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Abstract

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy disorder in reproductive age women. The symptoms of this disorder are the androgen excess seen with anovulation/oligoovulation or morphologically ovarian cysts. The aim of the study was to assess the efficacy of public health nutrition intervention designed to enhance healthy eating and lifestyle modification among PCOS patients attended the obstetrics and gynecology clinic at the American University of Beirut Medical Centre (AUB-MC) in Beirut, Lebanon.

A prospective hospital based public health nutrition intervention was proposed in which 76 women with PCOS were recruited in the pilot study and 588 women were recruited in the scale-up intervention divided between PCOS and non-PCOS. During the scale up phase non-PCOS women were recruited to study the effect of the nutritional counseling on them as a way to compare the outcome with PCOS women. Recruited population were divided into 8 groups; group A: overweight/obese PCOS patient's intervention (received weight management program with nutritional guidelines). Group B: overweight/ obese PCOS controls (received the usual heath care by the gynecologist), Group C: lean PCOS controls (received the usual heath care by the gynecologist), Group D: lean PCOS intervention (received weight maintenance program with nutritional guidelines), Group E: overweight/obese non-PCOS patient's intervention (received weight management program with nutritional guidelines) ,Group F: overweight/ obese non-PCOS controls, Group G: lean non- PCOS intervention (received weight maintenance program with nutritional guidelines), Group H: lean non-PCOS controls

Data were collected using a pre-validated questionnaire to capture socio-demographic variables, nutritional status, and physical activity, psychological and medical status. Blood analysis was carried out to determine biochemical indices. Assessment of study indicators were carried out at baseline, after 3 and 6 months from inception of intervention (pilot as well scale up). Patients in intervention groups attended a 6 month tailored nutrition counseling/education program (2 sessions per month), to enhance their understanding of their dietary intake and assist them with

weight management, physical activity, healthy cooking, lifestyle, and food shopping.

Following a six months pilot study intervention results have shown that 7% weight loss was achieved in overweight/ obese intervention groups and weight maintenance in lean intervention groups(Group A,B,C and D). There was a significant reduction in waist (-4.2 cm (±5.6)) and hip circumference (-3.1cm (±3.5)) with $P < 0.001$. There was no significant biochemical markers change (fasting blood sugar, CRP, LDL-C,HDL-C,TG,total cholesterol, fasting insulin, total testosterone,Vit D), however there was an increase in physical activity (3.1 hours/week (±1.5)) , and decrease in anxiety and depression score (BDI-II and BAD-7); -0.8 (±0.8) and -0.7 (±0.7) with P<0,001 compared to interventions.

Following six months scale up intervention, the results have shown a weight reduction among overweight/obese PCOS women (group A) who lost, on average, 8.2 kg (P=0.001). Whilst non-PCOS women lost, on average 11.6 kg (P<0.001)(Group E). Controls gained weight (Group B, D F and H).

The biochemical, psychological and reproductive profile showed significant improvements among PCOS women (P<0.001). Pregnancy rate increased to 70% among women trying to conceive.

The results of this study have shown this intervention to be effective in Lebanese women with PCOS, decreasing their initial body weight by 5%- 10% and improving their reproductive, metabolic and endocrine profiles. This suggests the need for a nutritional intervention (nutritional guidelines) for women diagnosed with PCOS patients as a first line treatment. The study results support the effectiveness of lifestyle modification diet for PCOS women.

Keywords: Polycystic ovary syndrome; Obesity; Lean; Physical activity; Weight management; Obesity; Lean; Physical activity; Weight management

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Author's declaration

I hereby declare that I am the sole author of this thesis and that all the materials contained in it are my work in collaboration with the gynecologists at the Department of Obstetrics and Gynecology at the American University of Beirut medical Center and was carried out in accordance with the Guidelines and Regulations of the University of Westminster.

The data presented in this thesis was obtained in a research carried out by The University of Westminster, London, United Kingdom in collaboration with the American University of Beirut Medical Centre, Beirut, Lebanon. I played a major role in the preparation and implementation of the study, and the data analysis and interpretation are completely by own work.

I am aware of and understand the university's policy on plagiarism and I confirm that this thesis is my own work, expect where indicated by referencing, and the work presented in it has not been submitted in support of another degree or qualification from this or any other university or institute of learning.

I declare that this is a true copy of my thesis, submitted for examination.
Any views expressed in this work are those of the author and in no way represent those of the University of Westminster.

FULL NAME: Caroline Hamadi

Signed: Date: March 18, 2017

Abbreviations

AE-PCOS	Androgen Excess and PCOS Society
AI	Aromatase inhibitor
AUBMC	American University of Beirut Medical Centre
ASRM	American Society for Reproductive Medicine
AE-PCOS	Androgen Excess and Polycystic Ovary Syndrome
AMH	Anti- Mullerian hormone
AUC	Area under curve
BMR	Basal metabolic rate
BMI	Body mass index
BDI-II	Beck depression inventory
CAM	Complementary and alternative medicines
CC	Clomiphene Citrate
COC	Cumulus-oocyte complex
CHO	Carbohydrate
CPA	Cyproterone acetate
CRP	C-reactive protein
E2	Estradiol
ESHRE	European Society of Human Reproduction and Embryology
FAI	Femoro-AcetabularImp ingement
FSH	Follicle stimulating hormone
FBS	Fasting blood sugar
FFQ	Food frequency questionnaire
FDA	Food and drugs association
GI	Glycemic index
DAD-7	Generalized anxiety disorder
GnRH	Gonadotropin releasing hormone
hCG	Human chorionic gonadotropin
HDL	High Density lipoprotein
IVF	In vitro fertilization
IVM	In vitro maturation

IMS	Indian Migration study
IRB	Institutional review board
ISD	Insulin –sensitizing drugs
LH	Luteinizing hormone
LDLD	Low density lipoprotein
TZD	Thiazolidinediones
MUFA	Monounsaturated fatty acids
NIH	National institute of health
OHSS	Ovulation hyperstimulation syndrome
OCP	Oral contraceptive
PA	Physical activity
PCOS	Polycystic ovary syndrome
PCO	Polycystic ovaries
PDR	Preconception Dietary risk score
PUFA	Polyunsaturated fatty acids
rFSH	Recombinant follicle stimulating hormone
RCT	Randomized control trials
SERM	Selective estrogen receptor modulator
SHBG	Sex hormone binding globulin
US	United States of America
VLDL	Very low density lipoprotein
WHW	Women's health weekly

List of publications

Journals

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Chapter 1
Introduction

Background to PCOS

Polycystic Ovarian (ovary) syndrome or PCOS first described in 1935 by Stein and Leventhal, is the most common endocrinopathy affecting 5-10% of women in their reproductive age worldwide (Pfeifer *et al*.,2009). Seventy-eight years were enough to dramatically change our understanding of PCOS pathophysiology. PCOS is defined by imbalanced hormones and irregular menses causing fertility problems. It has many short-term and long-term health implications varying from oligomenorrhea, amenorrhea, infertility, insulin resistance, diabetes mellitus and cardiovascular disease, to increased risk of endometrial cancer and excessive body hair (Pfeifer *et al.,*2009). Up till 1990, PCOS was a poorly understood condition when it was fully defined in the National Institutes of Health (NIH) conference of PCOS. The conference offered standard international criteria to serve the researchers and clinicians to more understand and manage this syndrome. It was followed by a consensus workshop in 2003 in Rotterdam in the Netherlands where a new diagnostic criteria was created, the Rotterdam Criteria that states that at least two of the following three features must exist: 1-oligomenorrhea/amenorrhea, 2-clinical and biochemical evidence of hyperandrogenemia, 3-polycystic ovaries documented on ultrasonography. The Androgen Excess (AE) and PCOS Society proposed the AE-PCOS Criteria in 2006. PCOS has been diagnosed in female as young as 6 years and in fact some girls are possibly born with this syndrome. The fact that PCOS is found more at adulthood is possibly due to the difficulty in diagnosing the disorder before puberty, a time at which seeking help for irregular menses or presence of acne do not take place. PCOS is affecting women in their reproductive age and thus causing these women to be unable to get pregnant. This has a major cost on the personal and social level, women with PCOS have lower chances of getting pregnant if left undiagnosed and thus untreated. The costs for this is being unable to have children, increasing the cost of medical treatments and thus putting the health status as barrier to marriage in few cultural norms.

Diagnostic Criteria for Polycystic Ovary Syndrome

The polycystic ovary syndrome (PCOS) was first described in 1935 by Stein and Leventhal as a combination of hyperandrogenism and oligo- ovulation. Over time,

several hypotheses have been proposed regarding the characterization and description of the disease, and in this regard three models are the most accepted by clinicians (Table 2)(De Paolo, 2012; Housman and Reynolds, 2014; Livadas and Diamanti-Kandarakis, 2012; Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2014).

The Rotterdam ESHRE/ASRM- Sponsored PCOS Consensus in 2003, the attendees of the workshops built the diagnostic criteria base on the NIH model in 1990 and the androgen excess and polycystic ovary syndrome society recommendation. The NIH model was revised and modified by a group sponsored by the European Society of Human Reproduction and Embryology (ESHRE) and The American Society for Reproductive Medicine (ASRM). In accordance, the PCOS does not represent a single entity, but rather occurs on a spectrum; for that reason, the inclusion criteria were widened in order to deflect missing certain patients. Consequently, the new diagnostic criteria include two of the following three symptoms: oligo – or anovulation, clinical or biochemical signs of hyperandrogenism, and polycystic appearing ovaries (PCO) on imaging, giving that other similar disease are excluded (Rotterdam consensus workshop,2003).

The assessment of the ovarian stromal volume as a ratio of the stromal area to total area of the ovary (S/A ratio) was also proposed as a parameter to be considered. However, even though this factor allowed for a better differentiation between females with and without PCOS, it was not adopted by any of the existing diagnostic criteria (Fulghesu e al.,2001) .

The anti- Mullerian hormone (AMH) was recently introduced as a new parameter that can replace the ultra-sonographic characterization of PCOS, and this will ensure a 97.1% specificity and 94.6% sensitivity if the Rotterdam model is used and a 97.2% specificity and 95.5% sensitivity using the NIH model (Eirlertsen *et al*.,2012) .

The diagnosis of hyperandrogenism in women is usually difficult to assess at the clinical as well as the biochemical assessment levels. Assays to measure androgen levels were designed for males and calibrations to develop commercial ones for females were not done. Furthermore, the diagnosis of clinical hyperandrogenism is subjective and dependent on the examiner.

Evidence of PCO morphology demonstrated by ultrasonography are not fully accepted in the diagnosis of PCOS. The reason for this is that PCO morphology is not only present in patients with PCOS, but rather it is found in 20-30% of the normal population, and thus it is considered as a manifestation of PCOS only when the other criteria are also present (Michelmore *et al* .,1999) .

PCOS is characterized by clinical or biochemical hyperandrogenism, chronic anovulation, and polycystic ovarian morphology seen by ultrasound (Refer table 2). Other symptoms might also be present but they do not necessarily indicate this disease; these include obesity, luteinizing-hormone elevations, and insulin resistance) (De Paolo, 2012; Newell-Price, 2014; Sedighi *et al*., 2014).

Table 1 Prevalence of PCOS according to NIH, Rotterdam criteria and AAE—PCOS criteria

Population	**NIH criteria**	**Rotterdam criteria**	**AE-PCOS criteria**
728 Australian women	8.7%	17.8%	12.0%
820 Iranian women	7%	15.2%	7.92%
929 Iranian women	7.1%	14.6%	11.7%
392 Turkish women	6.1%	19.9%	15.3%

Table 2 PCOS phenotypes based on the 2003 Rotterdam criteria (Housman and Reynolds,2003).

Phenotype	**Clinical features**
Severe PCOS	Irregular menses, polycystic ovaries, hyperandrogenemia, and hyperinsulinemia
Hyperandrogenism and chronic anovulation	Irregular menses, normal ovaries, hyperandrogenemia, and hyperinsulinemia

Ovulatory PCOS	Normal menses, polycystic ovaries, hyperandrogenemia, and hyperinsulinemia
Mild PCOS	Irregular menses, polycystic ovaries, mildly raised androgen levels, and normal insulin levels

Prevalence and Epidemiology of PCOS

PCOS is the most common endocrine disease affecting about 14.6%of females in the reproductive age (Tehrani *et al*.,2011). The prevalence of PCOS presents ethnic and regional variations and is mostly dependent on the diagnostic criteria adopted. To illustrate the truth of this, a retrospective birth cohort in Australia presented a prevalence of 8.7% using NIH criteria, 17.8% using Rotterdam criteria, and 12% using AES criteria. PCOS prevalence is higher among Mexican than American females indicating the importance of lifestyle in addition to the ethnic diversity in the occurrence of PCOS (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, 2004).

The prevalence of PCOS has been determined among women in different countries: Australia 8.7%, Spain, 6.5%, Greek Island of Lesbos 6.7%, the southeastern United States 4%,Sweden 4.8%, China 2.2%, Mexico 6%, and Pakistan 17.6% (Asuncion *et al*., 2000; Chen *et al*., 2008; Diamanti-Kandarakis at al., 1999; Fauzia *et al*., 2007; Knochenhauer *et al*., 1998, Lindholm *et al*., 2008; March *et al*., 2010; Moran *et al*., 2010). The difference in prevalence is due to the different diagnostic criteria used to study the prevalence. Each study used different criteria (Refer to table 1).

Prevalence estimates for PCOS, as defined by the NIH criteria, indicate that PCOS affects 4-8% (Sirmans and Pate, 2013). However, given the broader definition described in the Rotterdam criteria, the prevalence of PCOS has subsequently been noted to range from 15-18% (Housman and Reynolds, 2014).

Not surprisingly, women with menstrual abnormalities appear to have higher prevalence rate ranging from 37-90%. PCOS prevalence is also increased in the presence of certain diseases where it is shown to be greater in women with epilepsy than in women without epilepsy (Zacur, 2003).

Obesity in PCOS

Obesity can promote the occurrence of PCOS in predisposed women. Moran *et al*., in 2015 have shown that PCOS appeared after weight gain in some women. Obesity aggravates insulin resistance and thus insulin resistance cause excess androgens productions in the body. Further long term studies are needed to evaluate these correlations and the effect of weight management (weight loss and weight maintenance) on the treatment of PCOS.

The prevalence of obesity among PCOS patients (42-74 %) is higher than the general population, although it is not a feature of PCOS (Sedighi *et al*., 2014). The clinical presentation of the disorder is aggravated (reproductive and metabolic effects) by obesity (Sedighi *et al*., 2014). Obese PCOS women have hyperinsulinemia than normal weight PCOS women (Sedighi *et al*., 2014).Obesity aggravates the intrinsic insulin resistance among PCOS women. Furthermore, hyperinsulinemia can worsen hyperandrogenism and this due to the gonadotropin regulation and steroidogenesis (Sedighi *et al*., 2014). Hyperinsulinemia affects the sex hormone –binding globulin synthesis and cause excess androgen production (Pagan *et al*., 2003).

The prevalence of obese PCOS women with BMI > 30kg/m ^{2} is 40-60% (Hoeger, 2006). The prevalence of obesity and its clinical manifestations among PCOS women suggests the need for a weight management intervention as a lifestyle modification to significantly improve the metabolic effects. Weight loss among PCOS women reduces hyperandrogenism and thus improve ovulation (Hoeger *et al*., 2006) . Insulin resistance is reduced by physical activity regardless of weight reduction. The present literature didn't define clearly the role of physical activity on insulin resistance in PCOS women and the effect of physical activity on the clinical presentation of PCOS.

Scale of the problem and epidemiology of the disease

PCOS is a broad spectrum disorder that represents a serious health concern for women across their life span(Sedighi *et al*., 2014).Its effects go beyond the reproductive (menses disorders, failure to ovulate, late menopause), metabolic

(dyslipidemia, hypertension), physical (central obesity, acne, hirsutism, hair loss and baldness) and psychological consequences (depression, stress and anxiety); it is a leading cause of anovulatory infertility and a risk factor for endometrial dysfunction and uterine cancer. It also increases pregnancy complications and gestational diabetes risk (Lin and Lujan, 2014; Moran *et al*., 2015; Sedighi *et al*., 2014). Importantly, PCOS patients since their puberty are considered to have high reproductive and cardio metabolic risks. Women with PCOS are 2-4 times more likely to have metabolic syndrome than are women without PCOS. The metabolic syndrome among PCOS patients ranges between 40-50% worldwide (Eckel, 2012). These patients are four times more prone to develop type 2 diabetes mellitus at younger age, and have increased cardiovascular risk factors (Moran *et al*., 2015). They are more prone to gain weight which further exacerbates their condition. All of the mentioned facts highlight the need to target these women for prevention and management (Moran *et al*., 2015). The lack of knowledge about lifestyle modifications using nutritional guidelines is enlarging the scale of the problem causing obesity to increase among the PCOS population.

It is estimated that 105 million women suffer from PCOS universally (Sedighi *et al*., 2014). This syndrome occurs in a striking proportion of women who are in their fertile years, ranging from 6-18% worldwide (Lin and Lujan, 2014). The lifestyle modification focus on PCOS population can exert beneficial effects.

Prevalence of PCOS at regional and national levels

The prevalence of PCOS is well studied in the European and American populations. Despite of the few studies done on the Middle East women, there are no published data regarding the prevalence of this syndrome among the Lebanese women (Musmar *et al*.,2013 ; Al Khaduri *et al*., 2014 ; Hussein and Alalaf, 2013). As listed in appendix 1, two Iranian studies found PCOS prevalence to be 7.1% and 7% (Mehrabani *et al*., 2011; Tehrani *et al*.,2011).Regarding the Arab world, out of 137 study patients selected from female university students at An-Najah National University-Palestine, 10 students met the NIH criteria for diagnosis of PCOS making the prevalence rate to be 7.3% (Musmar *et al*., 2013In Lebanon, a descriptive review of cases of 160 females with hirsutism presenting to an

endocrine clinic showed that 72.6% are found to have PCOS (Zreik and Nasrallah, 2014). Till now, there is no identification study assessing the prevalence of PCOS among pure Lebanese women. The prevalence of PCOS in Lebanon is still scarce and presents a great challenge. Women in Lebanon does not present to the obstetrics and gynecology clinics unless they are experiencing irregularity of menses or infertility which is one of the very private cultural norms in our society. Lebanon shares common ethnic and socio-demographic factors with different Arab world populations. This could make us estimate that PCOS prevalence might be similar to the other studied Arab populations.

PCOS as a Public Health Problem

PCOS is an endocrine and reproductive syndrome which is of a clinical and public health importance. The NIH stated that worldwide the public is largely unaware of this condition and health care providers do not seem to fully understand it (De Paolo, 2012).

As mentioned earlier, PCOS patients have a likelihood of developing serious reproductive and metabolic consequences. This imposes a wide range of symptoms and life-long implications on women's health that subject them to undesirable mental health outcomes and impairment in quality of life at the emotional, physical, and social levels (Ghazeeri *et al*., 2013).

Evidence indicating a genetic influence includes a well-documented familial clustering of PCOS; an increased prevalence of its components, including hyperandrogenism and type 2 diabetes mellitus, in first-degree relatives of women with PCOS (Legro *et al*., 1998; Ehrmann *et al*., 2005). Genetic effect is estimated to explain approximately 65% of PCOS risk (Vink *et al*., 2006). In a Dutch twin study, the heritability of PCOS was estimated (Vink *et al*., 2006). Results demonstrate a large influence of genetic factors to the pathogenesis of PCOS and suggest that the syndrome's etiology is strongly heritable (Vink *et al*., 2006).

In Lebanese patients presenting with hirsutism, the family history was noted in 12.4% of the PCOS patients (Zreik and Nasrallah, 2014). This makes the syndrome to have probable implications on offspring and relatives (Baldani *et al*., 2013). Furthermore, this syndrome incurs heavy costs on healthcare systems in different

countries. For example, in 2004, the United States of America spent 4.36 billion dollars to treat PCOS and its complications. More than 40% of this cost was spent on infertility and menstrual period impairments and another 40% was spent on treatment and diabetes control (Sedighi *et al*., 2014).

Although in Lebanon there are no data regarding PCOS, it is a syndrome that necessitates public health awareness to control a lot of its preventable consequences on both the patient and the society.

Research aim and objectives

Aim

The aim of the study was to assess, design and implement the efficacy of public health nutrition intervention designed to enhance healthy eating and LSM among PCOS patients attending the obstetrics and gynecology clinic at the American University of Beirut Medical Centre (AUB-MC) in Beirut, Lebanon.

Objectives

The objectives of the study are:

i. To achieve and encourage a primary weight loss (from 5-10% of initial body weight) among overweight/ obese PCOS, and weight maintenance among lean PCOS patient.
ii. To design and optimize educational sessions tailored to PCOS patients in order to raise their nutritional awareness, enable informed healthy food choice and engage them in physical activity.
iii. To assess the rates of regularities of menses (examined by pregnancy onset and ovulation) in the participant groups and in matched control PCOS population receiving no intervention.
iv. To evaluate the efficacy of public health nutrition through the pre-intervention versus the post-intervention assessment employing the study indicators (positive deviation to healthy life style, adequate/balance dietary intake, healthy body weight, optimum physical activity, optimum blood pressure, improved biochemical indicators) via pilot/scale up intervention.

v. To develop and optimize the nutrition framework for people living with PCOS in Lebanon.

MPhil is presented through objective i, ii, and iii

PhD is presented through objective iv and v

Chapter 2

Literature review

2.0 Introduction

This chapter includes the literature review and current interventions and findings in the data as well as the nutritional intervention in PCOS. It shows and justifies how nutritional interventions improve some health concerns and reproductive outcomes among PCOS women and fill few gaps in the reproductive endocrinology area in Lebanon.

2.1 The Heritable Basis of PCOS

2.1.1. Familial Aggregation studies

Initially, studies have suggested an autosomal dominant mode of inheritance of PCOS with a relatively high prevalence rate (51- 66%) in the first degree relatives of a female presenting the disease. Accordingly, all of the symptoms, including the oligomenorrhea and hirsutism, were caused by a single gene (Carey *Et al.* .,1993). Moreover, the genome-wide association study (GWAS) on PCOS in a Han Chinese population identified variants mapping to three loci (2p16.3, 2p21 and 9q33.3) (Chen *Et al.*, 2011). A second GWAS in a larger cohort of Han Chinese PCOS patients discovered variants mapping to eight risk loci (2p16.3, 9q22.32, 11q22.1, 12q13.2, 12q14.3, 16q12.1, 19p13.3 and 20q13.2) for PCOS (Shi *et al*., 2012).Genetic approaches are rapidly uncovering new regions of the genome that appear to confer risk for PCOS. The strongest association was on chromosome 8q24.2, and other association signals were located at 4q35.2, 16p13.3, 4p12, 3q26.33, 9q21.32, 11p13 and 1p22. The strongest signal was located upstream of KHDRBS3 gene, which is associated with telomerase activity, and could drive PCOS and related phenotypes.

2.1.2The Candidate-Gene Era

Challenges of the Candidate Approach

The candidate approach was largely adopted in the study of PCOS; however, it was not very beneficial due to many factors. First, small to moderate cohorts were used in the studies where a single gene was investigated (Goodarzi *et al*.,2008). Consequently, false negative and false positive results cannot be prevented. Second, PCOS is associated with multiple genes each contributing to a small risk to

the disease, that is why a huge number of samples are needed for the study, and this was not available until recently (Goodarzi *et al*.,2011; Chua *et al*.,2012). The future treatment modalities are based on identifying the susceptible genes in PCOS drawing the pathophysiological pathways, and maybe preventing the disorder.

Epigenetics in PCOS

Epigenetics is the study of changes in an organism and is usually caused by modifications of the gene expression rather than alteration of the genetic code itself. Epigenetic changes can be influenced by many factors and its mechanisms include methylation, phosphorylation, acetylation (Muhonen *et al*.,2009).
PCOS was hypothesized to be caused by certain epigenetic changes leading to its pathogenesis (Li *et al*.,2008). The theory suggests that female fetuses exposed to increased androgen in utero will develop PCOS- like symptoms and might have permanent metabolic or reproductive abnormalities (Abbott *et al*.,2005).

2.2 Functional Underpinnings in Polycystic Ovaries

Androgens have been shown to cause alteration in the early folliculogenesis since a significant decrease in the follicle atresia was seen in follicles treated with androgens in ovo(Vendola et l.,1999; Qureshi *et al*.,2008). The primary follicles were the most affected since they are the ones with the highest amount of androgen receptors (AR) on their surface (Rice *et al*.,2007). Furthermore, the prenatal exposure to androgens has been associated with an adult PCOS phenotype (Forsdike *et al*.,2007)

2.2.1 Theca Dysfunction in PCOS

One important characteristic feature of PCOS is hyperandrogenism which is mainly of ovarian origin. According to the recent experiments, exaggerated steroidogenesis is present in the theca cells of PCOS, in addition to the increased responsiveness of these cells to LH (Gilling-Smith *et al*.,1997). This is due to the increased expression of the CYP17 gene that encodes for both the 17 alpha-hydroxylase and 17,20 lyase involved in the conversion of pregnenolone to 17 α-hydroxypregnenolone and then to DHEA, in addition to the increased expression of

the 3 ß HSD responsible for the conversion of DHEA to androstenedione and finally testosterone(Gilling-Smith *et al*.,1997)..

The hyperandrogenism is detected in females even before any ovarian change occurs implying the role of the androgens as a cause of PCOS and not a result (Gilling-Smith *et al*.,1997)..

To add to the intrinsic factors favoring the production of androgens, one can list both the LH and the hyperinsulinemia which are present in females with PCOS.

2.2.2 Ovulatory Dysfunction

Although numerous follicles are recruited in PCOS, their development stops once their size reaches 5-8 mm (Franks *et al* .,1985).The reason behind that dysfunction has been attributed to many factors one of them is the androgen overproduction. This androgen excess has been defined to be caused by an increase in the serum LH level resulting in premature luteinization (Franks *et al* .,1985). This hypothesis has been further supported by the fact that LH receptor mRNA in women with PCOS is overexpressed in granulosa cells (Franks *et al* .,1985).

