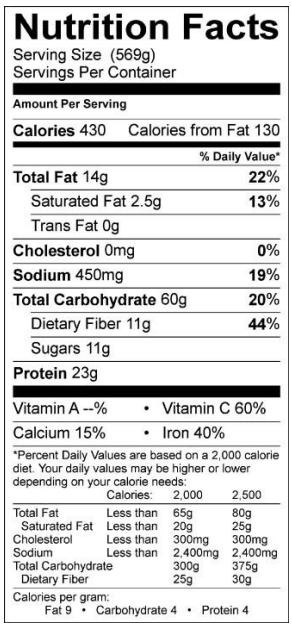
Directions:

1. Heat oil in a pot over medium heat. Add onion, carrots, celery and cabbage. Sauté for about 20 minutes, stirring often.

2. Raise heat to medium-high. Add garlic, tomato paste, bay leaves and dill seed. Sauté for another 1 to 2 minutes.
3. Add lentils, vegetable broth and water. Bring to a boil. Reduce heat to low, cover and simmer until lentils are cooked and vegetables are tender.
4. Add yogurt and salt. Mix well.
5. Portion out soup. Remember to remove bay leaves.
6. Freeze.

Serving Instructions:

Serve with 1 bun and 2 teaspoon margarine.



Nutrition Facts

Serving Size (569g)
Servings Per Container

Amount Per Serving

Calories 430	Calories from Fat 130
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 450mg	**19%**
Total Carbohydrate 60g	**20%**
Dietary Fiber 11g	**44%**
Sugars 11g	
Protein 23g	

Vitamin A --% • Vitamin C 60%
Calcium 15% • Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

ZIPPY MOROCCAN SOUP

Yield: 6 servings

Preparation time: 20 minutes **Cook time:** 1 hour and 10 minutes **Total time:** 1 hour and 30 minutes

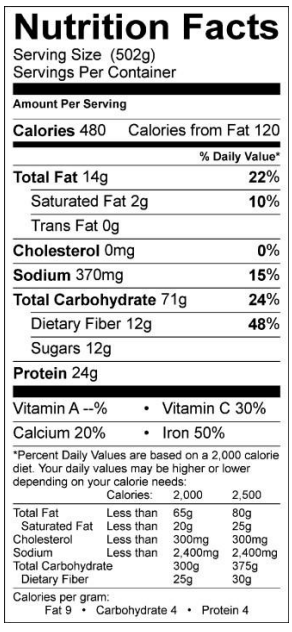
Ingredients:

- 1350 grams chickpeas, cooked and thawed
- 2 tablespoons olive oil
- 1 large onion, diced
- 3 tablespoons garlic, minced
- 3 teaspoons paprika
- 2 teaspoons cumin
- 2 teaspoons cinnamon
- ½ teaspoon cayenne pepper
- 4 cups low-sodium vegetable broth
- 1 796ml can diced tomatoes
- 1 tablespoon lemon juice
- ½ teaspoon salt
- fresh ground black pepper to taste
- ½ package spinach, frozen and thawed

Directions:

1. Heat oil in a pot over medium-high heat. Add onion and garlic. Sauté until onion is translucent, reducing heat if browning begins to occur.
2. Add spices. Sauté for another 1 to 2 minutes.
3. Add chickpeas, vegetable broth, tomatoes, lemon juice, salt and pepper. Add water until chickpeas are just covered with liquid if necessary.

4. Bring to a boil. Reduce heat to low and simmer until chickpeas are tender, about 45 minutes. Add more water if necessary.
5. Crush some of the chickpeas against the side of the pot using the back of a spoon.
6. Remove from heat. Stir in spinach. Allow to heat through until wilted, about 2 to 5 minutes.
7. Portion out soup.
8. Freeze.



Nutrition Facts

Serving Size (502g)
Servings Per Container

Amount Per Serving

Calories 480	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 370mg	**15%**
Total Carbohydrate 71g	**24%**
Dietary Fiber 12g	**48%**
Sugars 12g	
Protein 24g	

Vitamin A --%	• Vitamin C 30%
Calcium 20%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Salads

LENTIL QUINOA SALAD

Yield: 1 servings

Preparation time: 10 minutes **Cook time:** 20 minutes **Total time:** 30 minutes