Furthermore, abnormalities in the FSH secretions are another factor leading to ovulatory dysfunction since the selection and maturation of the follicle is FSH-dependent. Experiments have shown that FSH levels are lower in PCOS patients and granulosa cells have stronger binding and responsiveness to FSH (McNatty *et al*.,1982).

There is a strong relation between insulin resistance and anovulation; obese insulin-resistant females with PCOS started to have ovulatory cycles when they lost weight and they had improved insulin sensitivity parameters (Dunaif *et al*.,1997).

2.2.3 Energy Balance

During the division and the maturation of the follicles, energy derived from glucose through the glycolytic pathway should always be supplied through the follicular fluid which has a comparable glucose concentration to plasma. In PCOS, the insulin-mediated lactate accumulation is due to the weak glucose uptake and the reduction in the glycolytic pathway in the cells resulting in an impaired growth and development of granulosa cells (Oktem *et al*., 2008).

In that regard, metformin, the insulin- sensitizing drug used in the treatment of PCOS, was effective due to its role in increasing the glucose uptake and metabolism of the cells by increasing the amount of the GLUT-4 transporter in their plasma membrane (Tang *et al*., 2006).

2.3 The Role of the Adrenal Glands in the Hyperandrogenism Associated with Polycystic Ovarian Syndrome

Adrenal Androgens and PCOS

Till now, the source of the elevated androgens in PCOS patients is still unknown. Some studies have identified it as being the ovaries, others pretended it to be the adrenal glands, and finally some evidence supported the fact that both are contributing to the hyperandrogenism(Futterweit *et al*., 2006).

Both, the ovaries and the adrenal glands, contain certain steroid-producing tissues derived from the same embryonic origin; so the androgen production by the ovaries can affect the steroidogenesis in the adrenal glands, and the increased levels of androgens produced by the adrenal can cause increased steroidogenesis by the ovaries as well resulting in a subsequent increase in the LH (McGee *et al* ., 2012).

The Role of the Adrenal in Childhood and Subsequent Development of PCOS

PCOS after adolescence have been seen in children (<8 years) with the congenital adrenal hyperplasia (CAD) that is accompanied with excessive production of androgens. Studies have revealed that fetus or pubertal girls exposed to androgens might have subsequent alterations of the hypothalamo- pituitary- ovarian axis that is characteristic of PCO (Abbott *et al*., 2008). During this study obesity during adulthood wasn't observed as a factor of excess androgen production in order to make a correlation between obesity in adolescences and PCOS.

Children with premature adrenarche characterized by increased DHEA were also seen to develop PCOS subsequently. The increased DHEA might be caused by an increased activity of the enzyme CYP17A1 (P450c17 α) that is responsible for the 17 hydroxylation of pregnenolone and progesterone, and the deletion of C20 and C21 from the product to form DHEA and androstenedione due to its lyase activity(Abbott *et al*.,2009). Abnormalities in the lyase function of the enzyme cause

increased levels of DHEA and androstenedione as a result of the active conversion of the progesterone in the ovaries since the enzyme is active in the adrenal as well as the ovaries (Abbott *et al*., 2009).

Adult with PCOS and the Role of Adrenal Androgens

Once PCOS is diagnosed after the signs and symptoms are fully manifested, it would be very difficult to determine the source of the excess androgens. Certain researchers suggest that the excessively secreted androgens are converted to estrogen and then they act by feedback on the pituitary gland to increase LH; this is the case where the excess androgens originate from the adrenal. The other group assumes that the ovarian androgens block the hydroxylation of the 17 hydroxyprogesterone and the 11 deoxycortisol in the adrenal thus enhancing the channeling of the precursors into the androgen production; this how the ovaries can be the source of the androgen excess (Yoshida *et al*., 1978).

Kumar *et al*. have proved that the increased DHEA and especially DHEA-S are evidences supporting the adrenals being the origin of the hormonal excess in PCOS since DHEA-S is produced only by the zona reticularis of the adrenals while DHEA is produced by both, the ovaries and the adrenals (Kumar *et al*., 2005).

A more recent study has been done by Rosenfield (2011) showed that PCOS patients present typical and atypical phenotypes with ovarian or adrenal origin of the hyperandrogenism.

2.4 The Hypothalamus- Pituitary Axis in PCOS

Neuroendocrine Function in Normal Reproduction

The regulation of the menstrual cycle is accomplished by the hypothalamus and the pituitary through the GnRH, LH, and FSH, and the ovaries response to gonadotropins through ovulation and secretion of estradiol, progesterone, inhibin A, and inhibin B (Hall *et al*., 2012)

In a normal female, GnRH is secreted in a regulated pulsatile manner across the cycle to ensure repetitive cycles of the development of follicles.

To start a new cycle, the pulsatile LH-GnRH secretion increases from a frequency of 1 every 4h to 1 every 90 min early in the follicular phase. At the mid-follicular

phase, it increases to 1 every hour. Following the ovulation, GnRH secretion decreases back to 1 every 90 min and finally to 1 every 4h due to the increased level of circulating progesterone in the presence of high estrogen. Concerning FSH, the slow release of GnRH stimulates its synthesis, whereas the fast release of GnRH stimulates the secretion of LH.

What Causes the Neuroendocrine Abnormalities in PCOS

The Role of Androgens

Under the previously mentioned condition, the theca cells of the ovaries increase their production of androgen substrates. The co-presence of a low FSH level causes a deficiency in the aromatization of these androgens resulting in a milieu where the maturation of the follicles and the ovulation cannot occur.

The removal of the tumor cells producing the excess androgens, the wedge resection, or the ovarian drilling have proven to be effective in the restoration of the normal cycle (Dunaif *et al*.,1984).

The Potential Role of Altered Progesterone Negative Feedback

Progestin's use can transiently decrease the GnRH/LH pulse frequency and improve the LH/FSH ratio that is abnormal in PCOS patients. The progesterone binds to the PgRMC1 receptor present in GnRH neurons and exerts a negative feedback decreasing the LH secretion as a result of the reduction of the intracellular calcium. So in PCOS patients, the effect of progesterone on GnRH is reduced. Furthermore, the treatment with flutamide (antiandrogen) for four weeks restores the sensitivity to progesterone and the GnRH pulse becomes normal.

Consequently, one can say that the elevated levels of androgens in PCOS are the cause of the increased GnRH pulse frequency.

New evidences suggest that the decreased sensitivity to the progesterone is highly related to the hyperinsulinemia (Gill *et al*., 2001)

In PCOS females, the ovulatory defect can be corrected by the correction of the FSH levels through the use of estrogen receptor blockers that reduce the negative feedback exerted by estrogen (Brown *et al*., 2006).

In addition, studies have shown that the prenatal exposure to androgens elevates the LH levels and contribute to PCO morphology (Abbott et L., 2005).
Adolescent girls start their puberty as a result of an increase in LH and a mean FSH (Hall *et al*., 2005). GnRH secretion is influenced by the negative feedback of the diurnal increase in progesterone early in the morning. This is followed by an increased GnRH pulse frequency within 24h. The presence of increased androgen levels during this stage can lead to the impaired sensitivity to progesterone and subsequently to PCOS (Ibanez *et al*., 1994)

2.5 Modifiers of the Neuroendocrine Axis in PCOS

The Role of Obesity

Obesity is highly present in PCOS patients suffering from anovulation. BMI and percent body fat are inversely related to LH, so obese PCOS patients usually have a normal LH/FSH ratio.
It was thought that the effect of obesity in PCOS is exerted at the hypothalamic level; however, the GnRH pulse frequency was not affected by the BMI, and evidences support the fact that its effect is exerted at the pituitary resulting in a decreased LH responsiveness to GnRH.

Impact of Hyperinsulinemia

50-75% of lean and obese female with PCOS have insulin resistance and hyperinsulinemia.
Insulin resistance is not only affected by obesity.
Evidences suggest that insulin acts at the level of the pituitary. The insulin receptor in the gonadotropin-derived LßT2 cell line is present in the plasma membrane of the pituitary. Absence of the IRS-2 and IR causes abnormalities in LH levels, absence of ovulation, and decreases the sensitivity to GnRH (Navratil *et al*.,2009).

Role of Leptin

High levels of leptin are present in PCOS women and it is usually related to BMI. Its level is inversely related to LH amount. Those studies have revealed that leptin is the agent that mediates the effect of BMI on LH release.

Folliculogenesis and anovulation disorders are the results of neuroendocrine dysfunction in PCOS. Hyperandrogenemia is the cause of disturbed hypothalamic - pituitary axis. At the pituitary level, obesity and hyperinsulinemia cause neuroendocrine disorder. Thus ovulation is affected these abnormalities and insulin resistance. Intervening at the insulin and obesity level improve folliculogeneis and promote fertility.

2.6 Insulin resistance in PCOS

The main symptoms of Polycystic Ovary Syndrome (PCOS) are androgen excess and anovulatory infertility in women. However, it has been shown through numerous studies that other metabolic anomalies like metabolic syndrome, glucose intolerance, type 2 diabetes, endothelial dysfunction and others are more prevalent in the PCOS patients thus having a connection with it. One of the most recurrent associated conditions is insulin resistance. Altogether, managing these metabolic conditions can help with the manifestation of the syndrome. It has been decided by several scientific communities to diagnose PCOS if the patient has:

1. Hyperandrogenism
2. Dysfunctional ovaries in the form of oligo-anovulation and polycystic ovaries. Nonetheless, other common feature for PCOS patients are high BMI (Body Mass Index), insulin resistance (70% of women with PCOS) and compensatory insulinemia. Since not all PCOS patients have insulin resistance it is not clear if this symptom is a cause or a contributor to the syndrome, thus the necessity to determine its relation with PCOS.

Definitions of insulin resistance

Anatomically, Insulin is a hormone secreted by the pancreas. Two ranges of effects are in place: The metabolic effects and the mitogenic effects.

Its metabolic effects consist of promoting glucose intake, inhibiting lipolysis and glycogen synthesis in tissues sensitive to it like the liver, skeletal muscles and adipose tissue. This whole process depends on the levels of insulin in the blood and glucose in the blood.

The mitogenic effects consist of cell proliferation and differentiation.

When the tissues that are affected by insulin lose their sensitivity to it, insulin resistance is in place. Insulin can no longer execute its metabolic effects whereas mitogenic effects may remain intact. In other words, insulin resistance results in excess glucose levels in the blood since it is no longer picked up by the tissues in question resulting afterwards in an increase in insulin secretions (hyperinsulinemia)(DeFronzo *et al*.,1979).

Evolution of Insulin Resistance Knowledge in PCOS

Several studies across the years tried to uncover the relationship between insulin resistance and PCOS.

In 1920, Emile Charles Achard and Joseph Thiers were the first to associate insulin resistance to hyperandrogenism called then the Achard-Thiers syndrome defined as highly virilized women who developed type 2 diabetes (not called PCOS then)(Bellanger *et al*.,2012).

In 1980, the comparison between obese PCOS patients and healthy control women with the same BMI by Burghen and his colleagues determined a significant correlation between insulin levels and androgen circulating in the blood as well as insulin and testosterone or androstenedione (Burghen *et al*.,1980).

Another study by Dunaif in 1997 and colleagues suggested that insulin resistance in PCOS is not caused by high BMI but by the syndrome itself.

However, other studies that followed in which women with PCOS and healthy ones with different BMI were put in comparison showed that lower insulin sensitivity was present in obese PCOS patients but not as much as in lean PCOS women (Pawlak *et al*.,2002). Moreover, fit PCOS patients with insulin resistance lost this insulin resistance after a correction for truncal-abdominal subcutaneous fat distribution (Pawlack *et al*., 2002). Thus, concluding that the fat distribution is the main cause of Insulin resistance not the PCOS itself (Druce *et al*., 2005).

These differences in the studies' results are mainly caused by the heterogeneity of PCOS phenotypes across the studies (Lam *et al*., 2007). The findings are limited to certain genotypes. In conclusion, the literature across research reveals that insulin resistance and hyperinsulemia aggravate and may trigger PCOS in predisposed

subjects. The limitation of this conclusion is that PCOS can cause insulin resistance after excess weight gain and his needs to be further investigated.

Insulin and Hyperandrogenemia in PCOS

Insulin resistance as shown above is caused by factors affecting insulin signaling pathways in the insulin sensitive tissues. The resulting hyperinsulinemia leads to alterations in the androgen biosynthesis pathway so helping the PCOS phenotype to uncover.

In Vivo observations

Numerous studies tried to find the correlation between the role of insulin and androgen production.

In 1989, a reduction of free and total testosterone levels because of a diazoxide intake that lowered fasting and glucose-stimulates insulin levels was observed. The same result was observed with a treatment with acarbose (slows intestinal glucose absorption) for 6 months. In lean normo-insulinemic women with PCOS, the same treatment with diazoxide also caused a significant drop in free testosterone and androstenedione levels. Thus proving the relation between insulin and hyperandrogenemia even in lean PCOS patients (Hilll *et al*., 2006; Mozafffarian *et al*., 2011; Church *et al*., 2011). The study findings are limited to the diazoxide intake with no clear investigation about the nutritional intake of each participant.

Similarly, treatment with insulin sensitizing drugs such as metformin and rosiglitazone showed improved free-testosterone levels. Metformin was attributed the effect of lowering circulation insulin levels whereas rosiglitazone was said to lower androgens by restoring the androgenic response to insulin (Wren *et al*., 2001; Pereira-Lancha *et al*.,2010). There is limited data in the literature about the effect of a defined percentage of weight loss on androgenic response to insulin with no pharmaceutical agent's intervention.

A recent study reported that increased circulating progesterone and androstenedione levels was caused by GnRH-agonist stimulation during insulin infusion and after adrenal steroidogenesis suppression with dexamethasone, compared to when administered during saline infusion. However, LH and FSH hormone levels remained

the same between infusion conditions thus concluding that hyperinsulinemic condition potentiated ovarian androgenic response to gonadotropic hormone stimulation (Tosi *et al*.,2012).

In general, studies showed that a decrease in basal androgen production is not triggered by a lowering in insulin levels. In conclusion, it can be said that hyperinsulinemias in itself do not cause PCOS in a normal woman but can cause hyperandrogenemia in women with PCOS whether they are insulin-resistant or insulin-sensitive.

Another link between insulin and hyperandrogenemia is the sex hormone-binding globulin (SHBG) levels. SHBG binds to testosterone in the plasma which makes the testosterone unable to circulate freely. In PCOS women, the level of SHBG in the plasma is lower than for healthy women thus the high levels of testosterone circulating. The treatments with diazoxide and acarbose lower the SHBG levels so treating hyperandrogenemia(Farooqi *et al*., 2006; Speliotes *et al*., 2010).

In vitro Observations

Insulin resistance PCOS patients, alter the insulin signaling pathway. Normally, the molecules of insulin interact with its receptors on the insulin-sensitive tissue in a way that the receptor undergoes dimerization and autophosphorylation by its own tyrosine kinase activity (Baillargeon *et al*., 2003). This results in the activation of the first protein (IRS) in the signal transduction which in turn either activates the pathway which is responsible for the metabolic function of insulin or the pathway that activates the mitogenic effects of insulin.

Insulin resistance emanates from anomalies in those pathways (Baillargeon *et al*., 2003).

It is still scarce to confirm whether hyperandrogenemia is caused insulin resistance is the cause or the consequence of it. Both of them are factors of PCOS depending on the genotype. Insulin resistance contributes to PCOS differently between women and intervening on a prevention and treatment level of IR is a great challenge for scientists. Preventing IR to occur among PCOS women is mainly by genetic but without any factors such as glucose increasing food, IR can be prevented.

Lipotoxicity and the exposure to fat intake is a key contributor to IR thus decreasing

the body fat can decrease the risk and androgen levels. The exact cause of PCOS is unclear between metabolic and endocrine abnormalities however, it is important to study the implication of dietary intervention and the possible improvement to have a better insight to the PCOS mechanisms. The disorder characteristics are important in order to have a clear insight on the effect of nutritional support via weight loss in order to improve anovulation and hyperandrogenemia as a new therapeutic line. This line can better prevent, or treat PCOS development.

2.7 Obesity, Metabolic Dysfunction, and Inflammation in Polycystic Ovary Syndrome

Modern dietary habits, high intake of fats, salt and sugar, sedentary life and more recently discovered epigenetics related to the fetal environment have been shown to have contributed to the growth of the obesity pandemic in industrialized and developing countries. The excess adipose tissue present worsens the symptoms of PCOS like for example insulin resistance (Aubuchon t al., 2012). Additionally, adipose tissue stores energy and act as an endocrine and immunological organ.

Medical Disorders Associated with Obesity

Obesity has been linked to many other medical conditions:

- Impaired glucose tolerance (IGT) (Stein *et al*., 2007).
- Dyslipidemia: Lipoprotein (LDL, apolipoprotein B, VLDL) and triglycerides are elevated In the body of an obese person which delays hepatic clearance moreover, HDL tends to be low in their bodies (Stein *et al*., 2007).
- Hypertension: it is caused by the increase of circulating leptin, insulin, FFA which activates the sympathetic nervous system and the renin-angiotensin-aldosterone system which triggers excess sodium retention thus hypertension. Other cases face vasoconstriction because of endothelial dysfunction and nitric oxide decreased responsiveness thus causing hypertension (Stein *et al*., 2007).
- Type 2 diabetes mellitus: the insulin resistance in obese people causes an increase in the secretion of insulin in the pancreas and then contribute to the failure of the pancreatic Beta-cells (Stein *et al*., 2007).

- Cardiovascular diseases: increases proportionally to body weight (Stein *et al*., 2007).
- Risk of sleep apnea: related to the increase of body fat percentage and visceral fat (Stein *et al*., 2007).

Metabolic Dysfunction in PCOS: Prevalence and Risk

Women with PCOS show these medical disorders and symptom independently of obesity. Studies have shown that non-obese women with PCOS have higher LDL-C and higher total cholesterol than non-obese healthy women. Indeed these conditions are more likely to occur with a PCOS patient (Ford *et al*.,2008; Boutzios *et al*.,2013).

It is said that hyperinsulinemia affects the pulsatile patterns of FSH and LH and androgen production which is associated with anovulatory infertility (Ford *et al*.,2008).

Impact of Obesity on PCOS

Obesity is more probably to happen in PCOS patients (42-74%) than in the general population (25%) and insulin resistance is more prevalent in obese individual with PCOS or not than with normal-weight PCOS women. In fact, fit(normal BMI of 18-24.9 kg/m^2) PCOS patients have a milder form of insulin resistance that only results in postprandial hyperinsulinemia (Yildiz *et al*., 2008).

When obese women have PCOS the case of hyperinsulinemia worsens which causes excess androgen production and stops hepatic sex hormone-binding globulin (SHBG) synthesis, enhances LH pituitary secretion and enhances the androgenic response to LH.

When Obesity is superimposed with PCOS the woman shows more symptoms of hirsutism and menstrual disturbances than a normal weight PCOS patient. This is because of the greater elevations in circulating free testosterone cause by the low levels of SHBG found in obese PCOS women. Thus when treated for anovulatory infertility, obese women need more fertility drugs (Aubuchon *et al*., 2012). The limitation of this study is the generalizability to obese women with PCOS while lean women with PCOS are yet to be investigated.

The hypothesis that PCOS induces obesity is based on the fact that the PCOS patients have disordered hunger and satiety signals caused by ghrelin irregularities and that the superimposed obesity promotes more weight gain. However, the energy expenditure is the same for a PCOS patient and a healthy one with the same BMI (Aubuchon *et al*., 2012). The study findings are limited to the fact of similarity in energy expenditure among PCOS and healthy women and this is not clearly seen among other studies. There is a limited data about the exact energy expenditure among PCOS women. It is suggested to study the basal metabolic rate among PCOS and health women to detect the molecular activity in the metabolic rate.

Obesity-induced PCOS

It is hypothesized that the occurrence of PCOS is promoted by obesity in predisposed women (Aubuchon *et al*., 2012). Indeed, it has been shown that the disorder was encountered for some women after progressive weight gain while it should have been developed at menarche like it does for normally (Aubuchon *et al*., 2012). Weight loss triggered the suppression of the symptoms: acne, menstrual cyclicity disturbances and derangement in hyperandrogenemia (Abuchon *et al*., 2012). According to the study finding hirsutism remained after the change in the lifestyle but stopped progressing. In other words, if predisposed with PCOS the individual shows signs after weight gain. The study finding is limited to the phenotype with no further investigation about the biochemical level of androgens. It is suggested to study the effect of lifestyle modification and its relation to changes in biochemical and clinical levels of androgens.

PCO (polycystic ovaries) may be present in a menopausal woman (24-32%) but does not mean that PCOS has developed. If weight gain occurs the signs and symptoms start showing. If no weight gain is observed PCO resolve with age. So the insulin resistance of obesity affects ovarian functions when PCO are present at the time of the weight gain. Further, long term longitudinal studies are needed to evaluate these correlations.

Perpetuation of Medical Risks of Metabolic Dysfunction

Women with PCOS whether obese or not are more prevalent to develop chronic medical disorders (Aubuchon *et al*., 2012). The fat accumulation at the abdominal and visceral areas that happens in obese women with PCOS is correlated with insulin resistance, fat accumulation in the liver, reduced hepatic insulin clearance and induction of hepatic insulin resistance (Aubuchon *et al*., 2012).

2.7.1 Metabolic Inflammation in PCOS

Obesity and inflammation

Obesity is shown to be a prooxidant, proinflammatory state (Weisberg *et al*., 2003). The circulating MNC migrate to the adipose stromal-vascular compartment after the adipocyte death by hypoxia. Then these MNC become resident macrophages which induces membrane-bound nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity (Groemping *et al*., 2003). In turn this oxidation produces ROS a superoxide that activates the transcription factor nuclear factor Kb that triggers inflammation by its translocation on the DNA promoting the transcription inflammatory mediators. These events promote insulin resistance and atherogenesis (Baldwin *et al*.,2001).

The inflammation of the adipose tissue in obesity is superimposed with the PCOS symptoms.

Chronic low-grade inflammation in PCOS

Proinflammatory genes like those that encode TNFα and the type 2 TNF receptors that are the genetic basis for the chronic low-grade inflammation that is observed in PCOS. To test for chronic low-grade inflammation, it is necessary to measure CRP levels that should be normally less than 3 mg/L. However, only obese PCOS patients have more than 3 mg/L while fit PCOS individuals are in the norm (Aubuchon *et al*., 2012).

Diet-Induced Inflammation in PCOS

Dietary components as glucose and lipids are factors that contribute to a prooxidant inflammatory response (Aubuchon *et al*., 2012). In fact, ingesting glucose and lipids

increases in MNC-derived ROS generation and NFkB which are markers of oxidative stress and inflammation that eventually cause insulin resistance and atherogenesis. Promoting nutritional guidelines with health option sources of glucose and lipids can decrease the inflammatory response in PCOS women.

The Relative Impact of Excess Abdominal Adiposity in PCOS

Excess abdominal adiposity is present in PCOS patients regardless of weight however with a prevalence of 30% for fit individuals who trigger insulin resistance and atherogenesis. In normal-weight PCOS with no excess abdominal adiposity, the proinflammatory state is triggered by diet (glucose and lipid ingestion) (Carmina *et al*., 2007).

Thus, abdominal adiposity is not related to insulin resistance in normal-weight women with PCOS which would support the hypothesis that the amount of inflamed excess adipose tissue present in the abdomen in the absence of increased body weight may be insufficient to promote systemic insulin resistance (Gonzale *et al*., 2012).

Obesity with adipose tissue accumulation can increase inflammation and thus metabolic and endocrine disorders. Obesity worsens PCOS presentation such as hyperandrogenia and anovulation. Dietary behavior can contribute to inflammation in PCOS pathophysiology, and insulin resistance is underpinned by the oxidative stress induced by diet. The alterations at the molecular level can be the lead to ovulatory dysfunction and insulin resistance. In light to what is known till now, there is no study that assessed the effect of standardized nutritional guidelines and the needed period to decrease the inflammatory state in PCOS neither at the molecular or public health level.

2.8 Pharmaceutical therapy for PCOS

Managing the symptoms of PCOS by pharmaceutical agents is an essential part of the treatment and enhances their reproductive profile. The decrease in knowledge about dietary intake can lead to obesity, excess weight gain and unhealthy lifestyle.

Insulin-Sensitizing Drugs:

Hyperinsulinemia is a factor in disturbances of metabolic and reproductive functions in women; therefore, insulin-sensitizing drugs (ISDs) for women with PCOS have attracted a great deal of appeal over the past decade.

Metformin

Metformin is an FDA-approved biguanide used for the management of type 2 diabetes mellitus. Many of its physiological effects are found however its mechanism of action is still unknown (Lord *et al*., 2003). It is proposed that it lowers fasting serum insulin levels in insulin-resistant states averting hypoglycemia. Additionally, it induces in the reduction of insulin requirements in insulin-dependent and non-insulin-dependent diabetes (Matthaei *et al*., 2000).

Thiazolidinediones

Thiazolidinediones (TZDs) gain their status of ISD through their role in refining insulin signaling and raising glucose uptake in adipose and muscle tissue mainly. The drug ,however, causes so many complications such as cardiovascular problems that it was removed from the market (Baillargeone *et al*.,2003).

Role of Statins in PCOS Management

Statin therapy is a new suggested way of treating PCOS patients. In vitro and clinical evidence of its efficacy in treating PCOS while also describing the risks and limitations it presents. Statin therapy appeared to also lower cholesterol levels but not the triglycerides levels while reducing cardiovascular risk.

Medical Strategies for Achieving Ovulation

Clomiphene Citrate (Clomid, Serophene)

Being the first choice to treat infertility with PCOS patients, Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM). Its way of working would be to block the estrogen-mediated negative feedback on the hypothalamo-pituitary axis which results in an increased secretion of FSH from the anterior pituitary (Speroff *et al*.,2011) Then, follicular growth, development and selection to dominance are promoted. Tamoxifen can also be added to the treatment for the same effects.