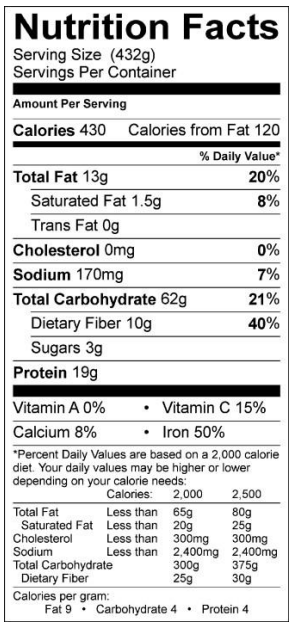
Ingredients:

- 150 grams lentils, cooked and thawed
- ¼ cup quinoa, dry
- ½ cup cold water
- ¼ teaspoon Dijon mustard
- ½ tablespoon red wine vinegar
- 1 tablespoon olive oil
- ½ teaspoon garlic powder
- ½ tablespoon lime juice
- ½ teaspoon lime zest
- ¼ teaspoon salt
- black pepper to taste
- 4 green onions, chopped
- 1 tablespoon fresh cilantro, chopped

Directions:

1. Combine water and quinoa in a pot. Bring to a boil. Reduce heat and simmer until cooked.
2. Whisk mustard and vinegar together in a small bowl. Drizzle in olive oil and whisk to emulsify.
3. Add garlic powder, lime juice, lime zest, salt and pepper, and whisk to combine.
4. Combine lentils, quinoa, green onions and cilantro in a large bowl.
5. Pour dressing onto salad and toss to coat.
6. Portion out salad.

7. Refrigerate.



Nutrition Facts

Serving Size (432g)
Servings Per Container

Amount Per Serving

Calories 430	Calories from Fat 120
	% Daily Value*
Total Fat 13g	**20%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 170mg	**7%**
Total Carbohydrate 62g	**21%**
Dietary Fiber 10g	**40%**
Sugars 3g	
Protein 19g	

Vitamin A 0%	• Vitamin C 15%
Calcium 8%	• Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

ORZO, LENTIL AND FETA SALAD

Yield: 4 servings

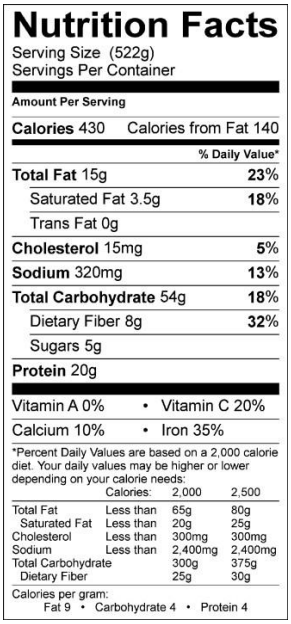
Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

Ingredients:

- 600 grams lentils, cooked and thawed
- ¾ cup whole wheat orzo pasta, dry
- 1 ½ tablespoon olive oil
- 1 ½ tablespoon canola oil
- 2 tablespoons red wine vinegar
- 1 teaspoon garlic, minced
- 1 cup cherry tomatoes, quartered
- ¼ red onion, diced
- 1/3 cup olives, pitted and chopped
- ¼ cup fresh dill, chopped
- 1/3 cup light feta cheese, crumbled
- 1/8 teaspoon salt
- black pepper to taste

Directions:

1. Bring a pot of water to the boil. Add orzo. Cook until al dente and drain.
2. Whisk olive oil, canola oil, red wine vinegar, garlic, salt and pepper together in a small bowl.
3. Combine pasta, tomatoes, red onion, olives, dill and feta cheese in a large bowl.
4. Pour dressing onto salad and toss to coat.
5. Portion out salad.
6. Refrigerate.



Nutrition Facts

Serving Size (522g)
Servings Per Container

Amount Per Serving

Calories 430 Calories from Fat 140

	% Daily Value*
Total Fat 15g	**23**%
Saturated Fat 3.5g	**18**%
Trans Fat 0g	
Cholesterol 15mg	**5**%
Sodium 320mg	**13**%
Total Carbohydrate 54g	**18**%
Dietary Fiber 8g	**32**%
Sugars 5g	
Protein 20g	

Vitamin A 0% • Vitamin C 20%

Calcium 10% • Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SANTA FE SALAD

Yield: 6 servings

Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

Ingredients:

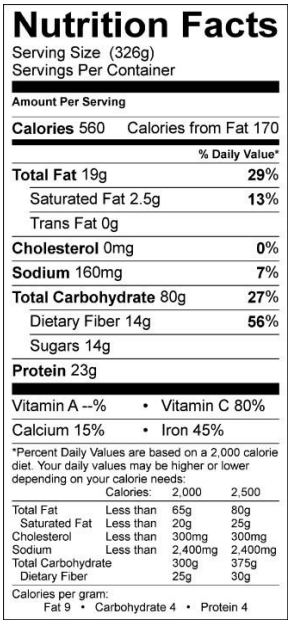
- 1350 grams chickpeas, cooked and thawed
- 1/3 cup olive oil
- 3 tablespoon lime juice
- ¼ cup cilantro, chopped
- 1 teaspoon cumin
- ¼ teaspoon salt
- black pepper to taste
- 1 red bell pepper, diced
- 1 ½ cup corn, frozen
- ½ cup red onion, chopped
- 1 jalapeno pepper, seeded and minced
- 1 ½ whole wheat pita bread

Directions:

1. Whisk olive oil and lime juice together in a small bowl.
2. Add cilantro, cumin, salt and pepper and whisk to combine.
3. Combine chickpeas, corn, onion, bell pepper and jalapeno in a large bowl.
4. Pour dressing onto salad and toss to coat.
5. Portion out salad.
6. Refrigerate.

Serving Instructions:

Serve with ¼ pita bread.



Nutrition Facts

Serving Size (326g)
Servings Per Container

Amount Per Serving

Calories 560 Calories from Fat 170

	% Daily Value*
Total Fat 19g	**29**%
Saturated Fat 2.5g	**13**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 160mg	**7**%
Total Carbohydrate 80g	**27**%
Dietary Fiber 14g	**56**%
Sugars 14g	
Protein 23g	

Vitamin A --% • Vitamin C 80%

Calcium 15% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

SUMMER BERRY SALAD

Yield: 1 serving

Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

Ingredients:

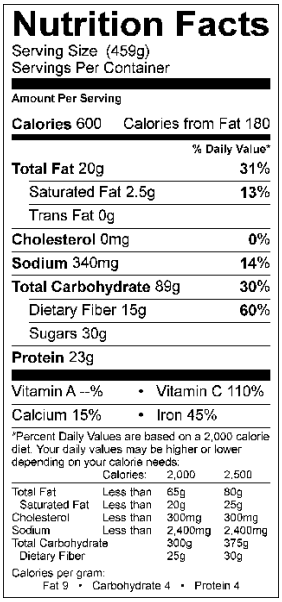
- 225 grams chickpeas, cooked and thawed
- 3 spears asparagus
- 1 cup baby spinach
- ¼ cup blueberries
- ½ cup strawberries, halved
- 2 tablespoons fresh mint, finely chopped
- 1 tablespoon extra-virgin olive oil
- 1 tablespoon apple cider vinegar
- 2 teaspoons honey
- pinch of salt

Directions:

1. Bring a pot of water to the boil. Cook asparagus until tender. Drain and chop into pieces. Allow to cool.
2. Combine asparagus, spinach, blueberries, strawberries and mint in a bowl. Mix well.
3. Whisk olive oil, vinegar, honey and salt together in a small bowl.
4. Portion out salad. Portion out dressing in a separate container.
5. Refrigerate.

Serving Instructions:

Serve with 1 container of dressing.



Nutrition Facts

Serving Size (459g)
Servings Per Container

Amount Per Serving

Calories 600 Calories from Fat 180

	% Daily Value*
Total Fat 20g	**31%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 340mg	**14%**
Total Carbohydrate 89g	**30%**
Dietary Fiber 15g	**60%**
Sugars 30g	
Protein 23g	

Vitamin A --% • Vitamin C 110%

Calcium 15% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

GREEK SALAD

Yield: 1 serving

Preparation time: 20 minutes **Cook time**: 10 minutes **Total time:** 30 minutes

Ingredients:

- 225 grams chickpeas, cooked and thawed
- ½ cup cucumber, chopped
- ½ cup cherry tomatoes, halved
- 1 tablespoon red onion, finely chopped
- 1 tablespoon light feta cheese, crumbled
- 1 ½ tablespoon light balsamic vinaigrette
- 1 teaspoon lemon juice
- 1 teaspoon dried oregano
- ¼ teaspoon salt
- black pepper, to taste

Directions:

1. Combine ingredients in a bowl. Mix until coated.
2. Portion out salad.
3. Refrigerate.

Nutrition Facts

Serving Size (377g)
Servings Per Container

Amount Per Serving

Calories 470	Calories from Fat 110
	% Daily Value*
Total Fat 13g	**20**%
Saturated Fat 2.5g	**13**%
Trans Fat 0g	
Cholesterol 5mg	**2**%
Sodium 390mg	**16**%
Total Carbohydrate 69g	**23**%
Dietary Fiber 11g	**44**%
Sugars 16g	
Protein 22g	