From a chemical point of view, CC is a racemic mixture of en- and zu-clomiphene with the en-clomiphene being the more active component leading to fertility and the one with the shorter half-life while the zu-clomiphene accumulating in the tissues for months after treatments.

From a general point of view, it can be said that CC mimics the physiological ovulatory cycle when administered in a way that minimizes the risk of ovarian hyperstimulation syndrome and multiple gestations.

Aromatase inhibitors

Aromatase inhibitors are an alternative treatment to CC therapy in case of resistance to CC. It is found in the ovarian granulosa cells of premenopausal women, adipose tissue, brain and skin fibroblasts (Pavone *et al*., 2013).

Its mechanism consists of decrease the aromatization of androgens into estrogens which would result in the pituitary release of FSH thus stimulating follicular development (Casper t al., 2006).

Gonadotropins

PCOS patients who failed to show effective result after treatment for 4-6 ovulatory cycles of CC or AI undergo gonadotropin therapy (Lunenfeld *et al*.,2004).

Gonadotropins for ovarian stimulation can be of a natural source from purified urine of postmenopausal women containing FSH and LH or from recombinant human FSH (rFSH). However, it comes with the negative effects of multiple pregnancies, OHSS and multi-follicular ovarian response which indicate that the treatment and the patient should be monitored closely.

In Vitro Fertilization

IVF is more successful than all the fertility treatments. However, it is costly and invasive. It is recommended for infertile women in general and more precisely for PCOS patients with tubal disease or severe male factor.

Even if PCOS patients undergoing IVF manifest longer stimulation period, higher number of developing follicles and higher number of cumulus-oocyte complexes (COC), the pregnancy, miscarriage and live birth rates are the same when compared to a non-PCOS woman undergoing the same treatment as shown by

studies (Heijnen *et al*., 2006). When the patient demonstrates limited ovarian response or exaggerated response (OHSS) the treatment is cut off.

In vitro maturation

The difference between IVF and IVM is that the oocytes in IVM are extracted immature then allowed to attain maturity in vitro over 24 to 48 h period. Since the first pregnancy through IVM in 1991 this technique has shown successful cases. When comparing it with IVF we can see that it has fewer side effects and less medication intake and monitoring.

Mechanism: after a natural or induced menstruation a baseline ultrasound is done (1-3 days after menstruation) to retrieve information about the follicles and their morphology as well as baseline monitoring of FSH and E2. On cycle day 6-8 early selection of dominant follicles and assessment of endometrial thickness is done. Then between days 8-10 the physician retrieves the follicles whether mature or not. When the dominant follicle reaches an appropriate size it is injected with hCG to begin the maturation process while on the other hand the endometrial priming is being undergone by the patients through injections of E2 and then progesterone support (Cha *et al*., 1991).

The success rates have been shown to be less than with the IVF procedure which even with more risk of OHSS has made IVF the preferred alternative.

Treatment of Hirsutism

A definitive solution for hirsutism is not present, when it comes to treating it means to slow it growth and make it less coarse. And this is what a patient should know clearly before beginning treatment.

Pharmacotherapy for managing hirsutism

Managing hirsutism means lowering the circulating androgen levels and limiting its action on the hair follicle itself. This type of therapy takes time and do not show results in the short term (McAvey B *et al*., 2014).

The treatment consists of oral contraceptive pills (OCPs) which suppresses steroidogenesis causing the lowering of androgen production. In response hepatic cells secrete (SHBG) which decreases testosterone in the blood. The use of OCP has positive effect on treating hirsutism and acne in general. The percentages of

improvement vary from patient to patient (McAvey *et al*., 2014).In addition to OCP, anti-androgens can also be used.

2.9 Nutrition role in PCOS :Lifestyle modifications and Weight Loss

High BMI (> 30 kg/m^2) in women has a negative effect on ovulation and fertility. Indeed, it is shown that the correlation of central obesity with fertility is stronger than the correlation of overall BMI and age with fertility. As a result, a weight loss would certainly result in improved chances of getting pregnant even after losing 5% of initial body weight (Sedighi *et al*., 2014). The limitation of this study is the generalizability of results with no further investigation about the role of weight loss on biochemical markers by incorporating a lifestyle modification and not an intervention study for a temporarily period.

In the case of fertility treatments, obese women need to take more medication and may not have a response to the treatment (Sedighi *et al*., 2014). Thus, losing weight before treatment is necessary. Thus this study did not assess the role of fertility treatment with tailored weight loss guidelines for PCOS women.

Treating PCOS means treating the medical conditions related to it like insulin resistance, hyperinsulinemia, obesity, dyslipidemia. However, in the cases of PCOS patients, lifestyle interventions can help with the symptoms of these conditions. Indeed, losing weight through hypocaloric diet and more importantly balancing the dietary macronutrients that can help with the disorder can altogether help restore insulin sensitivity and levels.

Why Diet Makes a Difference in Women with PCOS

The condition of insulin resistance consists of the inability of the insulin to regulate glucose levels in the blood which would in turn makes the pancreas secrete more insulin resulting in hyperinsulinemia. Moreover, in this state, glucose gets stored as fat in the body instead of being used to generate energy and the use of fatty acids is haltered. In the long run, the pancreatic beta cells that are secreting the excess insulin get impaired and stop functioning properly causing type 2 diabetes, a common symptom of PCOS (Hue *et al*., 2009). Promoting healthy food items with

45%-50% complex carbohydrates can decrease the risk of type 2 diabetes in PCOS women. It is suggested to promote healthy guidelines.
Other occurrences in a woman with PCOS are weight gain, craving for carbohydrates (CHO) and sweets and symptoms of hypoglycemia like dizziness, fatigue, headaches etc. Patients with PCOS encounter what is called reactive hypoglycemia: following the ingestion of carbs, the individual suffering from hyperinsulinemia secretes a large amount of insulin in his/her blood which would trigger a precipitous drop in glucose levels in the blood after the ingestion which would once again make the person feel hungry and craving for sweets (Hue *et al*.,2009). This study didn't assess the effect of complex carbohydrates to reactive hypoglycemia and thus this cannot be generalized to all PCOS women. Women with PCOS conditions should follow structured guidelines tailored o this population. For these reason, we can say that PCOS can be treated by a correct, appropriate diet. An appropriate diet would consist of:

- The right amount of (1200-1500 average caloric intake per day for weight loss and 1800 calories average caloric intake for weight maintenance) to take per day that is specific to each woman to help her lose weight or maintain it.
- The right time intervals between meals (3-5 hours) that would inhibits the effects of reactive hypoglycemia.
- Low glycemic index food and carbohydrates intake to lower insulin resistance and inflammation.
- A balance between carbs, proteins and fats (45-50%, 20% and 30 % respectively).

Dietary Carbohydrates

Mainly, the dietary carbohydrates (CHO) are originated from plant compounds (fruit, vegetables) and are used as fuel for the body. However, milk is the only animal-based food that contains CHO while other types of food like cookies, fruit juice, soft drinks, ice cream and candy bars contain unhealthy dietary CHO using high glycemic index CH such as white sugar. In fact, the more refined and processed the CHO is the faster is its digestion and get absorbed in the stomach and intestines

thus, refined carbs have more effect on the glycemic response thus on insulin release.

Glycemic Index of Foods

By definition the glycemic index (GI) is the measurement of the increase in blood glucose after ingesting the food. Each food has a GI and the numerical range makes it easier to compare between the foods. A low GI (50-70) means that the blood glucose response is slow, more satiety thus helping control appetite and weight. A high dietary fiber content of the food often slows down its absorption thus its GI. A high GI (70-100) refers to foods that contain CHO that is broken down more easily and rapidly with a much more effect on blood glucose.

A low-GI diet is adequate for a PCOS patients so that the insulin levels in the blood are regulated as well as the weight (Futterweit *et al*.,2006). Moreover, it is better than a high-GI diet for cardiovascular medical conditions. A study compared obese PCOS women who lost weight either with a low-GI diet or with a normal healthy diet with moderate GI: studies have shown that the women who followed the low-GI diet experienced a threefold greater improvement in whole body insulin sensitivity and menstrual cycle regularity (Mehrabani *et al*., 2012).

Limitations of Using the Glycemic Index for PCOS Patients

Several limitations present themselves when talking about PCOS patient following a low GI diet to treat the symptoms of PCOS:

1. Often GI charts present the GI of the food if it is eaten alone, however when combining the food while eating the individuals GIs are not precise anymore because of their effects on each other. For example combining dietary fat or fiber with high GI components lowers the GI of the whole meal. Thus rendering the GI system ineffective.
2. The physical state of the food (ripeness, cooking method, processing…) affects the GI. For example a brown banana has a higher GI than a yellow banana.
3. Nutritional content of the food is not related to the GI. A low GI food may not bring healthy amounts of macronutrients and vice versa

Guidelines for Dietary Carbohydrate

Since PCOS patients suffer from insulin resistance, obesity and likely type 2 diabetes reducing but not eliminating carbohydrates is necessary. Indeed carbohydrates cannot be eliminated because they also carry nutritional benefits. Generally, a carbohydrate-moderate diet for PCOS patients should include 40% of the calories from carbs in order to optimize circulating glucose and insulin while providing the benefits on the weight of a low-GI diet (Stamets *et al*.,2004).

Fruit and PCOS

Fruits contain CHO in good amounts (15g of CHO in 60g calories) and no proteins or fat. It also contains other benefits coming from vitamins, fibers, antioxidants and minerals which are good for the well-being. Generally only two servings of 30g should be consumed in a diet for a woman with PCOS. The latter should also avoid fruit juices because of their high CHO content without the other benefits and canned fruits in heavy syrup (Krystock *et al*.,2014).

Vegetables and PCOS

Vegetables are divided into 2 categories: starchy and non-starchy (refer to table 3). However, both of them are abundant in vitamins, fibers, minerals and antioxidants.

Table 3 The difference between non starchy and starchy food

Non starchy	Starchy
• Example: lettuce, spinach, broccoli, green beans, onions, mushrooms… • Low in calories and CHO • High in fibers • Promote satiety • Can be typically consumed in unlimited amounts (at least 3 servings in the diet)	• Example: corn, peas, winter squashes, plantains, potatoes… • High content of CHO • Bigger impact on insulin levels • Lowering but not eliminating them from the diet

Dietary Fiber and PCOS

Dietary fibers, the indigestible compound of plant-based food that passes through the gastrointestinal tract in its original form, contain lots of benefits. Food with high level (> 50 g of fibers)of fibers contribute to the feeling of fullness and satiety, delays gastric emptying, demonstrated a cholesterol lowering effect because of the products of its bacterial digestion in the colon and more importantly it helps stabilize glucose levels in the blood and prevent reactive hypoglycemia(Krystcok *et al*., 2014).

Recommendations for Fiber and PCOS

The general guideline for women with PCOS is to consume 25-30 g of fibers a day. Reading labels is a great help when in need of knowing if the food is a good source of fibers: >3g of fibers per serving is considered a good fiber source while >5g of fibers per serving is considered a high fiber source.

Protein

Proteins are very important for the body's structure; they are the building blocks of many components of the human body such as tissues, neurotransmitters, tendons, antibodies… It is recommended that a woman with PCOS takes 25-30% of the total calories consumed from proteins and to choose lean instead of high-fat sources of protein. The benefits of protein are to help the person feel fuller while helping with the regulation of insulin in the blood since they promote the decrease of CHO consumption.

Lipid Abnormalities in PCOS and Relevance of Dietary Fat

Two-thirds of women with PCOS experience dyslipidemia linked to hyperandrogenemia, central obesity, insulin resistance and hyperinsulinemia. The derangements in the lipid profile of the PCOS patients are much related when comparing it with type 2 diabetes. They are characterized by high levels of LDL (>130 mg/dL) and triglycerides (> 160 mg/dL) and low levels of HDL (<50 mg/dL) (Yildirim *et al*., 2003).

Triggered lipolysis and release of FFA into the blood are caused by hyperandrogenemia and hyperinsulinemia which trigger secretion of VLDL by the liver causing hypertriglyceridemia.

Dietary Fat and PCOS

Fatty acids are the basic unit of fats and oils and their different combination creates the different types of fats we know. Any type of fat consumed contains 9 cal/g which is higher than the number for CHO or proteins. Regulating the fat intake in quantity and quality for a PCOS patient can help her enhance the absorption of fat-based vitamins as well as feel more satiated while gaining sexual benefits since sex hormone are fat-based. The different types of fats are:

1. Monounsaturated fatty acids (MUFAs): consisting of certain vegetable oils like olive oil, vegetable oil, peanut oil; they can be a great help to regulate the cholesterol and insulin levels as well as aid in blood glucose control.
2. Polyunsaturated fatty acids (PUFAs): found in almonds, cashews, avocados fish and seafood, they are essential to human beings and can't be manufactured in vivo. Omega-3 and omega-6 are types of PUFAs.
    - Omega-3 fats: found in fatty cold-water fish, seeds, nuts, beans and leafy vegetable, they have anti-inflammatory functions when associated with low-grade systemic inflammation which is a great help for PCOS patients. Additionally, consuming omega-3 fats has other benefits on the bodily functions when it comes to cardiovascular, brain, mood and immune function.
    - Omega-6 fats: found in animal fats, bakery goods; they contribute to systemic inflammation. Their high consumption in western diet is bad for the health especially for PCOS patients. Thus their intake should be decreased while increasing omegs-3 fats.
3. Saturated fatty acids: found in high-fats cuts of meat, whole milk dairy products and chicken skin, coconut oil and palm oil; they raise cholesterol levels. It is recommended to be taken for less than 10% of total calories consumed per day.
4. Trans fatty acids: Processed vegetable oils to become semisolid at room temperature; they damage body tissues and compromise immune functions while promoting inflammation, various cancers and promoting coronary disease by raising LDL levels and lowering HDL levels(Katan *et al*.,1995).

Dietary Calcium

Having the effect of lowering blood pressure, skeletal health and enhancing insulin signaling, calcium is a necessary dietary component for PCOS patients. Calcium can be found mainly in dairy foods.

Dietary Sodium and PCOS

Sodium is found generally in most foods we consume and is often added to them. Its excess can worsen the predisposition of PCOS patients to have premature atherosclerosis and propensity of endothelial dysfunction (Dittas *et al*.,2007).

Caloric needs and PCOS

Harris Benedict equation is a method to calculate the individual's calorie needs in function of age, sex, weight, body composition and individual's own metabolism. Harris Benedict equation determines the BMR (1) which represents how many calories and individual burns at rest, and based on that and on the individual's activity level (2) the total allowed calories is determined.

1. BMR=655+(4.35×weight in pounds)+(4.7×height in inches)-(4.7×age in years)
2. Sedentary : BMR×1.2
   Lightly active: BMR×1.375
   Moderately active: BMR×1.55
   Very active: BMR×1.725
   Extra active: BMR×1.9

Behavior Modification in PCOS Management

Snacking and Optimal Meal patterns

Ideally, a person with PCOS should eat small snacks spaced every 2 to 4h while consuming breakfast 1h after waking up. The snacks should be around 60-100 calories containing 0-20 g of CHO, 0-7g of fat and a 2-8g of proteins. This pattern will help reduce cravings and fight the effect of reactive hypoglycemia.

Importance of Exercise for PCOS Management

The diet for PCOS women should be accompanied by regular exercising for more weight management, reducing insulin levels, optimizing lipid profile and improvement of insulin sensitivity. Additionally to physical benefits, mental health is

also benefited: working out boosts self-esteem, regulate mood swings and lower depression and anxiety.

Exercise Recommendations

It is recommended to work out more than 150 minutes a week of moderate-intensity exercise or 75 minutes of intense exercise (Krystock *et al*.,2014).

2.10 Current International interventions

International nutrition and weight loss intervention in PCOS have high potentials of alleviating the syndrome's symptoms and thus the disease itself. Everyday nutrition is a crucial aspect of healthy living, and its intervention in PCOS can improve its clinical spectrum. The elements of a diet have proven to be critical for the development and maturation of the ovarian follicles (Huijgen *et al*, 2015). The study findings didn't define the needs and the forbidden items needed for ovarian follicle maturation. There is still a lack in the literature about the nutraceutical food components need to ovarian follicles development. Lifestyle modification with weight loss for overweight/obese and maintain weight for lean PCOS women using nutritional guidelines adapted to the PCOS population provides healthy ovules and decreases both the risk of PCOS and manages its current treatment. Consequently, deficiencies in the diet link to improper programming of the ovarian follicles and contribute to the severity of PCOS phenotype.

Huijgen and his colleagues (2015) conducted a study to determine the association of diet inadequacies with the severity of PCOS. Their analysis showed a positive correlation such that an inadequate diet (measured using the PDR score) was significantly higher in PCOS than in controls (PDR score 3.7 vs. 3.5; $p = 0.017$), further linked to greater risks of the hyperandrogenic phenotype. However, the inadequacy in dietary behavior is different between obese and lean PCOS women. This should be further confirmed by a study assessing the nutritional behavior for different weight scale PCOS women. In addition to this, Rondanelli and her colleagues (2014) coordinated another study aiming at evaluating the nutritional correlates of PCOS and the optimum diet for these abnormalities. The study showed that PCOS patients exhibit instability in adipokines (adiponectin, leptin, visfatin) production and in omega6/omega3 PUFA ratio. This means that lifestyle

changes are the basis of PCOS treatment. The diet therapy includes a set of goals – insulin resistance modifications, adipokines secretion and reproductive function. All of these can be achieved by lowering the sugar consumption (lower glycemic index), portioning the food intake into small and frequent meals, and either increasing the fish intake to about 4 times a week, or using omega3 PUFA supplements. Vitamin D and chromium supplementations are also necessary in low serum level cases. This confirms that a lifestyle modification is the optimum choice for PCOS women rather than an interventional weight management for a defined period.

Not only can a balanced diet alleviate the symptoms of PCOS, but also weight loss, in overweight and obese women, improve their metabolic, reproductive health and regenerate fertility (Milone *et al*.,2016). Thus, weight loss is a type of lifestyle intervention, similar to the nutrient intake. In a recent study carried out by Legro and his associates, they examined differences in pregnancy outcomes among PCOS patients that used birth pills, and a second group of PCOS overweight or obese women that underwent lifestyle changes. Among the 49 women assigned to the birth control intervention, five gave birth compared to the 50 women in the lifestyle intervention group who delivered 13 babies (WHW, 2015).The most favorable weight loss diet aims at reducing excess body fat percentage, whilst maintaining the lean mass, altogether reducing the adipokines and the fatty acids released by the adipose (Kasim-Karakas *et al*, 2009). This has been seen to be achieved by following a low-carbohydrate (< 40 % total carbohydrates)/high-protein diet (> 30 % total protein), decreasing excess body fat (Phy *et al*.,2015). However, this study has different limitations such as using the elimination diet behavior by reducing carbohydrates and increasing protein intake. Elimination diet is not recommended for PCOS women who need a lifestyle nutritional management and not an intervention for a short period of time. A lifestyle modification should not eliminate any food component in order to be suitable for different PCOS women coming from different socio economic background. Any elimination of food group or reduction can thus reduce the vitamins and minerals intake and lead to deficiencies. It is suggested to have all food components with tailored energy intake. The study carried out by Kasim-Karakas showed that a hypocaloric diet, with protein

supplements caused greater weight loss and fat mass loss compared to a diet supplemented with simple sugars (Kasim-Karakas *et al*, 2009). The study method used elimination diet. The findings of this study confirmed weight loss but used an elimination diet that can't be incorporated into a lifestyle modification. This suggests filling the gap in the literature where no lifestyle modification nutritional guidelines are proposed and well assessed on the PCOS population.

Overall, PCOS remains a syndrome whose first line intervention remain the management of lifestyle, including a proper balanced diet, leading to weight loss in case of overweight or obesity.

2.11 Nutrition problems in PCOS

Obesity remains one of the most encountered nutrition problems worldwide. However, PCOS and obesity are seen to share a cause-effect relationship, where some studies indicate that PCOS women have a high tendency to develop nutrition problems of obesity and high levels of carbohydrate cravings (Jeanes et *al*.,2017). The findings of this study are limited to the fact that PCOS women have high tendency to obesity, but Jeanes and his colleagues didn’t study the effect of obesity itself on the onset of PCOS in controls who are not receiving any nutritional management.

Many studies have been developed to show that PCOS women are inclined to becoming obese. Worldwide numbers estimate between 38% and 88 % of the PCOS patients suffering from obesity (Hoeger *et al*, 2012). In Mumbai for example, 70-75% of the PCOS women are obese (Sen et *al*., 2014). Recently, a study was conducted, including 62 PCOS women and 23 control women with matching body weight. It was observed that all PCOS women, compared to the controls, had decreased basal metabolic rates (Hoeger *et al*, 2012). BMR is a representative of the energy expenditure while at rest, and its low values indicate a higher inclination towards weight accumulation. Thus, with low BMRs, women are more likely to become obese. In Lebanon there is no studies about the weight accumulation in PCOS women who represents a certain population with high social concerns about infertility and family.

It is clearly understood that PCOS and obesity are linked in one way or another. To understand the link, it is important to note that androgens have an important role in determining the composition of the body, where women normally accumulate more fat in the lower parts of the body. However, women with PCOS were shown to be more likely to develop upper body fat, as demonstrated by the high waist to hip ratio compare to same BMI controls (Sam, 2007). Thus, studies, although many remain contradictory prove that obesity in women is due to PCOS and is referred to as a nutrition problem that comes with it. It is yet needed to be investigated further about the link between obesity, PCOS and weight gain.

To understand why some PCOS women face problems of obesity, it is important to note that the polycystic ovary syndrome is associated with the inability of the body to use insulin. This leads to the accumulation of insulin and glucose in the bloodstream, also referred to as insulin resistance. Due to high levels of insulin, large quantities of the male androgen hormone are secreted, leading to hirsutism, acne, irregular periods, and weight gain (Ehrman *et al*.,2005). Consequently, the extensive weight gain leads to obesity. An alternative explanation for the fundamental system, in the development of obesity in PCOS women is a combination of genetic predisposition to obesity with an obese inducing environment that includes a poor diet and lack of exercise (Moran *et al*.,2015). Thus, the hormones play a role in the weight gain, but women who are predisposed to accumulating fat, are more likely to develop obesity with their PCOS.

In addition to all this, women with PCOS are known to suffer from excessive carbohydrate cravings. This can be due to the insulin resistance found in all PCOS women. The inability of the body cells to take in the glucose for muscle energy causes the body to constantly require more food, namely carbohydrates. The cravings therefore do not help with the aforementioned obesity problem, increasing the chances of weight gain, with eventual obesity, even more.

It is crucial to note however, that obesity can worsen both the clinical and endocrine features of PCOS, furthermore it results in the prevalence of irregular menses and menorrhagia, as well as increased serum androgen concentrations. Obesity additionally leads to a significant reduced response to ovulation induction regimens and reduces the chances of fertility treatments to succeed (Hoeger *et al*, 2012). All

the consequences discussed add to the already established complications of PCOS and thus understanding the connection between the two complex diseases- obesity and PCOS- aids in founding an appropriate treatment that targets the initial problem of weight gain, which has eventually lead to further complications.

2.12 Role of nutrition as interventional prime line to PCOS

To reverse the insulin resistance, the body needs to become re-sensitized to its own insulin. To do so, one must follow a diet with very few to no simple carbohydrates. If the amount of glucose in the bloodstream exceeds the body's daily needs, the cells can no longer absorb it. The blood glucose stays high and to try and bring it down, the pancreas secretes even more insulin Therefore, weight loss, by a low carbohydrate diet and physical exercise to burn fat, can restore the insulin sensitivity of the cells and tackle the other problems underlying insulin resistance, such as the factors of the metabolic syndrome affecting women with PCOS.

Losing the excess weight, by using a low glycemic index diet for example, will help recover the normal functioning of the cells, thus restoring insulin sensitivity. As a result, there would be no excessive glucose in the blood stream that could lead to high LDL cholesterol levels and blood pressure, furthermore reducing cravings and weight gain brought about by insulin resistance. With improved aspects of the metabolic syndrome, the risks of cardiovascular disease, stroke and diabetes are exponentially reduced.

Studies have shown that it takes a small amount of weight loss, as much as 5-10% of the initial body weight to see improvements in both the mild symptoms of PCOS as well as the risk factors mentioned previously (Moran *et al*.,2006). With a reduced BMI, the normal functioning of the ovaries can be reestablished, as well as normal hormone production. In PCOS patients, the androgen male hormones are increasingly secreted, due to insulin resistance, leading to acne, hirsutism, irregular periods and weight gain. With regular hormonal functioning, as a result of weight loss, the ovulation is restored, the menstrual cycle is regulated, fertility is improved, and the female self-esteem is boosted (Moran *et al*.,2006). Thus, weight loss not only helps with lethal risk factors but also with the symptoms of PCOS of excessive

hair growth, acne, and scalp hair loss. Moran and her colleagues concluded that 5-10% is sufficient to improve the PCOS symptoms following a structured weight loss plan. However, previous data have shown that weight loss can decrease the inflammatory states. Thus, Moran's study findings are limited and a further investigation need to be added to the scientific literature about the effect of nutritional guidelines on the long run for PCOS women. The limitations of Moran and her colleagues are the structured interventional approach to PCOS women and the short period of times used.

Weight loss thus remains the number one solution for the problems encountered in PCOS and it is considered as a treatment for the symptoms of the syndrome. Combined with physical exercise, a balanced, healthy diet reduces the lethal risk factors associated with the metabolic syndrome and plays a major role in PCOS (Palomba *et al*., 2010).

2.13 Nutritional Intake of Women with PCOS Worldwide and in the Middle East

The nutritional intake and diet of women with PCOS is an issue that is commonly studied since polycystic ovary syndrome is very common worldwide. There are several takes and perspectives considering the different regions of the world as well as the common nutritional trend that comes along with the disease itself.

A study looked at PCOS patients and whether they display altered dietary intakes and eating behaviors compared to controls (Larsson *et al*., 2016). It showed compliance with several other studies that BMI of women with PCOS is higher that than of women without PCOS (Larsson *et al*., 2016). However, that could have been explained by several contributing factors since PCOS (polycystic ovaries syndrome) is characterized by numerous symptoms. However, Larsson and colleagues didn’t compare the nutritional intake between obese and lean PCOS women sharing the same symptoms in order to further investigate the difference of intake and energy expenditure among them. A large study is needed to assess the difference in caloric intake among all weight scale range of PCOS women.

One that was commonly found in several studies is hyperinsulinemia and insulin resistance in both overweight and lean women with PCOS. This might push women to weight gain due to insulin’s effects in addition to changes in energy usage and

diet (Wright *et al*., 2004). The hyperandrogenism associated with PCOS can lead to more carbohydrate cravings and a more carbohydrate rich diet (Lindén Hirschberg *et al*., 2004). This study showed that intake of glycemic index foods is higher among PCOS than non-PCOS (Lindén Hirschberg *et al*., 2004). There is a large gap in the literature about the intake of food in the Middle Eastern society and this is needed to be further investigated.

In addition, it was also shown that a decreased (1232 versus 1964 pmol x min/l) secretion of CCK and the fluctuations in appetite regulation is associated with the increased (2.3 versus 1.4 nmol/l) testosterone which all aid in the increased occurrence of binge eating and weight gain in women with PCOS (Lindén Hirschberg *et al*., 2004).

A study that took place in turkey involving 94 adolescent women with or without PCOS to compare body dissatisfaction, eating attitudes and body esteem scales in both groups (Karacan *et al*., 2014). It showed that the scores on these tests of the group with PCOS are not significantly higher than patients without PCOS (Karacan *et al*., 2014).

Another study that involved 210 women with PCOS in the UK was based on a 7-day-estimated food and activity diary and questionnaire (Barr *et al*., 2011). It showed that women with PCOS showed a higher proportion of energy from fat (38 %) and a lower proportion of energy from carbohydrates (Barr *et al*., 2011). Another study that took place in the Middle East was a comparative study of 65 women with PCOS and without PCOS in Iran (Sedighi *et al*., 2014). It showed that women with PCOS have a different lifestyle and food habits than women without PCOS in the sense of paying more attention to nutrition and exercise (Sedighi *et al*., 2014).

In conclusion, a general trend in the eating habits and nutritional intake of women with PCOS worldwide was found, since there are consequences of the disease that link women from different countries to one another up to a certain extent. On the other hand, how the women deal with the weight gain and diets is also strongly affected by the personal, cultural and environmental factors that vary from one case to the other. By comparing all the previous study we conclude one point and it is the high caloric intake among PCOS women and this generalized as no study have investigated the highest food component intake using a food frequency

questionnaire adapted to their cultural regime. A more precise study is needed to use a more precise methodology and compare the food frequency questionnaire to their 24 hour recalls as part of control over the analysis.

2.14 Energy Requirement of women with PCOS

PCOS is a disease that affects the metabolism among several other effects; however, the metabolism is what affects energy production and requirements of women with PCOS.

In a study that took place in 2015, the BMI of women with PCOS is higher (28.5 versus 24.6 kg/m^2) than the BMI of women without PCOS that is congruent with most of the studies addressing the same topic (Larsson *et al*., 2016). The resting metabolic rate shows whether women with PCOS have a higher energy requirement due to their metabolic rate or not. The same study has shown that the resting metabolic rate is the same in women with or without PCOS after taking age and BMI into consideration; however, the respiratory exchange ratio was higher in women with PCOS in comparison to the controls (Larsson *et al*., 2016). In addition there were discrepancies in the sources of energy used by women with PCOS and without; such that a higher energy percentage was obtained from carbohydrates (49 % versus 44 %) while a lower energy percentage of alcohol (2.4 % versus 4.1%) was also obtained (Larsson *et al*., 2016).

There are some defects of endocrine origins associated with PCOS such as insulin resistance, hyperinsulinemia and metabolic syndrome. Other effects observed to be related to PCOS, is the increased leptin levels in women with PCOS. This showed to be related to weight loss since studies have shown that with weight loss of women with PCOS, came as a decrease in the leptin levels (Rondanelli *et al*., 2014).The previous studies used small sample size. However, these findings are still general and the discrepancies between hyperinsulinemia and increased leptin level facts should be concluded by a large sample size study.

2.15 The European Perspective on Weight Loss in PCOS

One of the main indicators of PCOS is usually weight gain. There is a degree of variance in diagnosis of PCOS in different countries of the world; however,

according to the European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine criteria, the occurrence of PCOS can be up to 15 to 20 percent (Sirmans and Pate, 2013)

It is crucial for weight loss to be included in the treatment strategies of PCOS. Physicians believe that it is involved in a vicious cycle that starts with androgen excess leading to abdominal fat deposition, which then triggers the insulin resistance and eventually hyperinsulinemia and consequently further increases ovarian androgen secretion (Harrison *et al*., 2011).

The European perspective aim on weight loss for women with PCOS suffering from obesity focuses mainly on lifestyle change, which includes exercise and diet (Conway *et al*., 2014). Diet and exercise aimed at weight loss is a very beneficial treatment for PCOS and has shown promising results by a follow up study involving obese women with PCOS following lifestyle programs of 1200-1400 kcal/day diet for the duration of 6 months as well as physical activity (Pasquali *et al*., 2011). Although there is a lot of variety in how women responded to the weight loss; more than one third of the women may have experienced full recovery from symptoms of PCOS (Pasquali *et al*., 2011). It was also shown that weight loss also aids in ovulation and aids in overcoming the reproductive adversaries imposed by PCOS (Consensus on infertility treatment related to polycystic ovary syndrome, 2008)(Tarlatzis *et al*., 2008).

Another mean for weight loss associated with obese and especially morbidly obese women with PCOS is bariatric surgery (Conway *et al*., 2014). It proved to be an efficient mean for weight loss as well as reversing some of the effects of PCOS in morbidly obese patients (Conway *et al*., 2014). Studies have shown that bariatric surgery can reverse some of the metabolic syndrome as well as provide reproductive assistance since weight loss is strongly linked to PCOS and hence might be quite therapeutic (Legro *et al*., 2012). Bariatric surgery also showed to help with other symptoms of PCOS through bringing back the hypothalamic–pituitary axis, decreasing cardiovascular risk and even improving pregnancy outcomes (Malik *et al*., 2012). However, the previous studies ' conclusion is crucial to many PCOS women where bariatric surgery can decrease the mineral and vitamins absorption. Bariatric surgery could be a lifetime solution for weight loss for

many obese PCOS cases. But this cannot be generalized and thus bariatric surgery cannot be a first time treatment to obesity in PCOS rather a lifestyle modification should be proposed with infertility treatment.

2.16 The right diet for PCOS

PCOS is a disease of over-nutrition and a primary management should focus on a nutritional approach.

An ideal diet should prevent nutritional deficiencies by providing adequate nutrients and energy for human development and reproduction. It should, also motivate the well-being and longevity by reducing the risks of diet-related chronic illnesses.

In the short term, the nutritional management of PCOS should focus on weight loss, and improvement of symptoms. In the long term, nutritional management must address the augmented risk of type 2 diabetes, CVD and certain cancers that are associated with PCOS. Figure 1 illustrates the process of nutritional intervention helping improving PCOS.

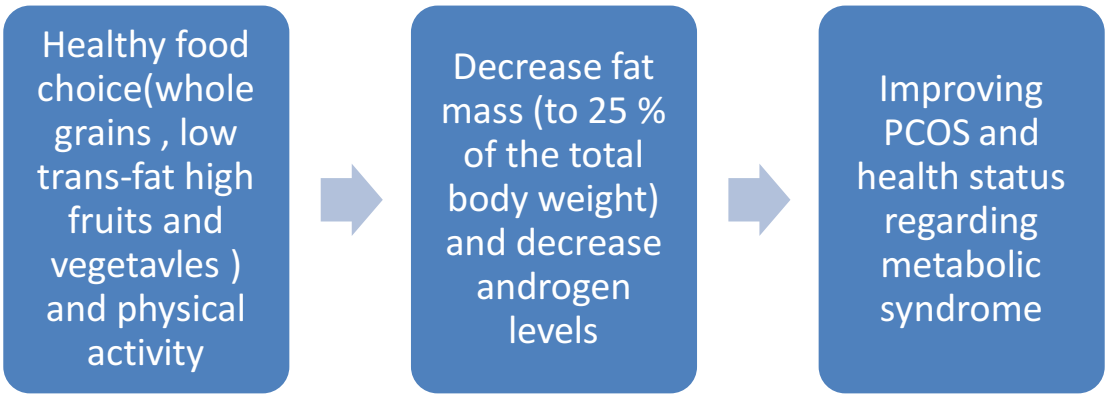


Figure 1 The effect of diet on PCOS

For a better response, the dietary management is preferred to be a combination of limiting the intake of a certain macronutrient and increasing physical activity.

Here is a literature review of the recommended dietary guidelines that this project will follow:

Calorie needs and restrictions for PCOS women

PCOS patients should follow a hypocaloric diet including all the macronutrients for a short-term and long-term effects. Restricting one macronutrient could affect the long-term commitment to keep weight in the ideal range.

To study the calorie needs, it is possible to check the individual calories intake allowance. Daily women's calorie allowance differs according to body weight in relation to age and physical activity. Dieticians recommend between 1200 calories to 2000 calories. A daily caloric decrease of 500 Kcal/day causes an average weight loss of 0.5 kg/week, and a caloric decrease of 1000 kcal/day causes an average weight loss of1 kg/week.

Fat intake

Fat is the most energetic dense macronutrient with 9 kcal/g compared to 4 kcal/g for proteins and carbohydrates. Hyperinsulinemic individuals have higher capacity to store fat in their body. Not all kinds of fat have the same effect. When consumed instead of carbohydrates or unsaturated fats (commonly found in non-hydrogenated vegetable oils), trans-unsaturated fats (commonly found in commercially fried and baked products) increase the risk of ovulatory infertility. The consumption of trans-fats instead of other macronutrients has been associated with greater insulin resistance, and risk of type 2 diabetes (Chavarro *et al*, 2007). Several studies have shown that excess fat intake is linked to insulin resistance (Vessby *et al*.,2001). Subjects with less than 37% of fat intake, and those with moderate consumption of unsaturated fatty acids, have improved insulin sensitivity (Riccardi and Rivellesse, 2000).

Although many studies showed the beneficial effects of polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaeonic acid are present mainly in fish oil) on diabetic patients, no evidence is found about their effect on PCOS patients (McAuley *et al*, 2005).

Overall, dietary fat should account for no more than 30% of the calorie content of the diet, with a maximum of 10% of calories coming from saturated fat. The remainder of the fat content should be as a balanced mixture of unsaturated fat including cooking oils and spreads (Norman *et al*, 2004).

Carbohydrate intake

Excluding carbohydrates is not efficient, but consuming limited quantities and replacing white carbohydrates sources (as white bread) with multi-cereals bread can cause an excellent shift to a healthy regimen as fibers inclusion is beneficial.

A low glycemic index (GI) diet may provide the greatest benefit for women with PCOS (Kennedy *et al*, 2005) where it is linked to enhanced insulin sensitivity, reduced postprandial hyperglycemia, increased HDL, and decreased triglycerides. Increased glycemic load (amount of carbohydrates taken multiplied by GI), caused by high GI food, and increases the risk for type 2 diabetes (Kennedy *et al*, 2005). Glycemic load can be reduced by following an isocaloric diet that replaces high GI foods with low ones. Following a low carbohydrate diet up to 6 months, people who have or are at risk of type 2 diabetes have shown to lose weight (Samaha *et al*, 2003). At the beginning, a carbohydrate restricted diet is essentially helpful but not effective if severe restriction is followed. On the long term, low-carbohydrate diet is associated with harmful effects on the lipid profile and should be used for short-term for better weight loss achievement (Moran *et al*, 2003).In general, carbohydrates should be 50% of the daily caloric intake.

Protein intake

Recent controversial studies have shown that high intake of protein leads to improved glucose and insulin responses. Proteins increase satiety and postprandial thermogenesis and lowers abdominal fat (Vessby *et al*, 2001). Moderate protein intake is essential to protect lean body tissue. Proteins should account for 20% of a diet's calories (Moran *et al*, 2003) consumed from lean meat sources and vegetarian sources such as beans which provide the patient with extra benefit from fiber contents.

Note that a high protein low carbohydrates diet is a type of weight loss plan that emphasizes consumption of high-protein-containing food. High-protein low-carbohydrate diet (for 1 month) was compared to low-protein high-carbohydrate diet (for 3 months) in PCOS patients. The comparison reported no significant differences in term of weight loss(Brehm *et al*, 2003).Another study showed that high protein diet resulted in minor differential improvements for high density lipoprotein (HDL-C, TC/HDL-C), AUC for glucose, and Femoro-AcetabularImp ingement (FAI) (Moran *et al*, 2003).

Eating behavior

Eating behavior is an important issue that is generally not well addressed, and more irregular eating behavior was noticed with more consumption of energy dense food. Studies have shown that regular meal consumption increases the postprandial energy expenditure, decreases calorie intake, and develops insulin sensitivity (Harnack *et al*, 2000).

Breakfast consumption is associated with less energy intake and improved insulin sensitivity (Kerver *et al*, 2006). It is also linked to weight gain and obesity. In general, low frequency of major eating meals is linked to high fat mass and leptin levels (Farshchi *et al*, 2004). Yet, no detailed studies regarding eating behavior in PCOS patients were noted.

In addition, a high intake of fruits, vegetables and whole grains shown to protect against CVD, diabetes and cancer.

Physical activity

Patients who exercise have increased lean body tissue and lower body fat; thus they have increased resting energetic expenditure which causes a higher metabolic function in women with PCOS (Jakcic *et al*, 2003).The recommendation for a healthy lifestyle is a 30 min of physical activity for at least 5 days weekly (Jeffery *et al*, 2003). Some studies showed that moderate-to-high intensity exercise for 60-75 min has a long-term greater effect on weight loss. Several exercise options could be taken into consideration such as aerobic exercise, endurance exercise, and resistance training (increases muscle and influence body mass and composition leading to an increase in the metabolic rate).

In conclusion, the best nutritional recommendation for PCOS patients is to follow a low caloric diet including all the macronutrients with emphasis on healthy choice and increasing physical activity. A combination of moderately calorie restriction with limited fat intake in parallel with physical activity is the best for achieving weight loss (Stern *et al*, 2004)

2.17 Rationale: The benefits of nutritional education in treating PCOS in Lebanon.

Several studies have been carried out to assess the benefits of LSM and the intervention of nutrition program on PCOS outcomes that include IR, ovulation induction, hyperandrogenism, fertility, menstrual frequency, and lipid profile (Karimzadeh *et al*.,2010 ; Hoeger *et al*.,2008 ; Tang *et al*.,2006). Furthermore, studies conducted on overweight and obese PCOS women have shown that weight loss, exercise and LSM are effective treatment methods (Legro *et al*., 2013 ; Vause *et al*.,2010).

Despite the fact that several studies have shown the role of adequate nutrition and exercise in enhancing the success of PCOS treatment, such methods of treatment are still far from application in Lebanon due to the heavy reliance of physicians in this field on medications.

It is fairly common in Lebanon to find that women who suffer from PCOS are also obese (BMI over 30 Kg/m^2). However, the nutritional approach in treating these patients is barely in action in Lebanon. A large study is needed to shed light on the vitality of introducing the nutritional aspect of PCOS treatment, and to study the influence of diet modification and nutritional counseling/education on PCOS outcomes. There is a limited literature in Lebanon about PCOS assessing the effect of short-term and long-term nutritional interventions on their metabolic ad endocrine features.

2.18 Research variables

- Nutritional investigations included FFQ and 24-hour recall (two 24-hours recall). Nutritional counseling was documented to exhibit operative commitment to study. Nutritional screening was carried out in a simple and non-invasive way primarily to evaluate the nutritional status. A range of both subjective and objective nutritional screening tools such as 24 hour dietary recall, food frequency questionnaire (FFQ) will be used. FFQ survey provides detailed data regarding eating patterns and history (DeBruyne *et al.*, 2011).

- Physical activity was assessed using the IMS- physical activity survey that permitted the study of the change in physical activity and behavior in PCOS patients.
- The anthropometric measurements taken were mainly weight, height, waist circumference, hip circumference and body composition analysis using ELG 3rd generation. This provides information on body analysis and obesity level information from which an individual plan of nutritional care can be devised and monitored.
- Hypertension measurements were taken to monitor the effect of healthy food choices on pre-hypertensive patients in PCOS women participant, for hypertension is a PCOS feature.
- Biochemical indicators: (Vitamin D, insulin resistance, blood glucose FBS, HDL-C, LDL-C, total cholesterol, triglyceride level, total testosterone, insulin resistance) in order to study the benefit and efficiency of the nutritional education/counseling on PCOS biochemical indicators. Refer to table 4 about the biochemical parameters and their cut-offs.
- Other PCOS conditions reported: regularities of menstrual cycle (seen by pregnancy onset, ovulation, and fertility rate)
- Hirsutism was measured using the Ferriman-Gallewey score.
- Psychological assessment using BDI-II. This is a very widely used scale and it is one of the oldest containing 21 item self-report measure of cognitive, behavioral, affective, and somatic components of depression, developed based on DSM-IV criteria of depression. Items on the BDI are rated on a 4 point type scale ranging from 0 to 3. Total scores are obtained by summing the highest ratings on each of the 21 items and may range from 0 to 69 with higher scores indicating symptom severity. The BDI has excellent 1 week test-retest reliability ($r=0.93$, $P<0.001$) and internal consistency ($\alpha=0.91$). Convergent validity for the BDI is satisfactory and was established through examining the correlation of its' scores with scores on the Revised Hamilton Psychiatric Rating Scale for Depression ($r=0.71$) (Beck *et al*., 1996).GAD-7 is another scale that will be used to score for anxiety in PCOS patient, a short screening tool and validated.

Each of the discussed variables was monitored and studied during the pilot and larger scale project at the beginning of the intervention, 3 months and 6 months after the intervention.

Table 4 : Research parameters and their cut- offs

Parameter	Unit	Normal Ranges	Rationale
Vitamin D	ng/ml	30-74 (1)	"Vitamin D plays a physiologic role in reproduction including ovarian follicular development and luteinization. Low Vitamin D levels may exacerbate the symptoms of PCOS, including insulin resistance, ovulatory, menstrual irregularities, infertility, hyper-androgenism, obesity and elevate the risk of cardiovascular diseases"(Lin *et al*.,2015).
Insulin	µU/mL	< 25 µU/mL	Women with polycystic ovary syndrome (PCOS) are profoundly insulin resistant, and the resultant hyperinsulinemia exacerbates the reproductive abnormalities of the syndrome. Agents that ameliorate insulin resistance and reduce circulating insulin levels could provide a new therapeutic modality for PCOS (Legro *et al*.,1998).
Blood glucose	mg/dL	126(4)	Women with polycystic ovary syndrome (PCOS) often develop type 2 diabetes mellitus (T2DM) (Boudreaux *et al*., 2006).
FBS	mg/dL	70 to	Fasting blood glucose, along with insulin

		99(1)	levels can be used to assess insulin resistance which is common in PCOS women (Carmina *et al*.,2004).
HDL-C	mg/dL	50-60(1)	Usually, women with PCOS, lower HDL-cholesterol than their non-PCOS counterparts even if they are not obese (Wild *et al*.,2012).
LDL-C	mg/dL	<130(1)	PCOS patients had very low LDL compared to non-PCOS patients (Wild *et al*., 1985).
Total Cholesterol	mg/dL	<200(1)	Commonly, women with PCOS dyslipidemia which leads to abnormal Total Cholesterol levels (Wild *et al*., 2012). .
Triglyceride	mg/dL	40-160(1)	On average, women with PCOS were higher triglyceride than their non-PCOS counterparts even if they are not obese.
Testosterone (female)	ng/dL	15 to 70(9)	PCOS patient have higher testosterone levels compared to non-PCOS patients.

2.19 Originality of the study

Being a developing country, Lebanon still lacks a rich peer reviewed research concerning PCOS. The data available about the knowledge and awareness of Lebanese PCOS patients on the important role of the adequate nutrition and physical activity in the PCOS treatment is still very scarce. Unfortunately, up until now, no research has been conducted in Lebanon concerning the public adopting healthy food choices under the influence of educational sessions that enhance LSM and weight loss although this issue might be the beneficial to many women seeking

PCOS treatment. Consequently, this study was the first to assess the benefits of non-pharmacological treatment of PCOS in Lebanon. What was therefore new about this project was to revisit the efficiency of balanced food choice and adequate nutritional framework in PCOS treatment in Lebanon. It is important to mention that an intervention was established and directed to discern the efficiency of this approach prior to proceeding to a final large scaled up intervention.

Chapter 3

Subjects, Materials and Methods

3.1 Introduction

The intervention is divided into three phases; planning, implementation and evaluation.

This chapter (3) will illustrate the major steps of the planning phase and these include: Sampling methods, Sample size calculation, Inclusion, exclusion criteria and confounding factors

However, to our knowledge no studies have assessed the lifestyle modification of PCOS on our target population.

3.2 The design and methodology of the study

The study that was conducted is a prospective public health interventional study specifically designed for the culture of Lebanese population, and fitted to study the influence of educational session's intervention in enhancing each of healthy eating choice and LSM on PCOS outcomes and health status of PCOS patients. Patients in this study included both obese and lean PCOS patients who attended the obstetrics and gynecology clinic of the American University of Beirut - Medical Center in Beirut, Lebanon. Institutional Review Board (IRB) at the American University of Beirut is responsible to give the ethical approval. Based on both the PCOS status and the physical and nutritional status of the PCOS patients, the development of an intervention was assessed. A primary pilot study was conducted on a small sample population size (N=76) that will to a great extent advance, enhance and assess the impact of nutritional counseling/education on PCOS patients but also to assess the acceptability of the program among PCOS patients. The results of the pilot study were used to plan a large scale up intervention program (N=294 PCOS women versus 294 non-PCOS women) that can be rolled out nationally in answer to the escalation in PCOS sufferers in the region. Please refer to figure 2 that illustrates the study process.

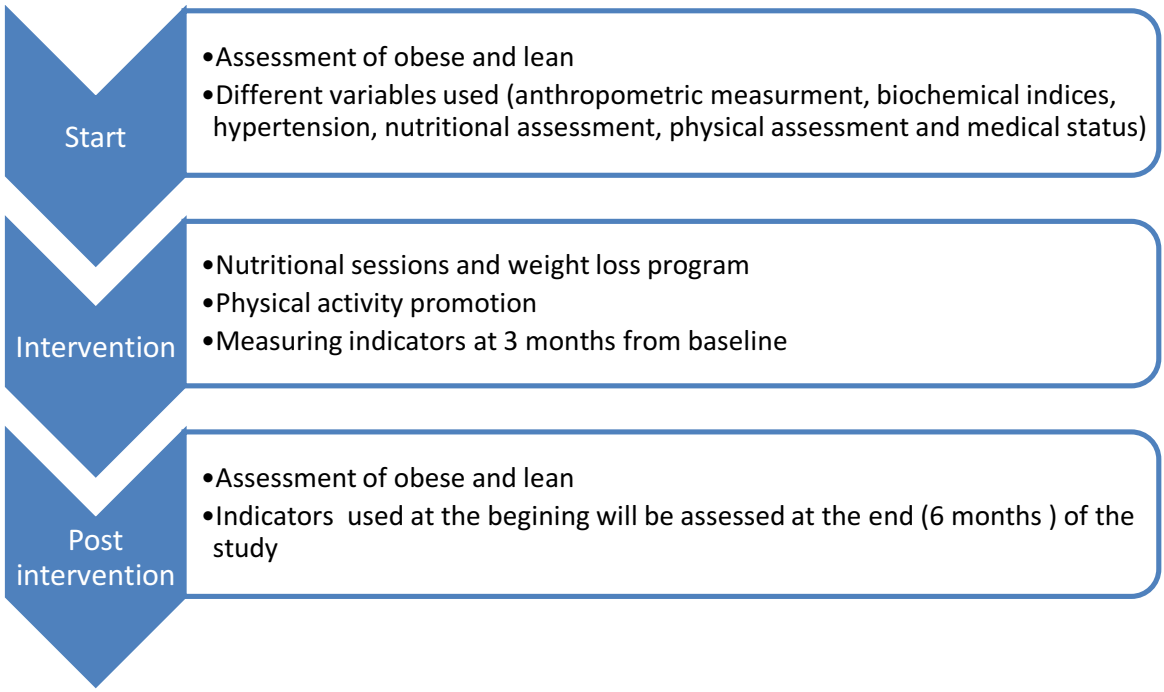


Figure 2 Flowchart showing the project stepwise process

3.2.1 Sampling Methods

Randomized control trials (RCTs) was used to test the efficacy of a public health nutrition intervention to enhance health eating and lifestyle modification among Lebanese PCOS women.

The 2003 Rotterdam in the Netherlands addressed new diagnostic criteria which states that at least two of the following three features must exist: oligomenorrhea/amenorrhea, clinical and biochemical evidence of hyperandrogenemia, and/or polycystic ovaries documented on ultrasonography. Women diagnosed with PCOS were approached by the physician first and then the researcher. Demographic characteristics such as age, gender, job status, education level, yearly income, past medical history, food intake, menstrual and ovulation dates were assessed.

Based on the sample size calculation, inclusion and exclusion criteria, patients (Pilot N=76; Scale-up N=588) were selected through simple randomization. During the pilot study PCOS women were recruited. During the scale up intervention PCOS and non-PCOS women were recruited from the Obstetrics and gynecology clinics. The purpose of recruiting non-PCOS women is to later compare PCOS data versus non-PCOS data. Patients were grouped according to their weight groups

(Group A-B: Overweight/obese (BMI> 24.9 kg/.m^2); Group C-D: Lean (BMI< 24, 9 kg/m^2)). The enrolled patients were randomly allocated into controls or interventions. Enrolled patients signed a consent form and were given the right to drop out of the study at any time. Patients diagnosed with PCOS were verbally informed by the gynecologist. The researcher provided patients, who participated, with an informed consent form including the name and address of the research facility and the investigators, the research topic and its purpose, the criteria of eligibility. A brief description of the procedure involved, benefits /risks, the time of participation required (6 months), the location of the research, the contact person for retrieval of further information, and an invitation for participation. Patients were asked to sign the consent form after having read all the terms and conditions for participation.