Vitamin A 0% • Vitamin C 25%
Calcium 15% • Iron 40%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HERBED POTATO SALAD

Yield: 1 serving

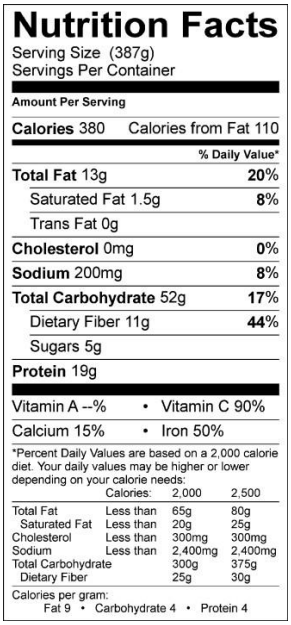
Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

- 150 grams lentils, cooked and thawed
- 1 small potato, peeled and diced
- 2 teaspoons olive oil
- 1 teaspoon garlic, minced
- ¼ teaspoon vegetable stock powder
- ¼ cup fresh parsley, packed
- ¼ cup fresh dill, packed
- ¼ cup green onion, chopped
- ¼ cup bell pepper, chopped
- 1 teaspoon cumin
- pinch of salt
- pinch of black pepper

Directions:

1. Bring a pot of water to the boil. Add potatoes. Simmer for 7 to 8 minutes. Drain.
2. Add olive oil, garlic, vegetable stock, parsley and dill to a food processor. Blend until a paste forms.
3. Combine lentils, potatoes, herb paste, green onion, bell pepper, cumin, salt and pepper in a bowl. Mix well.
4. Portion out salad.
5. Refrigerate.



Nutrition Facts

Serving Size (387g)
Servings Per Container

Amount Per Serving

Calories 380 Calories from Fat 110

	% Daily Value*
Total Fat 13g	**20**%
Saturated Fat 1.5g	**8**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 200mg	**8**%
Total Carbohydrate 52g	**17**%
Dietary Fiber 11g	**44**%
Sugars 5g	
Protein 19g	

Vitamin A --% • Vitamin C 90%

Calcium 15% • Iron 50%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LENTIL AND VEGGIE STUFFED PITA

Yield: 1 serving

Preparation time: 30 minutes **Cook time:** 15 minutes **Total time:** 45 minutes

Ingredients:

- 150 grams lentils, cooked and thawed
- ¼ cup bell pepper, chopped
- ¼ cup cucumber, chopped
- 1 ½ tablespoon light feta cheese, crumbled
- ¾ cup salad greens, chopped
- 1 tablespoon light balsamic vinaigrette
- 1 whole wheat pita bread, cut in half and opened

Directions:

1. Combine lentils, bell pepper, cucumber, and feta cheese in a bowl. Mix well.
2. Portion out salad. Top with salad greens. Portion out dressing into a separate container.
3. Refrigerate.

Serving Instructions:

Serve with 1 container of dressing and 1 pita bread.

Nutrition Facts

Serving Size 1 (350g)
Servings Per Container 1

Amount Per Serving	
Calories 485	Calories from Fat 37
	% Daily Values*
Total Fat 4g	**6%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 6mg	**2%**
Sodium 606mg	**25%**
Total Carbohydrate 84g	**28%**
Dietary Fiber 21g	**84%**
Sugars 7g	
Protein 26g	**52%**

*Percent Daily Values are based on a 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs.

	Calories	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2400mg	2400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

ORIENTAL PEA SALAD

Yield: 1 serving

Preparation time: 30 minutes **Cook time:** 30 minutes **Total time:** 1 hour

Ingredients:

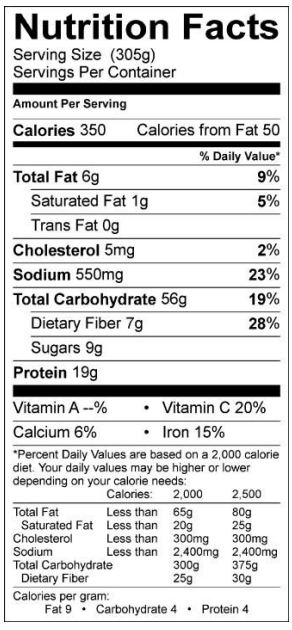
- 183 grams split peas, cooked and thawed
- ⅛ cup brown rice, dry
- ¼ cup water
- 1 small carrot, grated
- 1 stalk celery, sliced
- ¼ cup red bell pepper, diced
- 1 tablespoon green onion, sliced
- 1 teaspoon garlic, minced
- 1 teaspoon ginger, minced
- 2 tablespoons light Italian salad dressing
- ½ teaspoon sesame oil
- pinch of salt
- 1 ½ tablespoon. chow mein noodles, dry