The grouping procedure: Each patient had a code (randomization) used on each assessment form in order to ensure the anonymity of each subject involved in this study. To identify each patient, the code of each patient along with her name will be listed on a separate paper identifying their contact details in order to call them for follow-up. This list was placed in a locked closet that the researcher of this study has access to it.

1- The pilot study included 76 patients where the recruited population was divided into 2 groups: 38 patients in the overweight/obese group, and 38 patients in the lean group (each according to the BMI measured).Then each group was divided into subgroups receiving different treatments (Intervention or control) (Refer to Figure 3).

Each study participant had 3 sessions, divided into 2 sections: assessment and education.

An assessment form was filled by the candidate at each visit that include: medical assessment, nutritional assessment(measuring body analysis in term of weight, height, BMI, BMR, hip circumference, waist circumference and body fat), biochemical assessment (FBS, fasting insulin, total cholesterol, triglyceride, LDL,HDL,CRP, and total testosterone),hypertension, hirsutism score and psychological assessment (using BDI-II and GAD-7).Moreover, each patient filled in

questionnaires at each visit: food frequency questionnaire, 24 hour recall, physical activity questionnaire.

Qualitative data were collected from the study patients through:

Informal individual interviews on life style, adherence to the weight loss or weight maintenance programs and guidelines

- Continuous observation of patients ' response to the interventions
- Direct involvement of the researcher in ensuring that the study procedure is not compromised.

Sample size

The population of interest in this study included PCOS patients aged from 18 to 45 years (reproductive age).

Simple random sampling strategy was used in the selection of the different groups of the study patients according to the study design.

The outcomes which either decrease weight in obese PCOS or maintain weight in lean PCOS patients was assessed to be 76% although it is used for both groups. We took 76%, assuming there was no study before on weight maintenance in lean PCOS patients, never the less it is considerable to be 5% in the study to assess a difference of 14% between control and intervention to achieve the outcome.

For the pilot study and based on a previous study on obese PCOS patients we needed 76 PCOS patients (Hoeger *et al*.,2006).

This sample size calculation was used to determine the number of PCOS patients interviewed and assessed in order to get results reflecting the target population as needed. It also shows the level of accuracy in this sample size .

$$SS = \frac{Z^2 \times (p) \times (1 - p)}{C^2}$$

Z = Z-value (1.96 for a 95 percent confidence level)

P = Percentage of population expressed as decimal, in the case of this study we are using 76% = 0.76 (this value was based on previous studies on the weight loss percentage of PCOS patients) (Palomba *et al*.,2008).

C = Confidence interval, expressed as decimal (0.05 = +/- 5 percentage points)
Z-values (Cumulative normal probability table)= represent the probability that a sample will fall within a certain distribution. The Z-values for confidence levels in this case is 1.96 (guided by the cumulative normal probability table).

$$SS = \frac{1.96^2 \times 0.76 \times 0.24}{0.5^2}$$

$$SS = \frac{3.8416 \times 0.76 \times 0.24}{0.0025}$$

SS = 280

5% attrition will be added = (5/100 x 280) + 280

SS = 294

For the pilot study, 25% of the SS was considered = 25/100 x 294 = 73.5 = 76

Sample size (NSS) for pilot study =76

Thus sample size (NSS) for larger scale intervention = 294

The justification for the sample size was based on the 95 % confidence interval and a precision limit of 0.05 for the study.

In the scale-up intervention Two hundred nighty four PCOS and Two hundred nighty four non-PCOS women were recruited age and weight matched . Patients were approached, and those eight and age matched were accepted to participate in the study. Thirty PCOS women continued the study intervention from the study phase to the scale up phase. Non-PCOS women were recruited during the scale –up intervention. However, due to fall outs and some incomplete results during follow up, the total number of research patients that completed the six months scale – up intervention was five hundred eighty (580) (Refer to figures 3 and 4).The justification of the sample size is based on the 95% confidence interval and a precision limit of 0.05 for the study.

However, the prevalence of PCOS in Lebanon in this study was not conducted.

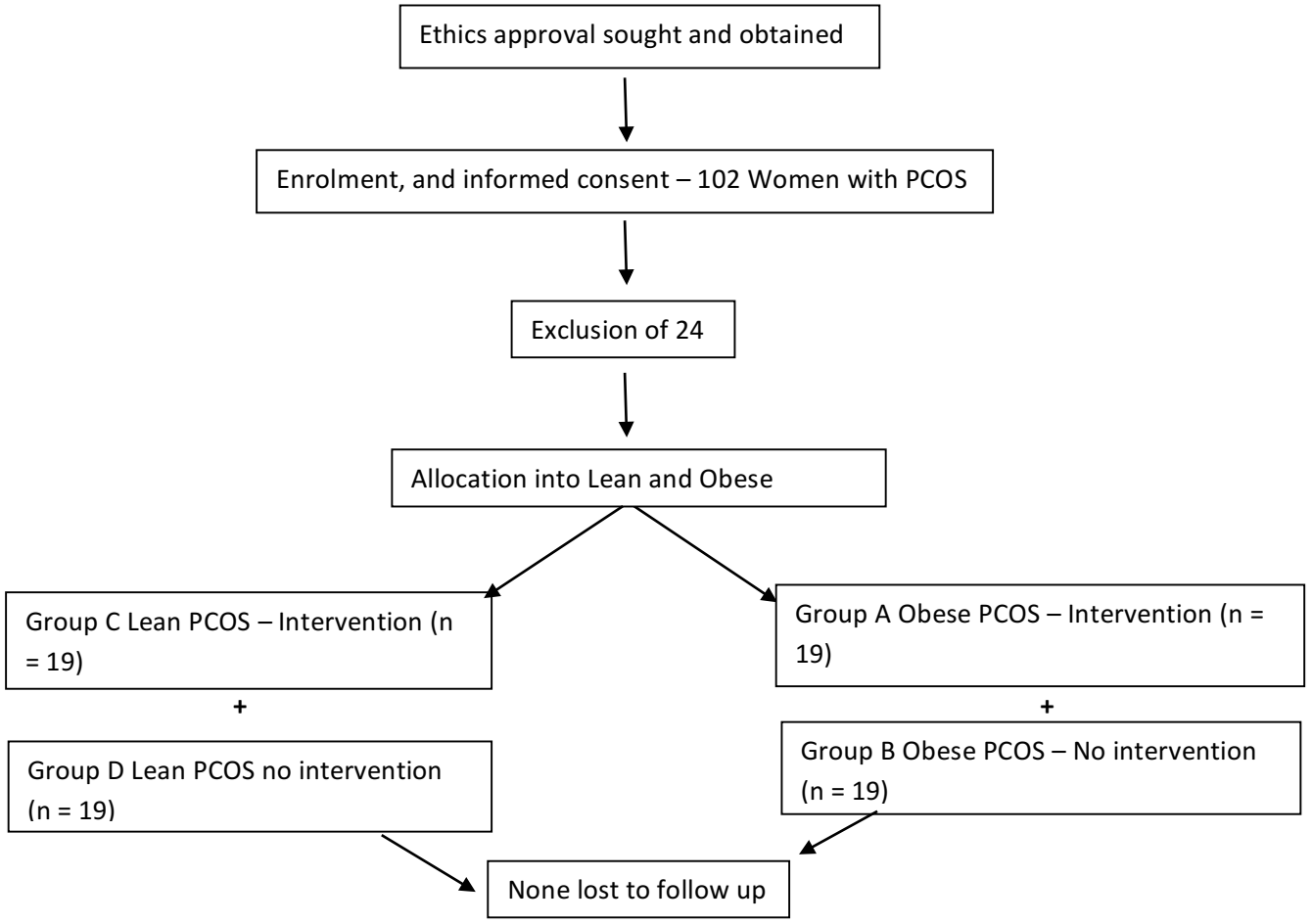


Figure 3 Consort 2010 flow diagram for Pilot study process

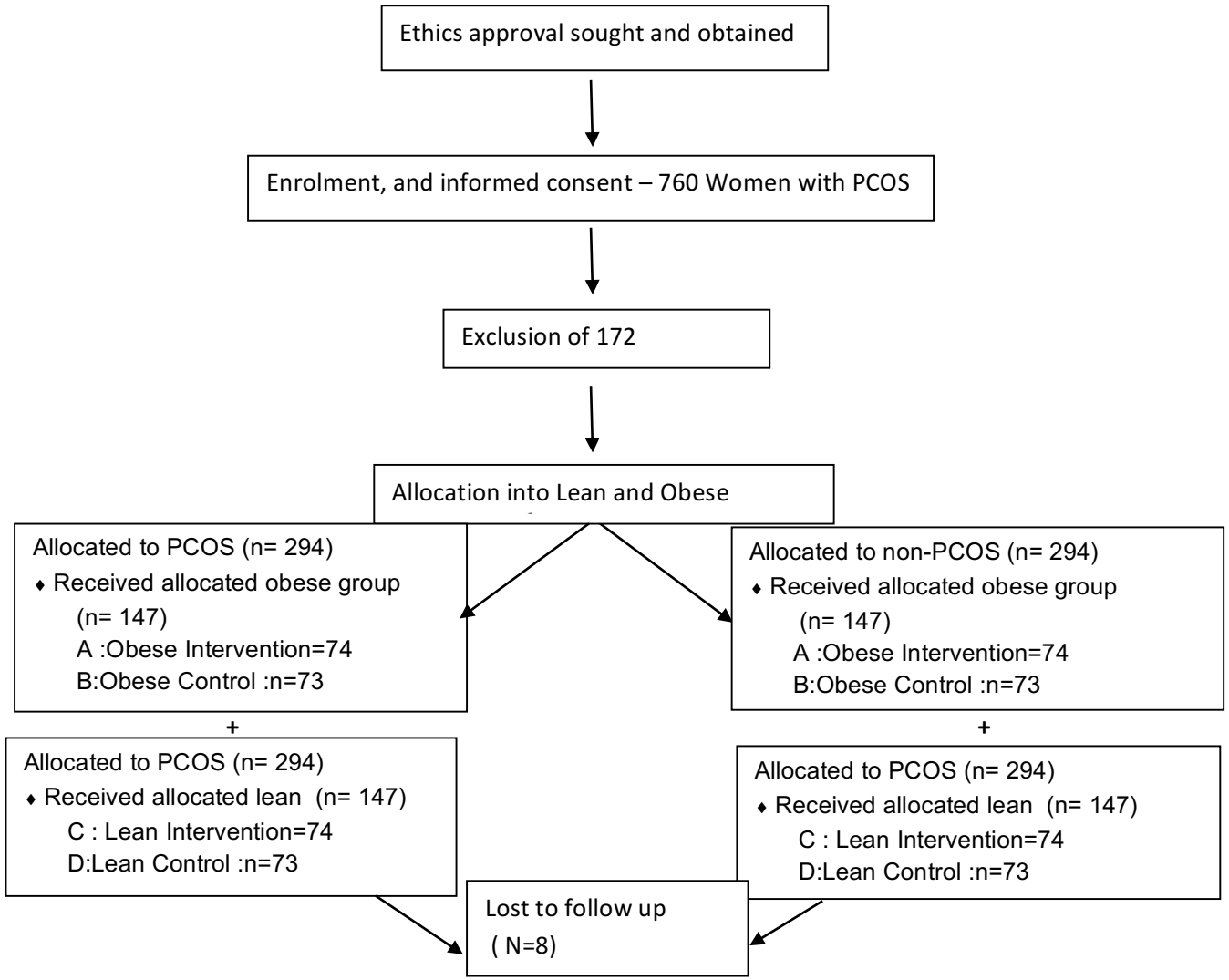


Figure 4 Consort 2010 flow diagram for scale-up process

3.2.2 Inclusion, exclusion criteria and confounding factors

The study was conducted in two phases (pilot and scale-up) employing the following criteria: Prospective study patients were briefed and selected using the inclusion criteria listed below as a prerequisite.

The eligibility criteria

- Patients must be aged between 18 years or above and below 45 years
- Diagnosed with PCOS according to the Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group, 2003.

Exclusion criteria

- Patients with Cushing's syndrome, thyroid disease, cancer, kidney or liver disease
- Patients following a structural weight loss program
- Women with history of daily physical activity more than 30 minutes 5 days weekly (in order to have matched controls and intervention groups).
- Patients on metformin medication (note that patients on metformin are excluded because metformin can cause weight loss which will give us false positive results).

3.2.3 Randomization of study patients

Life science researches need randomization. RCTs are planned experiment tailored to assess the efficacy of an intervention conducted on human beings by comparing intervention and control groups. Biases are reduced by randomization. In our study there was a specific plan to identify and enroll study patients. First patients are screened for PCOS and then the eligibility criteria is applied and thus who met these criteria were enrolled in the study.

The grouping procedure: Each patient had a code (randomization) used on each assessment form in order to ensure the anonymity of each subject involved in this study. To identify each patient, the code of each patient along with her name was listed on a separate paper identifying their contact details in order to call them for follow-up. This list was placed in a locked closet that the researcher of this study

has access to it. The purpose of this randomization was to eliminate any bias and make both groups equally distributed.

The two groups (weight loss or weight maintenance and controls) were randomly selected from the same population of PCOS diagnosed, receiving treatment and support at the OBS clinics at AUBMC, Beirut, Lebanon. They were not only statistically equivalent to the larger group; they were also statistically equivalent to each other. One group received weight loss intervention another group received weight maintenance intervention and the other 2 groups did not receive it and remained on the gynecologist usual health care. Simple randomized evaluation (with two groups) was that one group received the program that is being evaluated (Nutritional and guidelines intervention) and the other did not.

The design of this study ensures that the variables used are possible to use in order to determine the efficacy of this intervention on the PCOS women. The hypothesis was stated, the inclusion and exclusion criteria were different and the 3 follow-ups for assessment with the PCOS women were achieved, thus the study outcomes are accurate results to be discussed in chapter 4 and 5.

Randomization- Pilot study

The pilot study included 76 patients where the recruited population was divided into 2 groups: 38 patients in the overweight/obese group, and 38 patients in the lean group (each according to the BMI measured). Then each group was divided into subgroups receiving different treatments.

To explore more, the total of the pilot study was a 4 groups study where subjects (n=76 PCOS patients) were grouped into 2 groups: lean versus overweight/obese:

A. Lean group was sub grouped into 2 subgroups with equal number of patients:
    1- The intervention group (n=19): receiving an intervention by the researcher which include nutritional counseling/education, cooking sessions about healthy food, and the gynecologist treatment. Please refer to Appendix 8
    2- The control sub group (n=19): receiving the gynecologist treatment (drugs) with no nutritional intervention.

Indicators were measured at 0, 3 and 6 months

B. Overweight/Obese group was sub grouped into 2 subgroups:

1- The intervention group (n=19): receiving the tailored diet and weight loss nutritional intervention taking into consideration diabetes, high lipid profile and hypertension nutritional information. Please refer to Appendix 9.
2- The control sub group (n=19): receiving the gynecologist treatment (drugs) with no nutritional intervention.

Randomization- scale- up intervention

The pilot study included 588 women where the recruited population were divided into 8 groups:

The PCOS women group:

147 patients in the overweight/obese group, and 147 patients in the lean group (each according to the BMI measured).

Then each group was divided into subgroups receiving different treatments.

A. Lean group was sub grouped into 2 subgroups with equal number of patients:
    1- The intervention group (n=147): receiving an intervention by the researcher which include nutritional counseling/education, cooking sessions about healthy food, and the gynecologist treatment. Please refer to Appendix 8.
    2- The control sub group (n=147): receiving the gynecologist treatment (drugs) with no nutritional intervention.

Indicators were measured at 0, 3 and 6 months.

B. Overweight/Obese group was sub grouped into 2 subgroups:

1- The intervention group (n=147): receiving the tailored diet and weight loss nutritional intervention taking into consideration diabetes, high lipid profile and hypertension nutritional information. Please refer to Appendix 4.
2- The control sub-group (n=147): receiving the gynecologist treatment (drugs) with no nutritional intervention. Please refers to Appendix 9.

The non- PCOS women group:

294 patients in the overweight/obese group, and 294 patients in the lean group (each according to the BMI measured).

Then each group was divided into subgroups receiving different treatments.

C. Lean group was sub grouped into 2 subgroups with equal number of patients:

3- The intervention group (n=147): receiving an intervention by the researcher which include nutritional counseling/education, cooking sessions about healthy food. Please refer to Appendix 8.

4- The control sub group (n=147): did not receive any intervention.

Indicators were measured at 0, 3 and 6 months.

D. Overweight/Obese group was sub grouped into 2 subgroups:

3- The intervention group (n=147): receiving the tailored diet and weight loss nutritional intervention taking into consideration diabetes, high lipid profile and hypertension nutritional information. Please refer to Appendix 9.

4- The control sub-group (n=147): did not receive anything.

Figure 5 illustrates the distribution of the recruited population.

Indicators were measured at 0, 3 and 6 months.

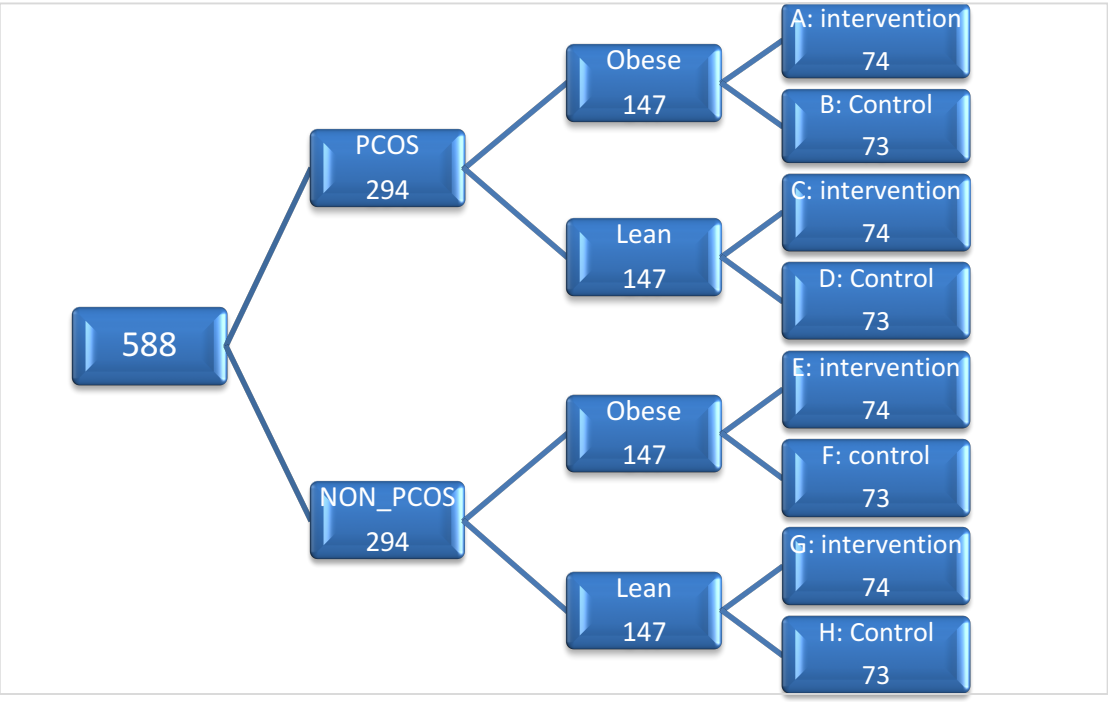


Figure 5 the study population group division

3.2.4 Confounding factors

Random assignment, as a result of confounding by indication, does not affect randomized trials (Johnston, 2001). Since the control characteristics (inclusion

criteria) of the various groups (overweight/obese Interventions and controls, lean interventions and controls) and subgroups (intervention versus Control) were the same, the probability of confounding was smaller in this study. By expanding the types and values of analogies performed in the analysis, devaluation in the possibility for the occurrence and confounding factors was obtained, such that these confounding variables can be classified by their origin. The choice of measurement instrument (operational confound), situational characteristics (procedural confound), or inter-individual differences (person confound). By augmenting the figures of comparisons executed in the proximate analysis, a contraction in the probability of happening and results of operational confounding factors were realized in this study. Equivalently, to guarantee the strength and validity of the findings and a reduction in the potential for operational confound, the data from anthropometric, 24 hour recall , and general assessment measurements were repeated twice and attested by another medical staff. To diminish situations of confounding, either before the study implementation or after the analysis, a peer review in the advancement of the theoretical scheme and at the end of the pilot intervention was used. The peer review likewise, depended on cumulative ability within medical colleagues in the care and support program for PCOS, to recognize possible shortcomings in the study design and analysis, containing methods where the data may depend on confounding. To lessen the incident of self-selection by patients or prejudice by the study planner, the people under study were randomly split after brief instructions, using the Randomized Control Trial (RCT) design. This methodology divides the members into groups (overweight/obese and lean) and subgroups (interventions versus control) significant to the criteria that match the research question.

3.3 Recruitment, study subjects and procedure

In this study, the patients were women who are in a range of age of 18 to 45 years old who were diagnosed with PCOS by the obstetrics and gynecology clinic of the AUB-MC.

3. 3.1 The eligibility criteria

- The patient's age must be included in the range of 18 to 45 years old.
- The patient must be diagnosed with PCOS according to the Rotterdam ESHRE/ASRM sponsored PCOS consensus workshop group, 2003.

3.3.2 Exclusion criteria

- Patients that have Cushing's syndrome, thyroid disease, cancer, kidney or liver disease
- Patients who are following a structural weight loss program
- Women with history of daily physical activity more than 30 minutes 5 days weekly (in order to have matched controls and intervention groups).
- Patients on metformin medication (this exclusion is done to avoid false positive result, for metformin may also lead to weight loss).

3. 4 Pilot study method

The gynecologist verbally notified patients of their diagnosis with PCOS. Prior to participation, the researcher provided patients, who wish to volunteer as a participant in the study, with an informed consent form composed of the following: the name and address of the research facility and the investigators, the research topic and its purpose, the criteria of eligibility, a brief description of the procedure involved, benefits /risks, the time of participation required (6 months), the place in which the study is conducted, the contact person for recovering further information, and an invitation for participation. After having read and discussed all the terms and conditions that relate to their participation in this study, patients were kindly requested to sign the consent form.

The method of grouping patients is as follows: For the sake of randomization, each patient had a code used on each assessment form in order to ensure the anonymity of each subject included in this study. To be able to call patients for follow up, the code of each patient along with her name were listed on a separate paper identifying their contact details. This list was be accessed by the researcher of the study and will be locked up in a closet.

Seventy six patients participated in the pilot study and were divided 2 groups: obese group containing 38 patients as well as a lean group including 38 patients (each according to the BMI measured). After that, each of the lean and obese groups was further divided into subgroups receiving different treatments.
In other words, the pilot study was a 4-group-study (n=76) PCOS patients:

A. Lean group consisted of 2 sub-groups each containing equal number of patients:
    1- The intervention group(n=19): receiving an intervention by the researcher which include nutritional counseling/education, cooking sessions about healthy food, informing about lycopene containing food and the significance of enhancing their lycopene containing food intake, as well as the gynecologist treatment.
    2- The control sub group (n=19): receiving the gynecologist treatment (drugs) with no nutritional intervention.

Indicators were measured at 0, 3 and 6 months.

B. Obese group were sub grouped into 2 subgroups:
    1- The intervention group (n=19): receiving the specially designed diet and weight loss nutritional intervention taking into account diabetes, high lipid profile and hypertension nutritional information.
    2- The control sub-group (n=19): receiving the gynecologist treatment (drugs). No nutritional intervention was present.

Indicators were measured at 0, 3 and 6 months.
The reason why the 2 main groups were divided into 2 subgroups was to study the significance of dietary treatment nutraceutical food component on PCOS outcomes. This aided the assessment of the effect of diet on both obese and lean PCOS patients after being subjected to nutritional education sessions focusing on promoting healthy food choice suitable for their condition, weight loss if needed and promoting physical activity.

Process of the study (pilot and scale up)

Every participant in the study had 3 sessions, divided into 2 sections: assessment and education.

At each visit, the candidate filled an assessment form including: medical assessment, nutritional assessment(measuring body analysis in term of weight, height, BMI, BMR, hip circumference, waist circumference and body fat), biochemical assessment (vitamin D(25)OH ,FBS, fasting insulin, total cholesterol, triglyceride, LDL,HDL,CRP, and serum testosterone),hypertension, hirsutism score and psychological assessment (using BDI-II and GAD-7). Moreover, each patient filled in questionnaires at each visit: food frequency questionnaire, 24 hour recall, physical activity questionnaire.

Patients who were in the intervention groups were then subject to nutritional education/counseling (following the proposed guidelines) as well as the structural weight loss program for obese patients.

The researcher, who is a licensed dietitian, proposed a weight loss diet guideline to the obese. This weight loss diet guideline is proposed by using 1200 -1500 calories daily menu plan calculated according to the obese patients BMR. Calories are assigned after calculating patient's daily recommended intake and subtracting 500 calories from their daily energy requirements. Lean patients; however, followed a weight maintenance diet proposed by the researcher after calculating their BMR, and proposing a daily diet menu according to their allowed calorie intake. Both obese and lean PCOS patients were advised to do physical activity of a minimum of 30 min per day, 3 times weekly.

After 3 and 6 months from the first sessions, each patient was asked to follow-up with the same questionnaires and assessments used. Consequently, there was a total of 3 blood samples taken over a period of 6 months (one 3 ml sample at each follow-up visit) for biochemical analysis [vitamin D(25)OH ,FBS, fasting insulin, total cholesterol, triglyceride, LDL,HDL,CRP, and total testosterone] in which the patient was required to fast before the blood test. Knowing that the food frequency questionnaire includes lycopene containing food choices, the lycopene servings consumed by the patient was counted at each visit (baseline, 3 and 6 months after education sessions) and then compared at the end of the study with the changes in testosterone levels. Moreover, obese patients were subjected to monthly follow-up with their diet and weight change.