Directions:

1. Combine brown rice and water in a pot. Bring to a boil. Reduce heat and simmer until cooked.
2. Combine carrot, celery, bell pepper, green onion, garlic and ginger in a bowl. Mix well.
3. Whisk salad dressing, sesame oil and salt together in another bowl.
4. Portion out salad. Portion out dressing and chow mein noodles into separate containers.
5. Refrigerate.

Serving Instructions:

Serve with 1 container of dressing and 1 container of chow mein noodles.



Nutrition Facts

Serving Size (305g)
Servings Per Container

Amount Per Serving

Calories 350	Calories from Fat 50
	% Daily Value*
Total Fat 6g	**9%**
Saturated Fat 1g	**5%**
Trans Fat 0g	
Cholesterol 5mg	**2%**
Sodium 550mg	**23%**
Total Carbohydrate 56g	**19%**
Dietary Fiber 7g	**28%**
Sugars 9g	
Protein 19g	

Vitamin A --%	• Vitamin C 20%
Calcium 6%	• Iron 15%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories:	2,000	2,500
Total Fat	Less than		65g	80g
Saturated Fat	Less than		20g	25g
Cholesterol	Less than		300mg	300mg
Sodium	Less than		2,400mg	2,400mg
Total Carbohydrate			300g	375g
Dietary Fiber			25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

TOMATO AND SWEET CORN SALAD

Yield: 1 serving

Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

Ingredients:

- 150 grams lentils, cooked and thawed
- ½ cup tomato, chopped
- ¼ cup cucumber, chopped
- ⅓ cup corn, frozen
- 1 tablespoon red onion, diced
- ¼ cup fresh parsley, finely chopped
- 2 tablespoons light cheddar cheese, shredded
- 2 tablespoons light balsamic vinaigrette
- 1 cup salad greens

Directions:

1. Combine lentils tomato, cucumber, corn, red onion, parsley and cheddar cheese in a bowl. Mix well.
2. Portion out salad. Top with salad greens. Portion out dressing into separate containers.
3. Refrigerate.

Serving Instructions:

Serve with 1 container of dressing.

Nutrition Facts

Serving Size (333g)
Servings Per Container

Amount Per Serving

Calories 410	Calories from Fat 150
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 5g	**25%**
Trans Fat 0g	
Cholesterol 15mg	**5%**
Sodium 260mg	**11%**
Total Carbohydrate 49g	**16%**
Dietary Fiber 9g	**36%**
Sugars 8g	
Protein 20g	

Vitamin A --%	• Vitamin C 30%
Calcium 15%	• Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

CARROT RAISIN SALAD

Yield: 5 servings

Preparation time: 20 minute **Cook time:** 15 minutes **Total time:** 35 minutes

Ingredients:

- 750 grams lentils, cooked and thawed
- 2 tablespoons olive oil
- 1 medium onion, sliced thinly
- 5 large carrots, peeled and sliced into coins
- 1 teaspoon cumin
- 1 teaspoon chili powder
- 1 teaspoon paprika
- 1 teaspoon red chili pepper flakes
- 1 teaspoon caraway seeds
- 1 teaspoon ground thyme
- ½ teaspoon salt
- ¾ cup raisins
- ½ cup part-skim mozzarella cheese, shredded
- 5 whole wheat buns
- 5 teaspoons margarine

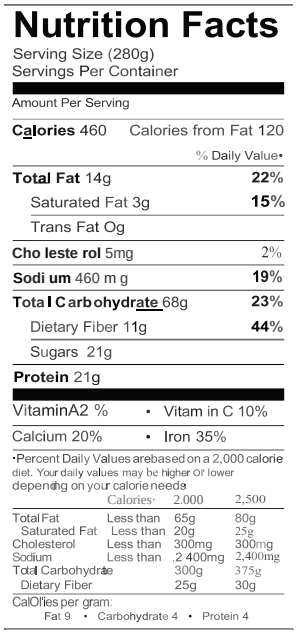
Directions:

1. Heat oil in a pan over medium heat. Add carrots. Cook until softened, about 30 minutes.
2. Add onions and raisins to the pan for the last 10 minutes.
3. Add spices. Cook for 1 minute more.
4. Portion out lentils. Portion out carrot mixture on top of lentils. Stir to combine.
5. Portion out mozzarella cheese on top of salad.

6. Refrigerate.

Serving Instructions:

Serve with 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (280g)
Servings Per Container

Amount Per Serving

Calories 460	Calories from Fat 120
	% Daily Value*
Total Fat 14g	**22%**
Saturated Fat 3g	**15%**
Trans Fat 0g	
Cholesterol 5mg	2%
Sodium 460 mg	**19%**
Total Carbohydrate 68g	**23%**
Dietary Fiber 11g	**44%**
Sugars 21g	
Protein 21g	

VitaminA2 %	• Vitamin C 10%
Calcium 20%	• Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

GRAPE WALNUT SALAD

Yield: 1 servings

Preparation time: 40 minutes **Cook time:** 20 minutes **Total time:** 1 hour

Ingredients:

- 150 grams lentils, cooked and thawed
- ½ cup green grapes, halved
- ¼ cup cucumber, chopped
- ¼ cup bell pepper, chopped
- 2 green onions, chopped
- 1 tablespoon walnuts, chopped
- 1 teaspoon extra-virgin olive oil
- 2 teaspoons fresh lemon juice
- ¼ teaspoon lemon zest
- pinch of salt
- black pepper to taste

Directions:

1. Combine lentils, grapes, cucumber, bell pepper and green onions in a large bowl.
2. Whisk olive oil into lemon juice in a small bowl. Add lemon zest, salt and pepper.
3. Portion out salad. Portion out dressing and walnuts into separate containers.
4. Refrigerate.

Serving Instructions:

Serve with 1 container of walnuts and 1 container of dressing.

Nutrition Facts

Serving Size (300g)
Servings Per Container

Amount Per Serving

Calories 440	Calories from Fat 140
	% Daily Value•
Total Fat 15g	**23%**
Saturated Fat 2g	**10%**
Trans Fat Og	
Cho leste rol 0mg	0%
Sodi um 360 m g	**15 %**
Tota l C arb ohydrate 62g	**21%**
Dietary Fiber 11g	**44%**
Sugars 14g	
Protein 19g	

Vitamin A O%	• Vitam in C 70 %
Calc ium 10 %	• Iron 35 %

•Percent Daily Values arebased on a 2,000 calorie diet. Your daily va lues may bc higher Or lower depending on your calorie needs:

	Calories·	2.000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	,2 400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

CalOries per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HONEY BROWN RICE SALAD

Yield: 1 servings

Preparation time: 20 minutes **Cook time**: 20 minutes **Total time:** 40 minutes

Ingredients:

- 150 grams lentils, cooked and thawed
- ¼ cup brown rice, dry
- ½ cup cold water
- ½ cup cherry tomatoes, halved
- ½ bunch green onions, chopped
- 1 tablespoon canola oil
- 1 tablespoon cider vinegar
- 1 ½ teaspoon honey
- ¼ teaspoon cinnamon
- pinch of salt

Directions:

1. Combine brown rice and water in a pot. Bring to a boil. Reduce heat and simmer until cooked.
2. Combine lentils, brown rice, tomatoes, and green onions in a bowl. Mix well.
3. Add oil, vinegar, honey, cinnamon and salt. Toss to coat.
4. Portion out salad.
5. Refrigerate.

Nutrition Facts

Serving Size (406g)
Servings Per Container

Amount Per Serving

Calories 450	Calories from Fat 140
	% Daily Value*
Total Fat 15g	**23%**
Saturated Fat 1.5g	**8%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 210mg	**9%**
Total Carbohydrate 65g	**22%**
Dietary Fiber 10g	**40%**
Sugars 12g	
Protein 17g	

Vitamin A --% • Vitamin C 30%
Calcium 6% • Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

		Calories: 2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

HUMMUS AND CRUDITÉS

Yield: 2 servings

Preparation time: 20 minutes **Cook time:** 20 minutes **Total time:** 40 minutes

Ingredients:

- 450 grams chickpeas, cooked and thawed
- ½ cup fresh parsley, packed
- 2 tablespoons garlic, minced
- 2 tablespoons olive oil
- 2 tablespoons fresh lemon juice
- ¼ cup water
- 2 teaspoons cumin
- ½ teaspoon red pepper flakes
- pinch of salt
- black pepper to taste
- ½ cup cherry tomatoes, whole
- ½ cup carrots, julienned
- ½ cup bell peppers, julienned
- ½ cup celery, julienned
- 1 whole wheat pita bread, halved

Directions:

1. Combine chickpeas, garlic, parsley, lemon juice, olive oil, water and spices in food processor. Process until smooth. Add water if necessary.
2. Portion out hummus. Portion out vegetables into separate bags.
3. Refrigerate.