Dropouts were also included in the research results in order to assess the compliance of the patients in the study.
After completing the pilot study successfully, (during MPhil) the intervention outcomes were assessed. More patients were included to undertake for a larger sample population to participate in the intervention clinical trial (for PhD).

Approach of the intervention

Food frequency questionnaire were developed using a valid FFQ but modified to suit the Lebanese culture and were used during the study to assess the nutritional intake of PCOS patients before and after intervention.
The plan was evaluated using statistical analysis. The characteristics of patients who had an improvement in PCOS outcomes and those who had unsuccessful outcome were compared in the 2 groups (obese versus lean). An independent sample t-test was used for the continuous variables and a chi-square was used for the categorical variables.
Differences in anthropometric assessment, nutritional assessment, physical activity questionnaire, hypertension measures, biochemical analysis, reproductive questions, hirsutism score and psychological assessment were measured at baseline, 3 months and 6 months from the beginning of the study.
A multivariate logistic regression analysis was carried out in order to study the association between the characteristics of the study patients and nutrition education/counseling and to check the statistical significance of an association between lycopene intake and testosterone levels. A two-sided value of $P< 0.05$ is considered statistically significant.

3.5 Intervention Setting, Assessment tools and Ethical consideration

3.5.1 Intervention Setting

The study was conducted at the American University of Beirut Medical Centre, Beirut, Lebanon. AUBMC is one of the leading medical institutions in the Arab region. The women's health center at the AUBMC is well known with the quality of care given to the women. Other research projects are being conducted in the same

center such as the periodontal disease and the in vitro fertilization outcomes, the Vitamin D status during pregnancy and bariatric surgery in PCOS women.

3.5.2 Measurements of research variables

Anthropometric, biochemical, nutritional, medical and psychological assessment and the nutritional counseling and education were undertaken at the Department of Obstetrics and Gynecology of the American University of Beirut Medical Centre, Beirut, Lebanon. Refer to table 5.

Table 5 The variables used and their frequency

Variable type	Variables	Frequency
Nutritional assessment	FFQ and 24-hour recall(two 24-hours recall	At baseline , after 3 and 6 months from baseline
Physical activity	IMS- physical activity survey	At baseline , after 3 and 6 months from baseline
anthropometric measurements	weight, height, waist circumference, hip circumference and body composition analysis	At baseline , after 3 and 6 months from baseline
Hypertension measurements	DP and SP	At baseline , after 3 and 6 months from baseline
Biochemical indicators	Vitamin D, insulin resistance, blood glucose FBS, HDL-C, LDL-C, total cholesterol, triglyceride level, serum testosterone, insulin resistance	At baseline , after 3 and 6 months from baseline
Fertility and gynecological assessment	regularities of menstrual cycle (seen by pregnancy	At baseline , after 3 and 6 months from baseline

	onset, ovulation, and fertility rate)	
Clinical hyperadrogenemia	Hirsutism score	At baseline , after 3 and 6 months from baseline
Psychological assessment	BDI-II and GAD-7	At baseline , after 3 and 6 months from baseline

3.5.3 Research assessment tool

The assessment of the PCOS patients was formulated to target the aim of the study and achieve a high quality of research. The different used tools are designed to insure correct reporting and deep analysis. After the pilot study (N=76) a critical evaluation was conducted for the tools used ensuring the quality of research and reporting in the scale up (N=588).

Critical appraisal of the assessment tool was ensured after the pilot study (n=76) to ensure adequacy of reporting detail on the data sampling, data collection and analysis in the scale- up (n=588).

3.5.4. Research indicators and their cut off

The cut off points of biochemical analysis are referred to in table 6.

Table 6 The different biochemical parameters and their cutt off values

Parameter	Unit	Normal Ranges
Vitamin D	ng/ml	30-74 (1)
Insulin	µU/mL	< 25 µU/mL
Blood glucose	mg/dL	126(4)
FBS	mg/dL	70 to 99(1)
HDL-C	mg/dL	50-60(1)
LDL-C	mg/dL	<130(1)
Total Cholesterol	mg/dL	<200(1)
Triglyceride	mg/dL	40-160(1)
Testosterone (female)	ng/dL	15 to 70(9)

3.5.5 Statistical analysis

All data was collected and analyzed using SPPS statistical software version 20. Descriptive statistics was performed using means and standard deviation for continuous variables, frequency and percentage for categorical variables.

Baseline characteristics were compared according PCOS using chi-squared test for categorical variables and student t-test for continuous variables.

Differences between baseline and 6 months for anthropometric assessment, nutritional assessment, physical activity questionnaire, hypertension measures, biochemical analysis, reproductive questions, hirsutism score and psychological assessment were tested using paired t-test after checking normality of the difference between the 2 periods (baseline and 6 months).

A multivariate logistic regression analysis was carried out in order to assess the association between the characteristics of the study patients and nutrition education/counseling. A two-sided value of $P< 0.05$ is considered statistically significant.

3.5.6 Training of the research team

The training for the research team involved in this study was designed to orient staff about the project implemented. The training involved the gynecologist at the Obstetrics and gynecology clinics and the nurses involved in the diagnostic procedure. The training included definition of the syndrome and diagnostic criteria used, and patient approach. The training has been organized to allow the team to know their tasks during this study.

3.6 Ethical consideration

Human studies project need a formal procedure of ethical consideration and approval. This project required ethical consideration from the University of Westminster and the collaborating institution in Lebanon where PCOS patient were recruited. Our research involves recruiting human beings, intervene with them and obtain sensitive and deep data, collecting blood 3 times and measuring the study variable outcomes at the end point (after 6 months from baseline). This study ensured decreasing the relative weights of the study's risks which involves the blood withdrawal where a skin discoloration could have caused on the site of withdrawal. The recruiting physicians have the right to withdraw any patients from study if they evaluated a high risk on the patients. All information about the patient from this research project was kept in a locked office. Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental

agencies have direct access to the medical records which may be audited without violating confidentiality , the name of the patients is never used.

Basic principles of ethical practice

1. Informed consent

   After being diagnosed with PCOS patients were approached about the study and an information sheet was given to every patient to provide them with all the needed information and details. The information sheet stated guarantee of collected data and its compliance with the Data protection Act and the University of Westminster's data Protection Code.
2. Patient's full volunteer and no pressure for participation
3. Confidentiality assurance
4. No risk of taking part of the study
5. Patient's option to stop at any time of the study.

Ethical approvals for this study were submitted to the Institutional review board the American University of Beirut on June 25, 2014 and the ethics committee at the University of Westminster on July 24, 2015. Both approvals were received before the initiation of the study.

Risks and discomforts

Polycystic ovarian syndrome patient on treatment visit the Women's health center every 6 months for blood tests and abdominal ultrasound. Blood withdrawal may cause a discomfort or blue discoloration at the site of blood withdrawal. Other risk such as the feeling of being forced to lose weight will cause an additional risk. Following a daily nutritional guidelines and weight loss or weight maintenance plan may put the PCOS women under pressure to follow it and be a source of stress to abide by the guidelines on a daily manner.

Measure to reduce risks and discomforts

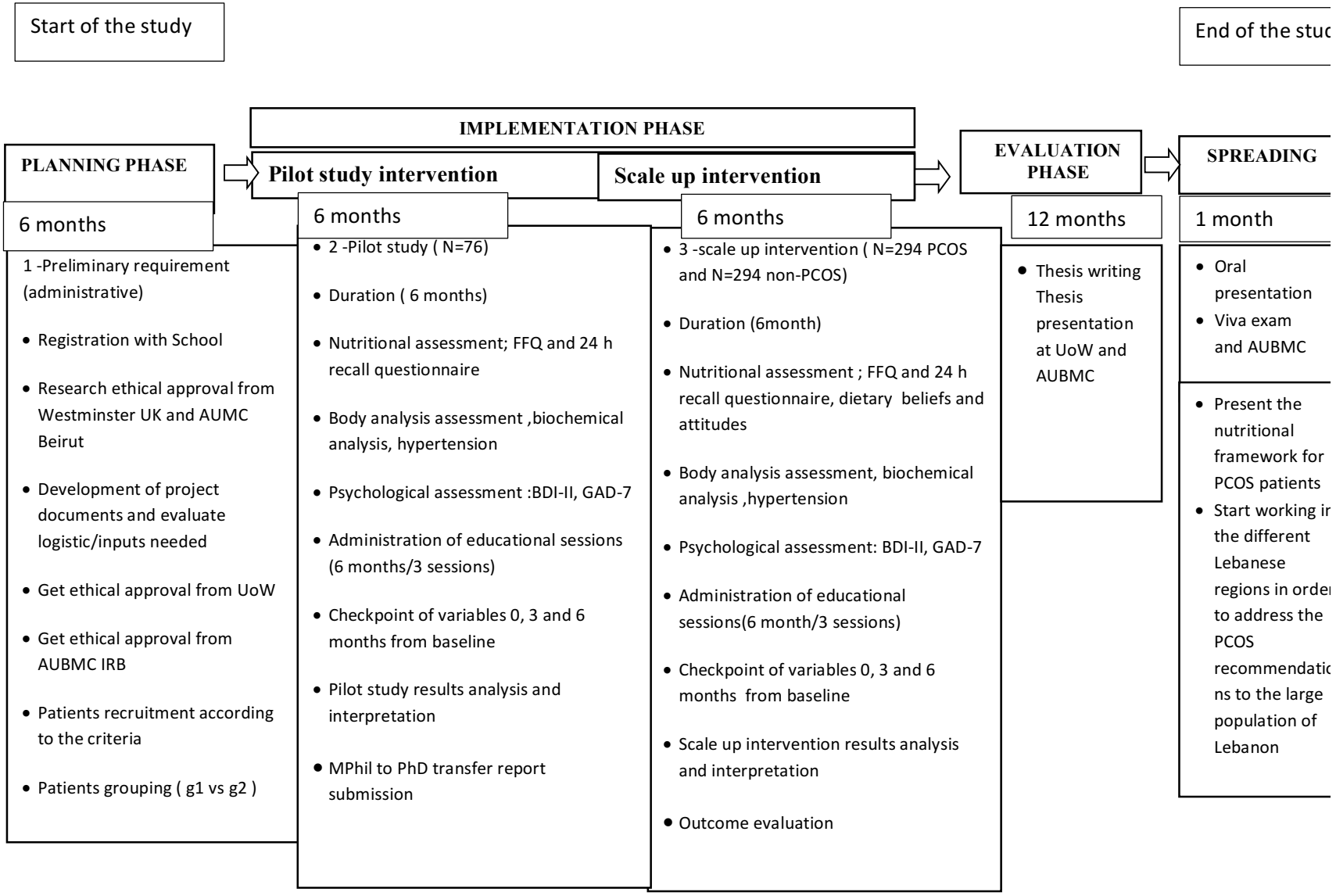
PCOS women who already had blood sample collected were asked to return on a couple of day in order to decrease any discomforts cause by blood withdrawal. The Medical laboratory team at the American University of Beirut Medical Center was responsible of blood withdrawal and analysis.

Regarding the compliance of nutritional guidelines PCOS women were informed and educated about the importance and benefits of adhering to these guidelines in order to improve their overall health status.
The research team was trained and always on reminder to approach diagnosed PCOS women.

Legal rights

Any women who participated in this study have not given up any of her legal rights by singing the informed consent form

3.7 Intervention, strategy-planning, implementation and evaluation phases



3.7.1. Planning phase

This phase was mainly focused on planning the research project, procedures and paper work. The aim was to create a plan for the timeline of the project and the activities included.

1- Administrative work

   An approval is needed before conducting any human being research and the documents need for the approval were prepared and submitted before the study initiation such as registering the at the UoW ,Registration document at the UoW , Ethical approval from the research ethics at Uow and IRB at AUBMC and the study details (sample size , recruitment and research team training).

2- Collaboration between the institutions in this phase included visiting the University of Westminster, and the American University of Beirut, Medical Center, Beirut, Lebanon. Then University of Westminster provided the workshop for PhD process and registration. The American University of Beirut, Beirut, Lebanon offers the facility to recruit patients, intervene with them and the Medical Laboratory analysis for blood samples. The Obstetrics and Gynecology department at the AUBMC offered a research course on how to undergo a research at the institution.

3.8.2 Intervention strategy –Implementation phase

Pilot study (N=76)

Pilot studies are normally carried out before scale-up interventions in quantitative research as a trial to evaluate the research and avoid any further investigation due to inadequacy in the designed project.

The pilot study population (n=76) were part of the scale up intervention population size. The pilot study is of valued vision because any missing detail from pilot study was added to the scale up intervention and thus improved the quality of outcomes.

Pre-intervention assessment – Pilot study

The following assessment were used

1. An assessment form approved by the IRB at AUBMC adapted to the aim and objectives of this research in a form of assessment tool (Appendix 3).
2. Medical and physical assessment; anthropometric measurement, medical history and demographic questions recorded in the appropriate section of the assessment form at baseline for all patients (Please refer to appendix 3).
3. Physical activity questionnaire (Please refer to appendix 4), Psychological questionnaires GAD-7 and BDI-II (Please refer to appendix 5), Hypertensive measurements, Food frequency questionnaire (Please refer to appendix 10), and biochemical assessment carried by the Medical Laboratory at the American University of Beirut medical center at baseline (Please refer to tables 4 and 5).
4. A 24 hour recall was conducted by phone call to the patient's one on a weekday and another one on a weekend and calories were calculated (Please refer to appendix 11).

3.8.3 Intervention phase: 6 months

The intervention phases included the following steps:

1: Randomization

Randomization of the PCOS women, and starting the nutritional intervention for overweight/obese group and the lean group for a period of 6 months.

2. Process checking to confirm that the project is following the ethical principles through the correct approach of the PCOS women, continuous feedback from the PCOS women to ensure their adherence to the allocated intervention and guidelines.

Weekly phone calls to the study patients to monitor their progress through the dietary intervention.

Follow-up calls to the patients to ensure their presence at the follow-up appointments and avoid any disturbance during the research timeline.

Routine checkup to the blood samples results to add them to the PCOS women data sheet.

3. Post-intervention assessment – Pilot study.

Assessments were conducted at baseline, after 3 months from baseline and after 6 months from baseline. Each assessment included all the variables used at baseline to ensure adequate data has been collected. Assessments were done to all groups (Interventions and controls).

Interventions groups were monitored every 2- 3 weeks at the hospital as a follow-up appointment to ensure their adherence to the guidelines and for any further questions.

4. Evaluation phase

The information collected before the intervention at baseline and after the intervention were compared. Data were entered after each assessment and after 6 months a statistical analysis was carried on.

Between pilot study and scale up intervention

After the pilot study the study patients progressed to the scale up intervention sample size. A number of study patients (N=30) of the pilot study patients continued their adherence to the guidelines for an extra 6 months which consisted a 1 year intervention.

Scale up intervention (N=588)

The pilot study results are evidence based and this determined by the scale up intervention were a larger sample size was used. During the scale up intervention non-PCOS women were recruited.

Pre-intervention assessment – Sale up

- A numerous of assessments were carried out at baseline
  An assessment form approved by the IRB at AUBMC adapted to the aim and objectives of this research in a form of assessment tool (Please refer to appendix3)
- Medical and physical assessment; anthropometric measurement, medical history and demographic questions recorded in the appropriate section of the assessment form at baseline for all patients.
- Physical activity questionnaire, Psychological questionnaires GAD-7 and BDI-II, Hypertensive measurements , Food frequency questionnaire , and

biochemical assessment carried by the Medical laboratory at the American University of Beirut medical center at baseline.

- A 24 hour recall was conducted by phone call to the patient's one on a weekday and another one on a weekend and calories were calculated.
- Biochemical indicators: (Vitamin D, insulin resistance, blood glucose FBS, HDL-C, LDL-C, total cholesterol, triglyceride level, total testosterone, insulin resistance) in order to assess the effectiveness of the nutritional education/counseling on COS biochemical indicators.
- Other PCOS conditions reported: regularities of menstrual cycle (seen by pregnancy onset, ovulation, and fertility)
- Hirsutism will be measured using the Ferriman-Gallewey score.
- Psychological assessment using BDI-II. This is a very widely used scale and it is one of the oldest consisting of 21 items self-report measure of cognitive, behavioral, affective, and somatic components of depression. Items on the BDI are rated on a 4 point type scale ranging from 0 to 3. Total scores are obtained by summing the highest ratings on each of the 21 items and may range from 0 to 69 with higher scores indicating symptom severity. The BDI has excellent 1 week test-retest reliability ($r=0.93$, $P<0.001$) and internal consistency ($\alpha=0.91$). Convergent validity for the BDI is satisfactory and was established through examining the correlation of its' scores with scores on the Revised Hamilton Psychiatric Rating Scale for Depression ($r=0.71$) (Beck *et al*., 1996a).GAD-7 is another scale that will be used to score for anxiety in PCOS patient, a short screening tool and validated (there is an Arabic version of it too).

Intervention phase – 6 months

The evaluation phase included randomization of the study patients for PCOS and non-PCOS women (weight matched), nutritional counseling and education using the dietary intervention and the proposed guidelines. This phase also included monitoring the scale up intervention groups for a period of 6 months.

Intervention strategy – Evaluation phase

Since scale up initiation evaluation was ongoing and study patients were called to ensure their attendance to the follow-ups appointments and assessment during the study timeline.

Non-PCOS women followed these proposed guidelines and results were compared to PCOS outcomes. After each data collection appointment, the data were entered. After the end of the scale up intervention assessment appointment data were analyzed and reported to MPhil report and Thesis.

Sustainability plan

Sustainability plan was intended after analyzing the outcome of the pilot study. The sustainability plan proposed is to blend the nutritional intervention program and guidelines after analyzing the outcome and finalizing the research.

The sustainability plan is to refer the PCOS diagnosed women at the Obstetrics and Gynecology clinics at the American University of Beirut Medical Centre, Beirut, Lebanon to be referred to the dietitian for counseling and assessment as part of their treatment along with any subscribed drugs.

Chapter 4

Pilot study results

4.1 Introduction

It allowed the researcher to adjust the aspects faced during the pilot study to be fully implanted in the scale up intervention. The pilot study allowed adjusting the project design, to meet the challenges faced in the field from patients' recruitment, to clinics.

4.2. Summary of Materials and Methods

There is no study up to date in Lebanon about the nutritional status for PCOS women and thus the need to map their intake and control it is crucial for their health status Patients prior to enrollment were subject to assessment (Appendix 3) using parameters such as personal and family medical history, anthropometric measurements, biochemical markers, menstrual cycle date marker, obstetrics and gynecology, medical history and socio-economic data. The patients were grouped according to their weight (normal weight and overweight/obese PCOS) and each group was divided into 2 subgroups the first was given nutritional intervention (nutritional counseling and intervention) and the second no intervention (controls) for a period of six months. The pilot study assessments were carried out three times (at baseline, after 3 and after 6 months) as mentioned in the study method.

4.3 Pilot study results at baseline, three months and six months follow up

4.3.1 Pilot study baseline characteristics: socio-demographic:

The pilot study included 76 PCOS patients divided equally between 4 groups, obese intervention , obese control , lean control ,and lean intervention (25%), having a mean age of 25.3 years. About 40% were married, with 31% were smokers, and 51% had a high-school educational level. Ninety six percent had irregular menstrual cycle, with a mean age at menarche of 12.4 years. Fifty percent were on oral contraceptives, and 72 % had acne. All baseline characteristics are summarized in table 7.

Table 7 Baseline characteristics of the recruited population

Character		Frequency(n=76)
Epidemiology of the disease among recruited PCOS patients	Age, mean (±SD)	25.3 (±5)
	Group, n(%)	
	A (obese intervention)	19 (25%)
	B (obese control)	19 (25%)
	C (lean control)	19 (25%)
	D (lean intervention)	19 (25%)
	Marital state, n(%)	
	Single	46 (60.3%)
	Married	30 (39.7%)
	Smoker, n(%)	24 (30.8%)
	Education, n(%)	
	< High school	13 (17.10%)
	High school	41 (59.94%)
	University-BS	19 (25%)
	Higher degrees	3 (3.8%)
	Home type, n(%)	
	Owned	66 (85.9%)
	Rented	10 (14.1)
	People living at home, mean (± SD)	4.2 (±1.3)
	Working, n(%)	37 (47.4%)
	Yearly income in $, mean (±SD)	44,835 (±20,791)
	Medical payment, n(%)	
	Private	40 (52.6%)
	NSSF	10 (13.1%)
	Insurance	17 (22.3%)
	HIP	9 (11.8%)

Gynecologic	Gravida, n(%)	
	0	58 (76.3%)
	1	8 (10.5%)
	2	7 (9.2%)
	3	3 (3.9%)
	Para, n(%)	
	0	66 (86.8%)
	1	7 (9.2%)
	2	3 (3.9%)
	3	2 (2.6%)
	Age at menarche, mean (± SD)	12.4 (±1)
	Period, n(%)	
	Irregular	74 (96.2%)
	Regular	2 (3.8%)
	Sexually active, n(%)	27 (34.6%)
	Contraception, n(%)	39 (50%)
	Hormonal therapy, n(%)	8 (10.3%)
	Acne, n(%)	56 (71.8%)

4.3.2 Pilot study anthropometric measurements

Anthropometric measures are shown in tables 8 in comparison between interventions and controls and their subgroups (overweight/obese and lean) between baseline and 6 months. The mean change in body weight after 6 months differed between the 2 groups where patients receiving intervention lost an average of 7.7Kg for overweight/obese patients and 0.5 for lean patients while controls gained about 3.6 kg for overweight/obese patients and 0.3 for lean patients with $p<0.001$ which corresponded to an average 9.4 % loss in body weight with overweight/obese interventions ,1 % with lean patients and 3.6% gain in body weight with overweight/obese controls and 1.3 % lean controls ($p<0.001$), noting that 19% of overweight/obese patients receiving intervention met the primary goal

of at least 5% loss in body weight compared to none of the controls (p<0.001). When comparing the subgroups of overweight/obese and lean patients, it was found that overweight/obese patients who took intervention lost an average of 7.7 Kg while controls gained an average of 3.1 Kg at the end of the study (p <0.001), whereas lean patients had no significant change in their weights whether they took intervention or not (p=0.084). Concerning BMI change, a significant difference occurred between interventions and controls where interventions had a decrease of an average 2.4 kg/m ^{2} of their BMI compared to controls who had an increase of 0.9 kg/m ^{2} in their BMI (p <0.001). This difference remained significant in subgroups of overweight/obese patients where interventions had an average decrease of 3.2 in their BMI (p <0.001) and in subgroups of lean patients the change was negligible. Also there existed a significant difference in waist and hip circumference between general interventions and controls and in overweight/obese subgroup patients (P <0.001) where overweight/ obese patients receiving intervention were having a significant decrease in these measures (-7.2 Kg and -5.1 Kg respectively) compared to those with no intervention (+2.0 and +1.3 respectively), but this change was not significant in lean patients. In addition, a significant difference occurred between interventions and controls regarding EBF (3.6% decrease in interventions compared to 1.1% increase in controls, p=0.002).

4.3.3 Pilot study gynecology results

Time to ovulation was measured at the time of intervention, as shown in table 8, and showed to be faster in overweight/obese patients receiving intervention (47.2 days) compared to overweight/obese controls (50 days). While lean interventions time to ovulation 42.5 days and lean controls 49 days. This difference was not significant statistically (p=0.648) between the 4 groups.

4.3.4 Pilot study biochemical markers results:

It was noted that no significant difference occurred between interventions and controls regarding the change in their biomedical markers, as shown in table 9, nor between the subgroups compared, however; testosterone showed to be in high levels (43 ng/dL), and Vitamin D in low levels (12.2-13.5 ng/dL).Moreover, it was

noted a higher mean decrease in LDL-C in obese control group compared to obese intervention (-22 vs -13 mg/dl).

4.3.5 Pilot study psychological status results:

Regarding the 3 scores used to measure psychological and physical activity (BDI-II, GAD-7, and PA), as shown in table 9 a significant difference occurred after providing the nutritional intervention ($p=0.014$, $p <0.001$, $p <0.001$ respectively). When taking obese patients separately, high depression and anxiety scores occurred, with a significant change when providing intervention ($p=0.002$ for GAD7), however for lean patients no significant difference occurred between interventions and controls with normal depression and anxiety scores.

4.3.6 Pilot study physical activity status results:

Regarding physical activity, obese patients receiving intervention had an average of 3.6 increase in their PA score, which was significantly different than controls ($p <0.001$), whereas lean patients had a borderline significant change ($p=0.056$) between interventions and controls.

Table 8 Variation in weight and reproductive characteristics between interventions and controls

Variable	Overweight/ Obese Interventions (n=19)	Overweight/ Obese Controls (n=19)	Lean Interventions (n=19)	Lean Controls (n=19)	p-value
Weight at baseline, mean (± SD)	81.2(±13)	84.5(±13	52.8(±3.3)	58.5(±5.9)	0.002
Individuals who lost weight, n(%)	19 (25 %)	0 (0 %)	12(15.7 %)	6 (7.9%)	**<0.001**
Individuals who gained weight, n(%)	0	14(18.4%)	0	5(6.5 %)	**<0.001**
Change in body weight(Kg),mean (± SD)	-7.7 kg (±5.5)	3.1 (±3.8)	-0.5 (±0.6)	0.8(± 2.3)	**<0.001**
% change in body weight, mean (± SD)	-9.4(±6.2)	+ 3.6 (±4.8)	-1 (± 5.5)	+ 1.3 (± 4.2)	**<0.001**
Achieved 5% loss in body weight,n(%)	19 (25 %)	0 (0 %)	0 (6.5%)	0 (0 %)	**<0.001**
Time to ovulation (number of days), mean (± SD)Kg	47.2 (±32.1)	50(±31.6)	42.5 (±33.4)	49 (± 32)	0.648

Statistically significant results are shown in **boldface.**

Student-t-test was done for continuous variables to get the mean and Chi ^{2} for binary variables to get percentages.