Serving Instructions:

Nutrition Facts

Serving Size (392g)
Servings Per Container

Amount Per Serving

Calories 540	Calories from Fat 150
	% Daily Value*
Total Fat 17g	**26%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 400mg	**17%**
Total Carbohydrate 80g	**27%**
Dietary Fiber 13g	**52%**
Sugars 16g	
Protein 23g	

Vitamin A 2%	• Vitamin C 40%
Calcium 15%	• Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Serve with 1 bag of vegetables and ½ pita bread.

LENTIL CONFETTI SALAD

Yield: 1 servings

Preparation time: 35 minutes **Cook time:** 25 minutes **Total time:** 1 hour

Ingredients:

- 750 grams lentils, cooked and thawed
- 2 tablespoons brown rice, dry
- ¼ cup cold water
- ¼ cup cherry tomatoes, halved
- ¼ cup cucumber, diced
- ¼ bell pepper, diced
- ½ bunch green onions, diced
- 2 tablespoons fresh parsley, finely chopped
- 2 tablespoons Italian salad dressing
- ½ teaspoon extra-virgin olive oil
- pinch of salt
- ½ cup romaine lettuce
- 1 whole wheat buns
- 1 teaspoon margarine

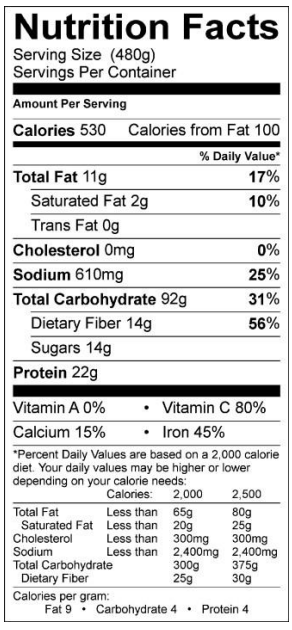
Directions:

1. Combine brown rice and water in a small pot. Bring to a boil. Reduce heat and simmer until cooked.
2. Combine lentils, rice, tomatoes, cucumber, bell pepper, green onions and parsley in a large bowl. Mix well.
3. Whisk salad dressing, olive oil and salt in a small bowl.
4. Portion out salad. Top with romaine lettuce. Portion out dressing into a separate container.

5. Refrigerate.

Serving Instructions:

Serve with 1 container of dressing, 1 bun and 1 teaspoon margarine.



Nutrition Facts

Serving Size (480g)
Servings Per Container

Amount Per Serving

Calories 530	Calories from Fat 100
	% Daily Value*
Total Fat 11g	**17**%
Saturated Fat 2g	**10**%
Trans Fat 0g	
Cholesterol 0mg	**0**%
Sodium 610mg	**25**%
Total Carbohydrate 92g	**31**%
Dietary Fiber 14g	**56**%
Sugars 14g	
Protein 22g	

Vitamin A 0% • Vitamin C 80%

Calcium 15% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

AUTUMN SALAD

Yield: 2 servings

Preparation time: 30 minutes **Cook time: -** **Total time:** 30 minutes

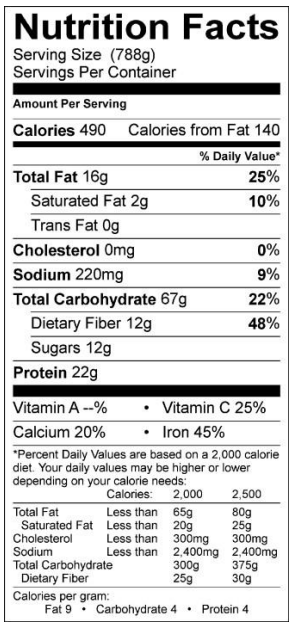
Ingredients:

- 450 grams chickpeas, cooked and thawed
- 2 shallots, thinly sliced
- 1 teaspoon garlic, minced
- 1 tablespoon extra-virgin olive oil
- 2 tablespoons red wine vinegar
- pinch of salt
- 100 grams carrot, grated
- 100 grams beet, peeled and grated
- ¼ cup dried cranberries
- 1 tablespoon extra-virgin olive oil
- ¼ cup fresh parsley, chopped
- fresh ground black pepper to taste

Directions:

1. Preheat oven to 450°F.
2. Combine shallot, garlic, olive oil, vinegar and salt in a large bowl. Set aside to mellow.
3. Combine chickpeas, carrot, beet, cranberries and olive oil on a baking sheet. Stir to coat. Roast in oven until vegetables are lightly browned.
4. Pour chickpea mixture into shallot mixture. Add parsley and pepper. Toss to coat.
5. Portion out salad.

6. Refrigerate.



Nutrition Facts

Serving Size (788g)
Servings Per Container

Amount Per Serving

Calories 490	Calories from Fat 140
	% Daily Value*
Total Fat 16g	**25%**
Saturated Fat 2g	**10%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 220mg	**9%**
Total Carbohydrate 67g	**22%**
Dietary Fiber 12g	**48%**
Sugars 12g	
Protein 22g	

Vitamin A --%	• Vitamin C 25%
Calcium 20%	• Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

LEMONY LENTIL SALAD

Yield: 1 servings

Preparation time: 20 minutes **Cook time:** - **Total time:** 20 minutes

Ingredients:

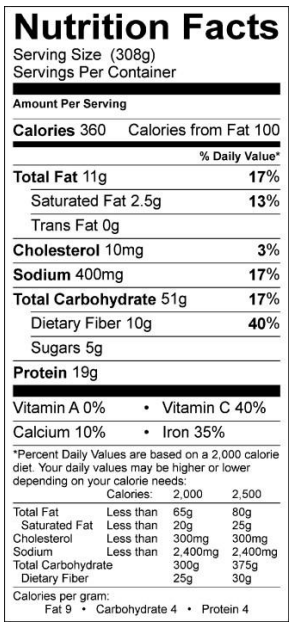
- 75 grams lentils, cooked and thawed
- 1 tablespoon fresh dill, chopped
- ¼ teaspoon Dijon mustard
- 1 tablespoon fresh lemon juice
- pinch of salt
- fresh ground black pepper to taste
- ½ tablespoon olive oil
- ¼ cup red bell pepper, diced
- ¼ cup cucumber, diced
- 1 tablespoon red onion, minced
- 1 ½ tablespoon feta cheese, crumbled
- 1 cup romaine lettuce, shredded
- 1 whole wheat pita bread

Directions:

1. Combine dill, mustard, lemon juice, salt and pepper in a large bowl. Gradually whisk in olive oil.
2. Add lentils, bell pepper, cucumber, onion, and feta cheese. Toss to coat.
3. Portion out salad. Portion out lettuce on top of salad.
4. Refrigerate.

Serving Instructions:

Serve with 1 pita bread.



Nutrition Facts

Serving Size (308g)
Servings Per Container

Amount Per Serving

Calories 360	Calories from Fat 100
	% Daily Value*
Total Fat 11g	**17%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 10mg	**3%**
Sodium 400mg	**17%**
Total Carbohydrate 51g	**17%**
Dietary Fiber 10g	**40%**
Sugars 5g	
Protein 19g	

Vitamin A 0% • Vitamin C 40%

Calcium 10% • Iron 35%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

TOASTED CHICKPEA SALAD

Yield: 2 servings

Preparation time: 15 minutes **Cook time:** 15 minutes **Total time:** 30 minutes

Ingredients:

- 450 grams chickpeas, cooked and thawed
- 2 tablespoons ground cardamom
- ¼ cup green olives, drained
- 1 tablespoon red wine vinegar
- 2 tablespoons extra-virgin olive oil
- ½ cup grapes, halved or quartered
- pinch of salt
- fresh ground black pepper to taste
- 4 cups lettuce, chopped

Directions:

1. Heat a large pan over medium heat. Add chickpeas. Cook until toasted, about 12 to 15 minutes. Add ground cardamom while cooking.
2. Combine olives and vinegar in food processor. Process until smooth. Stream in olive oil while processing.
3. Combine chickpeas, olive dressing, grapes, salt and pepper in a large bowl. Toss to coat.
4. Portion out salad. Portion out lettuce on top of salad.
5. Refrigerate.

Nutrition Facts

Serving Size (421g)
Servings Per Container

Amount Per Serving

Calories 510	Calories from Fat 170
	% Daily Value*
Total Fat 19g	**29%**
Saturated Fat 2.5g	**13%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 480mg	**20%**
Total Carbohydrate 68g	**23%**
Dietary Fiber 14g	**56%**
Sugars 12g	
Protein 22g	

Vitamin A --% • Vitamin C 60%
Calcium 15% • Iron 45%

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Saturated Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4