Table 9 Variation in anthropometric, biochemical, psychological and physical activity parameters between the study population groups

Factor	A Overweight/Obese intervention (n=19)		B Overweight/Obese control (n=19)		p-value	C Lean control (n=19)		D Lean intervention (n=19)		p-val
	Mean at baseline	Average change after 6 months	Mean at baseline	Average change after 6 months		Mean at baseline	Average change after 6 months	Mean at baseline	Average change after 6 months	
Weight (kg)	81.2(±13)	-7.7(±5.5)	84.5(±13)	3.1(±3.8)	**<0.001**	58.5(±5.9)	0.8(±2.3)	52.8(±3.3)	-0.5(±0.6)	0.08
BMI (kg/m ^{2})	31.7(±6.1)	-3.2(±2.4)	32.6(±5.1)	1.2(±1.5)	**<0.001**	22.2(±1.7)	0.4(±1.1)	20.3(±1.5)	-0.3(±0.5)	**0.04**
Waist circumference (cm)	103(±11)	-7.2(±5.4)	106(±12)	2.0(±2.5)	**<0.001**	85.5(±7.8)	0.6(±1.2)	82.2(±5.1)	0.25(±1.1)	0.45
Hip circumference (cm)	116(±13)	-5.1(±3.3)	115(±10)	1.3(±1.4)	**<0.001**	99.2(±7)	0.4(±1.1)	92.4(±3.8)	-0.1(±0.3)	0.22
CRP (mg/L)	8.3(±8.6)	-3.6(±6.6)	6.5(±5.9)	-0.7(±3.4)	0.154	1.4(±1.9)	-0.2(±1.0)	1.5(±2.2)	-0.6(±1.4)	0.46
LDL (mg/dl)	108(±38)	-13(±34)	127(±37)	-22(±39)	0.505	96.1(±54)	-12(±27)	94.7(±23)	-20(±40)	0.60
HDL(mg/dl)	52.2(±13)	1.0(±4.7)	50.9(±13)	1.0(±4.5)	0.978	61.0(±15)	-0.8(±14)	59.9(±13)	-4.0(±7.2)	0.58

TG (mg/dl)	75.2(±28)	-2.0(±9.1)	120(±53)	-9.1(±33)	0.375	69.4(±30)	-6.5(±22)	99.6(±44)	-25.0(±56)	0.40
T Chol(mg/dl)	176(±44)	-12(±31)	202(±38)	-23(±40)	0.441	171(±64)	-14(±30)	174(±34)	-29(±51)	0.43
Testosterone (ng/dL)										
Vitamin D										
BDI-II	3.08(±0.9)	-0.9(±1.1)	3.5(±0.8)	-0.1(±0.6)	0.062	2.57(±1.0)	-0.2(±0.6)	3.22(±0.8)	-0.6(±0.5)	0.15
GAD7	2.92(±0.5)	-1.1(±0.8)	2.79(±0.6)	0.1(±0.5)	**0.002**	2.14(±0.9)	0.0(±0.4)	2.50(±0.7)	-0.4(±0.5)	0.06
PA (hours)	0.23(±0.5)	3.6(±1.7)	0.23(±1.0)	0.07(±0.3)	**<0.001**	0.64(±0.8)	0.9(±2.1)	1.5(±2.7)	2.5(±1.0)	0.05

- Statistically significant results are shown in **boldface.**
- The p value refers to comparison of mean change between baseline and 6 months.
- BMI: Body Mass Index, CRP: C - Reactive Protein, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, TG: Triglycerides, T. Chol: Total Cholesterol, BDI-II: Beck Depression Inventory-II, GAD-7: Generalized Anxiety Disorder 7, PA: Physical Activity.

4.3.7. Food Frequency Questionnaire and 24 hour recall:

Food intake was measured by FFQ at 0, 3, and 6 months in order to study effect of nutritional intervention on food intake, and these are shown in table 10.FFQ was analyzed by calculating the serving sizes. Average caloric intake via 24 hour recall was analyzed using NutriPro software. Patients who received intervention in the overweight/obese had a significant decrease in number of portions of most high calorie food items including bread and carbohydrate portions ($p<0.001$), fruit portions ($p=0.027$), fats and oils whether saturated or unsaturated ($p=0.040$ and 0.028respectively), high fat meat ($p=0.030$), and sweets ($p=0.010$), and an increase in lean meat and vegetables. However, overweight/obese controls had no change in most types of food items except for an increase in bread portions by 3 portions), in saturated fat (+ 1), high fat meat (+ 2) and in sweets .The difference between overweight/obese interventions and controls is significant in bread intake.

In the lean intervention groups there was a decrease in portion size in bread (- 1), fruits (- 1), saturated fat (- 2) and sweets. The lean controls group had an increase in portion in the bread (+1), high fat meat (+1), saturated fat (+1) and sweets (+1). There was no statistical significance between the lean intervention and control groups.

Table 10 Variation in food serving size between the study groups employing FFQ

Food items	Overweight/obese (n=38)		p-value	Lean (n=38)		p-value
	Interventions	Controls		Interventions	Controls	
Breads (30g)	- 3	+ 3	**< 0.001**	- 1	+ 1	**0.025**
Dairy products (30g)	0	0	0.890	0	0	0.890
Fruits (1 serving)	- 3	0	**0.027**	- 1	0	0.035
Fats/Oils						
MUFA/PUFA	+2	0	**0.070**	0	0	0.950
Saturated	- 2	+1	**0.028**	-2	+1	0.035
Meat(90 g)						
High fat	- 2	+ 2	**0.030**	0	+1	0.047
Medium fat	0	0	0.790	0	0	0.920
Lean	+2	0	**0.045**	+1	0	0.043
Vegetables(1cup)	+3	0	**0.025**	+1	0	0.038
Sweets	- 3	+ 3	**0.010**	-1	+1	**0.029**
Beverages	0	0	0.910	0	0	0.880

Statistically significant results are shown in **boldface.**

Student-t-test was done to get the mean number of serving (rounded to the nearest ones).

4.4 Discussion

The justification of recruiting non-PCOS women was to assess the effect of the researcher intervention on non –PCOS women and compare the outcomes with PCOS women. This helped us determining the effect of diet on both overweigh/obese and lean PCOS patients after being subjected to nutritional education sessions focusing on promoting healthy food choice suitable for their condition, weight loss if needed and promoting physical activity and the effect of this intervention on non – PCOS women.

Weight management among interventions

A significant number of studies have investigated the effect of weight loss programs in synchronization with pharmaceutical agents rather than weight loss/maintenance unaided.

In this pilot study, the weight loss seen among the overweight/obese intervention group who followed weight management sessions was higher (7.7Kg (±5.5)) than the lean weight maintenance group (0.5 Kg(±0.6)) . On the other side, overweight/obese control group had more weight gain (3.1 Kg (±3.8)) than the lean control group (0.8 Kg (±2.3)).

The changes seen between the four groups were due to the obedience of the intervention groups to the nutritional intervention (a tailored weight management plan) and the weight gain amongst control groups who did not follow any dietary intervention. Consequently, the dietary intervention was potentially dynamic in term of weight loss/management for a period of time (6 months) to endorse a targeted 5-10 % weight loss of initial body weight. A recent study on overweight/obese PCOS patients following a low carbohydrates/low dairy diet for eight weeks showed a weight loss higher than the weight loss seen in our study (Phy *et al*.,2015).

By linking these results to the pilot study results, the low carbohydrates/low dairy diet was more effective in the short term (8 weeks) than the hypocaloric diet followed in the pilot study. However, in the long run to preserve a sustainable weight loss, the six months hypocaloric diet seemed effective for PCOS patients who were vulnerable

to weight gain. During the scale-up intervention, the-pilot-study-overweight/obese - intervention-group will be followed for an extended period to confirm the maintenance efficiency of the hypocaloric six months weight loss diet.

Another study by Moran *et al* in 2006 indicated a similar average weight loss of 5.7 Kg, but on a shorter period of intervention (8 weeks) (Moran *et al*.,2006). This is due to the difference in type of diet management (meal replacement versus hypocaloric –pilot study), where the caloric intake is controlled by meal replacement, while the hypocaloric diet is not controlled due to differentiation in meal preparation among the PCOS patients. So practically on the long term, hypocaloric diet is more efficient, which will be tested further in the scale-up intervention.

Anthropometric changes pre- and post-intervention

A significant reduction was seen in waist circumference ($P<0.001$) amongst the overweight/obese intervention group, which was comparatively equal to the low starch/low diary 8 weeks diet (Phy *et al*., 2015) ($P < 0.0001$). A variation in the anthropometric indices like waist circumference can be seen in low starch/low diary weight loss group due to the exclusion of carbohydrates and simple sugars from the regime (Phy *et al*., 2015). This is why a quicker modification in waist circumference was seen in association to the pilot study results.

Low starch/low diary diet management is effective in reducing waist circumference and abdominal obesity amongst PCOS patients. This pilot study gives evidence that a non-elimination diet with hypocaloric dietary management can offer similar results even for a longer period of time (6 months). The intervention patients in the lean group gained fewer centimeters in waist circumference compared to control group This pilot study demonstrates that a six months dietary intervention gives an improvement in the body composition of overweight/obese PCOS patients and decrease the progress towards obesity in lean PCOS patients.

The BMI in intervention overweight/obese PCOS patients decreased and in control patients increased by significantly ($P < 0.001$) and this is due to the weight loss accomplished after the intervention. In the weight loss group, the BMI decrease is clinically significant in reducing diabetes and metabolic syndrome in the global non-PCOS population, which improves fertility issues in the PCOS population (Moran *et*

al., 2006). The change seen in BMI pre and post-intervention is similar to other studies, but on a shorter period of time (8 weeks) (Phy *et al*., 2015), which is due to the low starch/low diary diet followed by fast weight loss after weight management in relation to biochemical changes.

The 7% weight loss via nutritional intervention showed non-significant changes in biochemical markers (Fasting blood glucose; LDL-C, HDL-C, TC, TG, total testosterone, CRP and fasting insulin levels) with P values (0.40, 0.50, 0.55, 0.47, 0.74, 0.837, 0.13 and 0.64) respectively. These results are similar to Moran's *et al*. (2007) study outcomes, where no reduction in CRP levels was seen after 4% weight loss. This pilot study adds to the scientific data that a 7 % weight loss shows non-statistically change in CRP levels (P= 0.13). Even though former literature demonstrates that weight loss decreases CRP levels (Moran *et al*., 2007), it is not perceived in women with PCOS.

The lipid profile factors (total cholesterol, LDL-C, HDL-C, and triglyceride) also showed a non-statistically significant P-value reduction amongst overweight/obese group with P values (0.60, 0.59, 0.43 and 0.4) respectively. The outcomes after the study accomplishment indicated that a 7% weight loss was not adequate to alter the lipid profile parameters in PCOS patients after a hypocaloric weight loss diet. However, a 7.7 Kg weight loss in this pilot study was seen too minor to employ beneficial effects on the metabolic profile. In comparison, Phy's *et al*. (2015) research outcome presented that low starch/low dairy eight weeks diet statistically reduced total testosterone ($p = 0.008$) and fasting insulin ($P < 0.001$).

Consequently, insulin sensitivity is affected by reducing carbohydrate intake in PCOS patients in a short period of time (8 weeks) even though, with the comparatively equivalent weight loss compared to this pilot study. PCOS patients are at increased risk of cardiovascular disease and raised inflammatory indicators, and this requires an additional weight loss to exert metabolic benefits. A higher mean reduction in LDL-C was noted in the overweight/obese control group compared to the obese intervention, who were on LSM, due to high statins medication intake in this group (45.5 % of the total population) (Refer to appendix 14; figure 7).

Additionally, HDL-C was seen to be higher in obese control and this is due to reduction in LDL-C caused by statin intake. This might be the event for a longer

period of intervention need to improve the metabolic syndrome parameters in overweight, obese PCOS women. However, non- significant change in lipid profile except in HDL-C (P< 0.001) was seen after a 1-year lifestyle intervention for PCOS adolescents, which is due to altered population age range (adolescents PCOS girls) (Lass *et al*., 2001).

Hirsutism is clearly related to hyperandrogenism, and results recommend that total testosterone and SHBG are critical parameters in PCOS. Total testosterone is linked to hyperinsulinemia according to Lass *et al*. (2011). The alteration in weight seen in overweight/obese intervention group was not seen to be associated to the equivalent change in insulin and total testosterone level. The hypothesis stating that obesity and PCOS are linked to insulin resistance is supported in the pilot study outcomes. These effects will be evaluated further on the scale-up intervention. Long term suitability (long period weight loss plan and lifestyle change with promoting physical activity) and obedience are required to be examined in the scale-up intervention when a sub-population of the pilot population will continue for an extra 6 months on the weight management intervention and later compared to the population who follow a 6 months intervention.

Menstrual Regularity post intervention

In overweight/ obese group nutritional education and weight loss/maintenance have revealed a cost-effective intervention in terms of time to ovulation between intervention and controls. The time to ovulation in this study has confirmed to be different between intervention (overweight/obese and lean) and controls with no statistically significant difference and a P =0.648.

Time for ovulation was less in the intervention groups (overweight/obese and lean) due to the weight loss and increased physical activity while a longer time for ovulation was seen in controls. Lass *et al*. (2011) has shown equal results over a longer period (1 year) where amenorrhea reduced by 42% (P< 0.001) in the weight loss group. The study results are biased by the fact of ovulation induction medications intake by some PCOS women as part of the usual care have biased this factor, thus, an extra variable (number of menstrual period during the study duration) will be counted in the scale-up intervention. The pilot study results propose that a hypocaloric weight loss diet

with physical activity promotion and a 7.7 Kg average weight loss can reestablish menstrual cycle in overweight/obese women.

Many PCOS women in the population studied were looking to conceive following the intervention proposed, but obesity, and insulin resistance can alter their pregnancy outcomes and cause adverse effects such as preeclampsia. Consequently, a weight loss before pregnancy plan is desirable in order to conceive in a healthy BMI range.

Deviation to a healthy lifestyle

Significant results appeared in the overweight/obese intervention groups with an increased consumption of vegetables lean meat MUFA and PUFA when comparing the food frequency questionnaire before and after intervention. Major decrease seemed in saturated fat as well as sweets, in fruits, bread, high fat meat, causing a decrease in the weights of the patients. The lean intervention group results showed a decrease in high caloric food such as bread, saturated fat and sweets. This decrease has prevented them from proceeding to gaining weight by excess caloric intake compared to the lean control group who showed increase in unhealthy food consumption.

The intervention (Lean or Obese) groups were educated to consume healthy food items such as fruits and vegetables, lean proteins and MUFA/PUFA fats that can limit the likelihood of nutritional deficiencies, which may stop them from conception or adverse effects. This is the first study to offer a balanced nutritional weight loss plan. Thus, women who are concerned about their reproductive health can follow the proposed nutritional guidelines for the PCOS (overweight/obese and lean). The originality of this study is diverse from education on healthy lifestyle, food options, to physical activity promotion and tools to be used in the long term and not just for interventional purposes.

All PCOS population can use the proposed nutritional guidelines as tools in the future. The compliance of the PCOS patients to the pilot study's nutritional guidelines was seen in the changes seen in their food items intake suggested.

On the other hand, a significant increase of bread ($P=0.025$) and sweets intake due to their uncontrolled eating plan was seen amongst the control groups. These results are vital to promoting the awareness of the implication of nutritional guidelines and dietary behaviors for the PCOS patients.

In addition, menstrual regularity was altered by the significant increase in total physical activity among the overweight/obese intervention. A significant improvement was seen in psychological status between both intervention groups and both control groups with a P value 0.014 and a BDI-II with a P value < 0.001. This is the first study to assess the physiological state changes among all PCOS patients and to compare those following a weight loss/ maintenance diet. After study completion , it was lifestyle modification (weight loss/maintenance and physical activity) can improve the psychological status (anxiety, depression, and mood swings) amongst intervention groups (overweight/obese and lean PCOS). Considerably weight loss/maintenance is an important factor for PCOS patients in self-esteem restoration. The psychological scores were improved as a result of their compliance and in the future a possible sustainability indicator. Thus, this pilot study highlights the positive effects of physical activity and weight loss nutritional interventional programs in PCOS patients with psychological status disturbances, (anxiety and depression).

Pilot study results improving scale up design

This pilot study is a potential stage towards preventing or limiting obesity among PCOS population. The outcomes highlighted the inconvenience faced during body temperatures measurement at the different menstrual cycle stages to indicate the ovulation dates. Therefore, data collection in the scale up was changed to dates recording and life birth rate recording. It is suggested to count the number of cycles rather than the body temperature measurement in the scale up intervention. Another limitation seen in the pilot study is the single centered study population recruitment (one medical center as a setting of the study) at the capital of Lebanon. A single center study does not include different socio-economic and cultural status patients with. So in the scale up intervention four centers in different socio-economic settings and areas were included.

- All research assessment tools were refined to adapt to the higher population size in the scale up intervention. The monitoring plan was developed during the pilot study.

- The research team including the gynecologists were trained about the patient approach, data collection and diagnosis. The purpose of re-training them is to ensure good data collection with minimum bias.
- Data was reviewed following after each patient visit at the study site by the doctoral researcher and the gynecologist to assure completeness of data.
- Continuity of field check-ups by the researcher for monitoring purposes and detection of any potential hazards that may affect the flow of the study.
- Monitoring the effect of nutritional guidelines on patients' adherence and its results on anthropometric measurements and biochemical analysis.
- Sustainability plan (from outcome of conclusion and discussion) and guidelines follow-up by all gynecologists in order to guide their PCOS patients on the importance of nutritional intervention and lifestyle medication in AUBMC and another women’s health center in Lebanon.

Chapter 5

Results: Scale-up intervention

5.1 Introduction

The scale up intervention (n=588) outcomes involve the recruitment of pilot study patients (n=76) in the scale up intervention with an additional four hundred twenty-two patients. Patients are recruited based on the calculation method used. A sub-sample (n=30) of the pilot study patients were monitored for a total of 12 months' period of time. The scale up intervention was well organized to monitor the changes and sustainability plan involving the research team consisting of the researcher, the hospital staff team and the patients involved for a period of six months. Data collection was collected over this scale up period of time.

Objectives

i. To evaluate the effectiveness of public health nutrition through the pre-intervention versus the post-intervention assessment employing the study indicators (positive deviation to healthy life style, adequate/balance dietary intake, healthy body weight, optimum physical activity, optimum blood pressure, improved biochemical indicators) via pilot/scale up intervention.
ii. To design and optimize educational sessions tailored to PCOS patients in order to raise their nutritional awareness, enable informed healthy food choice and engage them in physical activity.
iii. To assess the rates of regularities of menses (examined by pregnancy onset and ovulation) in the participant groups and in matched control PCOS population receiving no intervention.
iv. To evaluate the efficacy of public health nutrition through the pre-intervention versus the post-intervention assessment employing the study indicators (positive deviation to healthy life style, adequate/balance dietary intake,

healthy body weight, optimum physical activity, optimum blood pressure, improved biochemical indicators) via pilot/scale up intervention.

v. To develop and optimize the nutrition framework for people living with PCOS in Lebanon.

Diagnosed women with PCOS (n=76) were approached about the study and invited to participate in the research at the clinics of Obstetrics and Gynecology of the American University of Beirut Medical Center, Beirut, Lebanon(during 2016-2017). Upon the applied sample size calculation, inclusion and exclusion criteria, 588 patients were designated over simple randomization. Patients diagnosed with PCOS and accepted to participate in the study were subject to an assessment form including medical history, obstetrical and gynecological history, anthropometric measures, menstrual regularity diary, biochemical parameters and socio-demographic indices. Patients who participated in the study were PCOS and non-PCOS. Each women group was subdivided into lean and over/obese group. PCOS women recruited were weight-matched with non-PCOS women. Study patients were grouped into interventions and controls according to their randomization number. Study patients received a nutritional assessment, counseling and education. Patients over 6 months were subject to a total of 3 educational sessions. Assessment was carried on at baseline, 3 months and 6 months after baseline as mentioned in the study methodology (details in Chapter 3).
The following data are presented for study after 6 months and then after 12 months.

5.2 Results

I. Baseline characteristics
    a. Socio-demographics
    b. Gynecology

II. Changes after 6 months
    a. Anthropometric measurements
    b. Gynecology
    c. Biomedical markers
    d. Psychological status and physical activity

e. 24-hour recall
f. Comparison between obese and lean patients
g. FFQ changes

III. Changes after 12 months
a. Gynecology
b. Anthropometric measurements
c. Biomedical markers
d. Psychological status and physical activity
e. FFQ Changes after 12 months for PCOS patients

5.2.1 Baseline characteristics

Socio-demographics

The study included 588 patients, half of which had PCOS (294). Table 11 summarizes the socio-demographic baseline characteristics. Most patients in this study were in the middle age group (20-30 years) (80.4%), patients were matched in age and weight between PCOS and non-PCOS groups, where similar percentages occurred in the 2 groups with a non-significant p-value. More than 60% of the patients were single, and this distribution occurred similarly between the 2 groups with a non-significant p-value. About 13.9% of the females in general were smokers, but there was a significant difference between the 2 groups where 20% of PCOS patients were smokers compared to only 2.4% of the non-PCOS patients. About quarter of the patients were alcoholics and no difference between whether they had PCOS or not. Most of the patients had high school or university educational levels, and lived in owned homes with an average of 4 people living at home. About half of the patients were working (53%), with an average yearly income of about 40,501$ (44,770 for PCOS patients and 34,168$ for non-PCOS patients), and most paying privately their medical payments followed by health insurance plan payments.

Table 11 Baseline characteristics of all baseline scale up patients

Character	Total (N=588)	PCOS (n=294)	Non-PCOS (n=294)	p-value
Age, n(%)				
18-20	56 (9.6%)	27 (9.2%)	29 (9.8%)	
20-25	247 (42%)	106 (36%)	110 (37.4%)	0.677
25-30	226 (38.4%)	126 (42.8%)	123 (41.8%)	
30-35	59 (10%)	35 (11.9%)	32 (10.8%)	
Marital state, n(%)				
Single	393 (66.8%)	202 (68.7%)	186 (63.3%)	0.380
Married	195 (33.2%)	92 (31.3%)	108 (36.7%)	
Smoker, n(%)	82 (13.9%)	59 (20.1%)	7 (2.4%)	**<0.001
Alcoholic, n(%)	152 (25.9%)	75 (25.5%)	78 (26.5%)	0.887
Education, n(%)				
< High school	45 (7.7%)	33 (11.2%)	0 (0%)	
High school	240 (40.8%)	110 (37.4%)	139 (47.2%)	*0.002
University-BS	199 (33.8%)	90 (30.6%)	118 (40.1%)	
Higher degrees	104 (17.7%)	61 (20.7%)	37 (12.6%)	
Home type, n(%)				
Owned	431 (73.3%)	263 (89.4%)	128 (43.5%)	**<0.001
Rented	99 (16.8%)	29 (9.9%)	87 (29.6%)	
Family	58 (9.9%)	2 (0.7%)	79 (26.9%)	

People at home, mean (± SD)	4.0 (±1.4)	4.47 (±1.3)	3.14 (±1.1)	**<0.001
Working, n(%)	312 (53.0%)	142 (48.3%)	183 (62.2%)	0.036
Yearly income in $, mean (±SD)	40,501 (±17,526)	44,770 (±17,209)	34,168 (±16,106)	**<0.001
Medical payment, n(%)				
Private	240 (40.8%)	106 (36.0%)	39 (51.3%)	
NSSF	110 (18.7%)	60 (20.4%)	11 (14.5%)	*0.002
Insurance	55 (9.3%)	40 (13.6%)	0 (0%)	
HIP	183 (31.2%)	88 (30.0%)	26 (34.2%)	

Gynecology

As shown in table 12, more than 90% of patients had nulliparous, lower menarche age for PCOS patients (Mean age of 12.1 years for PCOS compared to 13.9 for non-PCOS patients). Fifty-three percent had irregular cycle (53.2%), however when comparing between the 2 groups, seventy-two percent most PCOS patients (79.2%) had irregular cycle compared to none in non-PCOS patients. Patients having PCOS were more sexually active (19%) compared to non-PCOS patients (6.1%)PCOS patients had more acne (81.6%) compared to non-PCOS patients (6.4%), lower average number of cycles (3.78 compared to 5.67) and longer time to ovulation (43.9 days compared to 12.6 days for non-PCOS patients).

Table 12 Gynecological baseline characteristics for all baseline scale-up patients

Character	Total (N=588)	PCOS (n=294)	Non-PCOS (n=294)	p-value
Gravida, n(%)				
0	540 (91.8%)	258 (87.8%)	294 (100%)	
1	19 (3.2%)	14 (4.8%)	0	*0.005
2	22 (3.7%)	16 (5.4%)	0	
3	7 (1.2%)	6 (2.0%)	0	
Para, n(%)				
0	561 (95.4%)	267 (90.8%)	294 (100%)	
1	15 (2.5%)	15 (5.1%)	0	0.062
2	8 (1.4%)	8 (2.7%)	0	
3	4 (0.7%)	4 (1.3%)	0	
Age at menarche, mean (± SD)	12.7 (±1.4)	12.1 (±1.0)	13.9 (±1.3)	**<0.001
Period, n(%)				
Regular	275 (46.8%)	61 (20.8%)	294 (100%)	**<0.001
Irregular	313 (53.2%)	233 (79.2%)	0 (0%)	
Sexually active, n(%)	87 (14.8%)	56 (19.0%)	18 (6.1%)	*0.007
Contraception, n(%)	176 (30.0%)	92 (31.3%)	84 (28.6%)	0.872
Acne, n(%)	335 (57%)	240 (81.6%)	19 (6.4%)	**<0.001
Number of cycles, mean (±SD)	4.99 (±2.7)	3.78 (±1.1)	5.67 (±1.0)	**<0.001
Time to ovulation in days, mean (±SD)	32.3 (±28.2)	43.9 (±28.8)	12.6 (±11.8)	**<0.001

I. Changes after 6 months

5.2.2 Improved anthropometric indices

Initially patients were weight and age matched for the different groups and for PCOS and non-PCOS patients. In the PCOS group, the average initial weight was 86.4(±2.7) for intervention and 81.7(±6.4) for controls in the overweight/obese group. As for the average initial weight for in the intervention group was 53.5(±3.4) and 56.8(±3.9) for controls in the lean group. The difference between these groups was statistically significant with P= 0.001. In the non- PCOS group, the average initial weight was 87.1(±8.2) for intervention and 82.6(±9.1) for controls in the overweight/obese group. As for the average initial weight for in the intervention group was 54.4(±6.5) and 51.0(±2.0)) for controls in the lean group.

However, when studying the change in the body weight after the 6 months between the different groups, as shown in tables 13 & 14 there was a low statistical significance in this change whether in the PCOS group or the non-PCOS group (P=0.002 and P=0.005). PCOS patients who got the intervention lost about 8.2 Kg in the overweight/obese group and 1.5Kg in the lean group .Whereas, the control ones in the PCOS overweight/obese group gained about 4.96 Kg and the lean 3.13 Kg. As shown in tables 13 & 14 there was a statistical significance between the PCOS groups (P= 0.001).In the non-PCOS group patients who got the intervention lost about 11.6 Kg in the overweight/obese group and 0.81 Kg in the lean group. Whereas, the control ones in the PCOS overweight/obese group gained about 1.22 Kg and the lean 0.23 Kg. The difference was statistically significant in both PCOS and non-PCOS groups (P=0.001; P=0.001).

When studying the % change in the body weight, PCOS patients who got the intervention lost an average of 10 .03% in the overweight/obese group and 2.8 % in the lean group of their body weight. In the PCOS controls groups 90% of PCOS

overweight/obese and 16.3% of lean interventions met the primary goal of 5% loss in body weight. In comparison to the controls PCOS group patients gained an average of 5.74 % in the overweight/obese groups and 5.5 % in the lean group with no patients in the control groups meeting the 5 % loss in their weights (P-value significant in all cases). Whereas non-PCOS patients who got the intervention lost a slightly higher percentage of their weights than PCOS ones (13.3 Kg for overweight/obese group and 1.48 Kg for lean group) and 100 % of the overweight/obese group met the 5 % loss in body weight while only 16.7% in the lean maintenance group. Note that the intervention patients met the 5 % loss in body weight in more than. 50% of cases whether they had PCOS or not, with a statistical significance (P =0.001; P=0.001).

Most body composition parameters decreased significantly with intervention patients but stayed the same or increased with the control patients in both PCOS and non -PCOS groups.

PCOS overweight/obese patients who got the intervention showed a decrease in waist circumference of 4.5 cm (compared to an increase 2.0 cm for controls). Non-PCOS overweight/obese patients who got the intervention showed a decrease in waist circumference of 6.7 cm (compared to no change for controls). As for the PCOS lean patient who got the intervention showed a decrease in waist circumference of 0.3 cm (compared to an increase of 4.2 cm for controls). The difference between these group is statistically significant with P=0.001. While the non PCOS lean had a decrease in waist circumference of 0.3 compared to no change for controls with no statistical significance between the non-PCOS group.\

Note that the non-PCOS patient who got the intervention had a bigger decrease in most parameters more than PCOS ones but the difference was no statistically significant.

5.2.3 Improved reproductive profile

As shown in tables 13 & 14 , there no significant difference between the intervention and control groups in the change in hirsutism score for both PCOS and non-PCOS patients where the score decreased slightly in the intervention groups

(by 0.14 for overweight/obese PCOS, 0.30 for lean PCOS, 0.01 for overweight/obese non-PCOS and 0.1 for lean non -PCOS) slightly decreased for PCOS groups and didn't change for non-CPS group.

On the other hand, time to ovulation and number of cycles changed significantly between groups in the PCOS and non - PCOS patients.

Time to ovulation was longer PCOS overweight/obese and lean control groups in comparison to non-PCOS patients and overweight/obese intervention groups. PCOS patients who got the intervention had a shorter time to ovulation (34.6 days for overweight/obese groups and 38.1 for lean group), than those who didn't with (52, 4 days to overweight/obese group and 53.6 days for lean group). The difference between the PCOS groups is statistically significant with $P < 0.001$. As for the non-PCOS who got the intervention time to ovulation different in the 4 groups with 17.4 days for overweight/obese intervention and 10.1 for controls , while the lean groups had 9.6 days for intervention and 13.1 for controls. The difference between the groups was statistically significant with $P<0.001$.

Similarly PCOS patients who got the intervention had more average number of cycles during the 6 months study duration (4.3 cycles) for overweight/obese group and (4.1 cycles) for lean group in comparison to the control groups with average (3.1 cycles for overweight/obese group and 3.6 for lean group). The difference between the PCOS groups is statistically significant with $P< 0.001$. On the other hand, the non -PCOS who received the intervention or not had an equal number of cycles with non -statistical significance between the groups ($P= 0.0.3$).

Regarding the pregnancy rate for PCOS patients , it was shown that it increased significantly with intervention patients in all groups compared to controls (from 0% to 70% compared from 0% to 15 % respectively)as shown in figure 6 below.

5.2.4 Biomedical and metabolic profile changes

The CRP decreased slightly for both intervention and control patients who had PCOS or not, but the most decrease was for the PCOS patients in the overweight/obese group who got the intervention where CRP decreased by 1.7 mg/l

(Refer to tables 15 & 16). However, FBS decreased for PCOS significantly in the overweight/obese and lean by 5.5 mg/dl and 3 mg/dl respectively compared to an increase in the FBS in the control groups by 7.4 mg/dl and 3.7 mg/dl for overweight/obese and lean groups respectively. The difference between PCOS gross was statistically significant with P<0.001. In comparison to non-PCOS patients FBS decreased for both interventions in the overweight/obese and lean groups by 5.5 mg/dl and 3.2 mg/dl respectively while controls showed an increase with significant P-value of <0.001.

Regarding the lipid profile, PCOS patients who got the intervention had a decrease in all lipid parameters (LDL, HDL, TG and total cholesterol) more than the control patients, but this difference was significant for LDL where P<0.001. Total cholesterol decreased by 13.5 and 19.7 for overweight/obese and lean PCOS intervention respectively compared to overweight/obese and lean controls with a decrease in 10.9 and an increase for lean control by 1,6. The difference was statistically significant with P<0.001 (Refer to table 15). Similarly non-PCOS patients who got the intervention had a decrease in lipid profile more than controls who had a slight increase in most lipid parameters, with a significant difference between intervention and controls for Total cholesterol that decreased by 6.2 for overweight/obese intervention and 0.6 for lean intervention and decreased by 0.6 and increase by 1 for overweight/obese and lean groups respectively(Refer to table 16).

Testosterone as indicator of hyperandrogenemia, decreased for PCOS by 3.2 ng/dL for overweight/obese group and 2 ng/dL for lean groups who got the intervention but increased by 1.9 ng/dL and 1.5 ng/dL respectively for overweight/obese and lean groups who didn't receive the intervention. The difference is statistical non-significant with P=0,120. However, non-PCOS patients had a slight decrease whether they got intervention or not (-0.01 for overweight/obese group, -0.03 ng/dL for lean group; and -0.06 ng/dL for control in the overweight/obese group and -0.04 ng/dL for lean controls).

Looking for insulin levels, PCOS patients who got the intervention had more decrease than controls (- 1.3 µu/mL for overweight/obese group and -0.6 µu/mL for lean group) and an increase for controls overweight/obese group by 0.5 µu/mL).

Whereas, non-PCOS patients who got the intervention had higher decrease than PCOS ones (- 3.8 µu/mL for overweight/obese group and -1.2 µu/mL for lean group compared to slight change for controls. The difference was not statistically significant.

Vitamin D levels improved slightly in PCOS patients whether they got the intervention or not (+0.71 for overweight/obese intervention group, +0.92 for overweight/obese controls, +0.59 for leas intervention and +0.80 for lean controls). Whereas, the change in non-PCOS patients was negligible.

5.2.5 Enhancement of psychological status and physical activity

Studying psychological status, 2 tests were used; first the BDI-II which decreased significantly between intervention and controls in the PCOS groups (P<0.001)(Refer to table 15), but the change was not significant between the non-PCOS patients (P=0.006)(Refer to table 16).

PCOS who got the intervention showed a decrease in BDI-II by 1.2 for overweight/obese intervention and 0.69 for lean intervention compared to an increase of 0.16 for overweight/obese controls and 0.08 for lean controls. Whereas, the non-PCOS groups who got the intervention showed a decrease by 1.6 for the overweight/obese group and 0.45 for lean group and an increase by 0.56 for the overweight/.obese control group and no change for lean control groups. The second score, GAD-7, showed a decrease by 0.9 units for overweight/obese intervention and by 0.2 units for lean intervention in the PCOS groups while controls showed an increase by 0.08 for overweight/obese controls and 0.06 units for lean controls. The difference was statistically non-significant (P= 0.027). Whereas, non-PCOS patients showed a decrease in score by 2.1 units for overweight/obese and a decrease of 0.5 units for lean intervention group, however, an increase for overweight/obese group was seen by 0.08 units and 0.45 units for lean. The difference was statistically non-significant (P=0.039).

A third score studied being the physical activity showed a significant increase for patients who got the intervention in general and for PCOS in specific. PCOS patients who got the intervention showed an increase in their physical activity by 3 hours for overweight/obese group and 2.22 hours or lean group compared to

controls who showed only 0.06 hours for overweight/obese and lean controls with P=0.001. Similarly, non-PCOS patients who got the intervention showed an increase in their physical activity (2.5 hours for overweight/obese group and 0.6 for lean group) compared to controls (0.08 hours for overweight/obese groups and 0.45 for lean group).

Table 13 Average changes in anthropometricand reproductive parameters among the 4 groups from baseline to 6 months in PCOS women

Factor	PCOS				p-value
	Obese		Lean		
	Intervention A (n=147)	Control B(n=147)	Intervention C(n=147)	Control D(n=147)	
Initial Wt (kg)	81.7(±6.4)	86.4(±2.7)	53.5(±3.4)	56.8(±3.9)	**<0.001**
Weight change	-8.2(±7.1)	4.96(±3.9)	-1.5(±2.1)	4.73(±3.45)	**0.001**
% change in weight	-10.03(±6.7)	5.74(±4.8)	-2.8(±3.6)	5.5(±6.4)	**<0.001**
Met 5% weight loss, n(%)	90%	0%	16.3%	0%	**<0.001**
BMI	-3.7(±3.01)	1.1 (±1.5)	-0.3(±0.5)	1.9 (±1.2)	0.025
Waist circumf. (cm)	-6.7 (±5.8)	2.0 (±2.5)	-0.08(±1.0)	4.2 (±2.5)	**<0.001**
Hip circumf. (cm)	-4.5 (±3.3)	1.3 (±1.5)	-0.3(±0.9)	2.8 (±2.0)	0.015
Hirsutism	-0.14(±1.1)	-0.06(±1.9)	-0.30(±1.6)	-0.03(±1.7)	0.13
Time to ovulation	34.6(±26.1)	52.4(±26.0)	38.1(±28.6)	53.6(±24.1)	**<0.001**
Number of cycles	4.3(±0.8)	3.1(±0.7)	4.1(±1.2)	3.6(±1.9)	**<0.001**

Table 14 Average changes in anthropometricand reproductive parameters among the 4 groups from baseline to 6 months in non –PCOS PCOS women

Factor	Non-PCOS				p-value
	Obese		Lean		
	Intervention E(n=147)	Control F(n=147)	Intervention G(n=147)	Control H(n=147)	
Initial Wt (kg)	87.1(±8.2)	82.6(±9.1)	54.4(±6.5)	51.0(±2.0)	**0.001**
Weight change	-11.6(±3.6)	1.01(±0.3)	-0.81(±3.4)	0.12(±1.0)	0.005
% change in weight	-13.3(±2.3)	1.22(±0.5)	-1.48(±3.9)	0.23(±2.0)	**0.001**
Met 5% weight loss, n(%)	100%	0%	16.7%	0%	**0.001**
BMI	-3.5 (±0.5)	2.9 (±0)	-0.5 (±0.8)	0.8 (±0)	0.020
Waist circumf. (cm)	-4.5 (±1.5)	0.0 (±0)	-0.3 (±2.0)	0.0 (±0)	0.008
Hip circumf. (cm)	-4.4 (±1.0)	0.0 (±0)	-0.4 (±0.7)	0.0 (±0)	0.025
Hirsutism	-0.01(±0.4)	0(±0.8)	-0.1(±0.0)	0(±0.0)	0.16(±1.1)
Time to ovulation	17.4(±14.2)	17.4(±12.3)	9.6(±3.2)	13.1(±3.4)	**<0.001**
Number of cycles	5.6(±1.4)	5.6(±1.9)	6.0(±0.0)	6.0(±0.0)	0.003

Table 15 Average changes in biochemical, psychological and nutritional parameters among the 4 groups from baseline to 6 months in PCOS

Factor	PCOS				p-value
	Obese		Lean		
	Intervention A (n=147)	Control B(n=147)	Intervention C(n=147)	Control D(n=147)	
CRP (mg/L)	-1.7 (±4.9)	-1.0 (±4.0)	-0.9(±1.6)	-0.08(±0.5)	**<0.001**
FBS (mg/dl)	-5.5 (±1.2)	+7.4(±3.1)	-3.0(±1.4)	+3.7(±0.3)	**<0.001**
Insulin (µu/mL)	-1.3(±0.2)	+0.5(±0.01)	-0.9(±0.02)	-1.2(±0.1)	0.033
Testosterone (ng/dL)	-3.2(±1.3)	+1.9(±0.4)	-2.0(±0.4)	+1.5(±0.7)	**0.001**
Vitamin D (ng/mL)	+0.71(±1.5)	+0.92(±0.1)	+0.59(±2.3)	+0.80(±1.7)	0.120
LDL (mg/dl)	-16.5 (±22)	-10.5 (±41)	-4.5(±17)	1.7 (±20.1)	**<0.001**
HDL (mg/dl)	0.61 (±3.4)	1.26 (±4.4)	-0.95(±5.0)	-0.13(±8.6)	0.071
TG (mg/dl)	-7.5 (±7.0)	-4.8 (±7.8)	-8.8(±7)	0.02(±1.6)	0.023
T-Cholesterol	-13.5 (±2)	-10.9 (±4)	-19.7 (±3)	1.6 (±1)	**<0.001**
BDI-II	-1.2 (±1.1)	0.16(±0.6)	-0.69(±0.4)	0.08(±0.3)	**<0.001**
GAD7	-0.9 (±0.8)	0.08 (±0.5)	-0.2(±1.6)	0.2 (±0.2)	**<0.001**
PA (hours)	3.0 (±1.5)	0.06 (±0.3)	2.22(±0.9)	0.06 (±1.3)	0.038
24 hour recall 1	-579(±319)	-55(±288)	-314(±157)	165(±141)	**<0.001**
24 hour recall 2	-688(±312)	-60(±219)	-421(±140)	0.5(±128)	**<0.001**

Table 16 Average changes in biochemical, psychological and nutritional parameters among the 4 groups from baseline to 6 months in PCOS

Factor	Non-PCOS				p-value
	Obese		Lean		
	Intervention E(n=147)	Control F(n=147)	Intervention G(n=147)	Control H(n=147)	
CRP (mg/L)	-0.25(±0.25)	0.0 (±0)	0.0 (±0)	-0.05(±0.0)	0.056
FBS (mg/dl)	-5.5(±2.5	+3.2(±3.2)	-3.2(±1.4	+2.2(±0.5)	**0.001**
Insulin (μu/mL)	-3.8(±0.8)	+0.2(±0.0)	-1.2(±0.3)	+0.1(±0.0)	0.024
Testosterone (ng/dL)	-0.01(±0.0)	-0.06(±1.2)	-0.03(±0.8)	-0.04(±0.5)	0.186
Vitamin D (ng/mL)	-0.002(±1.8)	0.003(±1.5)	-0.001(±2.3)	0.003(±1.8)	0.132
LDL (mg/dl)	-0.2 (±0.2)	-0.4 (±1.3)	-2.0 (±3.4)	-1.0 (±0)	0.034
HDL (mg/dl)	0 (±0)	0 (±0)	-0.45 (±1.1)	2.0 (±0)	0.536
TG (mg/dl)	-7.5(±5.1)	2.08(±2.2)	-1.8 (±4.3)	0.0 (±0)	0.002
T-Cholesterol	-6.2 (±0.8)	-0.6 (±1.6)	-1.5 (±4.0)	1.0 (±0.0)	**<0.001**
BDI-II	-1.6 (±0)	0.56 (±0.5)	-0.45 (±0.9)	0.0(±0)	0.006
GAD7	-2.1 (±0)	0.0 (±0)	-0.5 (±0.6)	0.0 (±0)	0.039
PA (hours)	2.5 (±0.5)	-0.08 (±0.2)	0.6 (±1.3)	0.45(±0.5)	0.021
24 hour recall 1	-461(±108)	-178(±85)	-210(±427)	116(±0)	**<0.001**
24 hour recall 2	-714(±183)	-31 (±144)	-204(±486)	-107(±0)	**<0.001**

All values represented as mean (±SD), except for met 5% loss in body weight as percentage

*p-values of comparison between PCOS groups and non-PCOS groups patients.

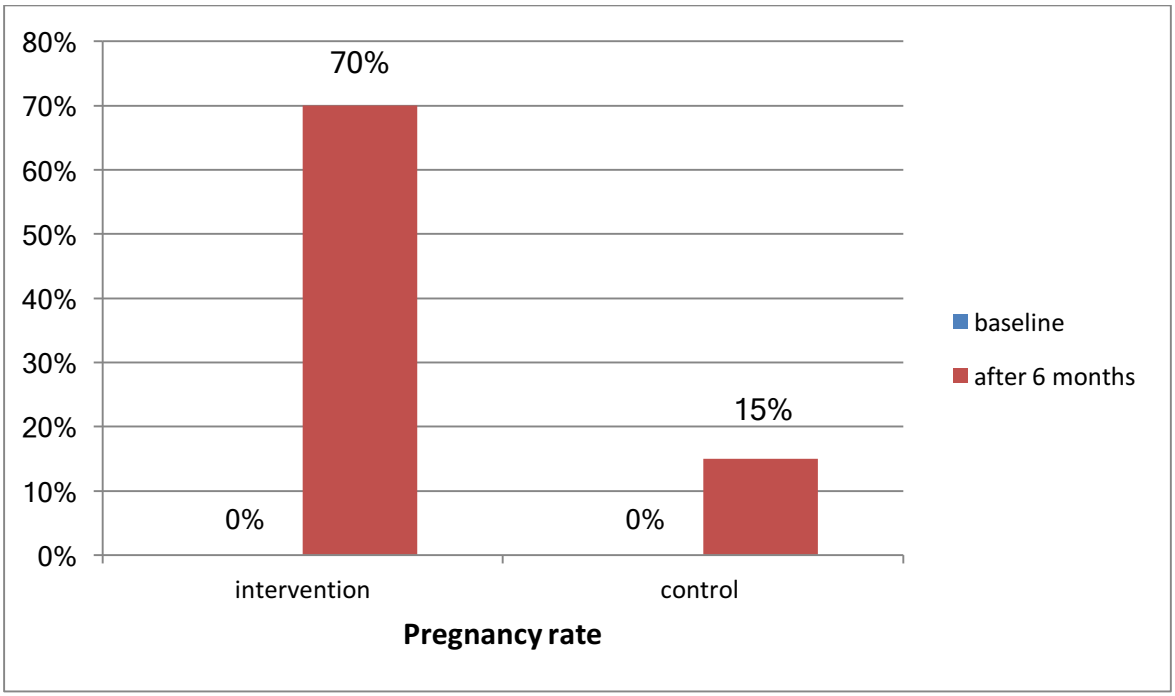


Figure 6 The graph depicts pregnancy rate in intervention and control group at baseline and after intervention

Table 17 Average change in values between baseline, 6 and 12 months for a subpopulation of PCOS

Factor	Between 0-6 (n=30)			p-value	Between 6-12 (n=30)			p-value	p-value*
	Intervention	Control	Total		Intervention	Control	Total		
Hirsutism score	-2.1 (±0.2)	0.3 (±0.7)	-0.8 (±0.3)	**<0.001	-2.0 (±0.2)	0.0 (±0.1)	-1.0 (±0.2)	**<0.001	0.089
Number of cycles	2(±0.3)	1(±0.4)	3(±0.7)	**<0.001	3(±0.2)	1(±0.5)	3(±0.7)	**<0.001	**<0.001
Waist circumference	-1.0 (±2.0)	1.5 (±2.5)	0.25 (±2.6)	*0.003	-4.0 (±4.1)	5.5 (±3.6)	0.75 (±6.1)	**<0.001	*0.034
Hip circumference	-3.0 (±3.0)	2.0 (±2.0)	-0.5 (±3.6)	**<0.001	-3.0 (±3.0)	5.5 (±1.5)	1.2 (±4.9)	**<0.001	**<0.001
Waist/Hip ratio	0.010 (±0.00)	-0.009 (±0.009)	0.0003 (±0.01)	**<0.001	-0.005 (±0.015)	0.010 (±0.03)	0.0025(±0.02)	0.074	**<0.001
% Body fat	0.0 (±0.0)	0.5 (±0.5)	0.25 (±0.4)	**<0.001	-0.05 (±0.5)	1.1 (±1.1)	0.55 (±1.0)	*0.001	**<0.001
% EBF	-3.0 (±3.0)	0.9 (±0.9)	-1.02 (±3.0)	**<0.001	0.0 (±0.0)	0.2 (±0.2)	0.10 (±0.1)	*0.001	**<0.001
% BM	0.1 (±0.1)	0.4 (±0.4)	0.25 (±0.3)	*0.007	0.4 (±0.3)	0.2 (±0.2)	0.30 (±0.2)	*0.030	0.518
BMI	-2.3 (±0.5)	0.7 (±0.7)	-0.8 (±1.6)	**<0.001	-2.8 (±2.9)	1.3 (±0.3)	-0.7 (±2.9)	**<0.001	**<0.001
BMR	10.0 (±13.3)	3.0 (±4.1)	6.5 (±10.3)	*0.046	4.5 (±4.6)	-5.0 (±6.1)	-0.2 (±7.2)	**<0.001	*0.004
Change in weight (kg)	-3.5 (±3.6)	2.0 (±2.0)	-0.75 (± 4.0)	**<0.001	-7.0 (±7.2)	4.0 (±1.0)	-1.5 (±7.5)	**<0.001	**<0.001
% change in weight	-3.6 (±3.7)	2.5 (±2.5)	-0.59 (±4.4)	**<0.001	-7.9 (±8.1)	6.5 (±3.1)	-0.68 (±9.5)	**<0.001	**<0.001
CRP (mg/L)	-0.8 (±0.0)	0.2 (±0.0)	-0.13 (±0.4)	**<0.001	-0.3 (±0.0)	0.1 (±0.1)	0.0 (±0.2)	**<0.001	**<0.001

FBS (mg/dl)	-7.5 (±1.5)	-2.0 (±4.1)	-5.7 (±4.8)	**<0.001	-3.0 (±2.0)	2.5 (±2.5)	-0.2 (±3.6)	**<0.001	**<0.001
LDL(mg/dl)	-7.5 (±3.6)	-10.5 (±12.8)	-9.0 (±9.4)	0.347	-11.0 (±0.0)	12.5 (±0.5)	0.7 (±11.9)	**<0.001	0.268
HDL (mg/dl)	0.0 (±1.0)	2.5 (±1.5)	1.2 (±1.8)	**<0.001	0.0 (±0.0)	0.0 (±0.0)	0.0 (±0.0)	-	-
TG (mg/dl)	-3.0 (±3.0)	0.5 (±2.5)	-1.2 (±3.3)	*0.001	-9.0 (±11.3)	3.0 (±2.0)	-5.5 (±12.5)	**<0.001	0.707
Testosterone (ng/dL)	-0.8 (±0.1)	-1.1 (±1.0)	-0.9 (±0.7)	0.189	-3.2 (±0.4)	-1.9 (±2.0)	-2.5 (±1.6)	*0.014	**<0.001
Insulin (μU/mL)	-0.9 (±0.7)	-0.3 (±0.4)	-0.6 (±0.5)	*0.013	-1.4 (±0.3)	-0.1 (±0.0)	-0.4 (±0.0)	*0.004	*0.034
Vitamin D (ng/mL)	2.2 (±0.0)	0.3 (±0.3)	0.96 (±0.93)	**<0.001	0.06 (±0.04)	0.00 (±0.10)	0.03 (±0.09)	*0.031	0.304
BDI-II	-0.5 (±0.5)	1.0 (±0.0)	0.5 (±0.5)	**<0.001	-2.0 (±0.0)	1.0 (±0.0)	-0.5 (±1.5)	<0.001	**<0.001
GAD-7	-2.0 (±0.0)	0.5 (±0.5)	-0.7 (±1.3)	**<0.001	-0.01 (±0.0)	-0.08 (±0.0)	-0.04 (±0.0)	<0.001	**<0.001
Physical activity (hours/week)	3.0 (±0.7)	0.0 (±0.0)	1.5 (±1.6)	**<0.001	1.2 (±0.4)	0.0 (±0.0)	0.6 (±0.7)	<0.001	**<0.001

5.2.6 24-hour recall

Final factor studied was the 24-hour recall that showed a significant change between intervention and control patients whether they had PCOS or not, however no significant difference occurred between PCOS and non-PCOS patients. Patients who got the intervention had a significant decrease in the 24-hour recall during weekdays (579 calories for PCOS overweight/obese intervention and 461 calories for non-PCOS patients overweight/obese intervention) and during weekends (688 calories for PCOS overweight/obese interventions and 714 calories for non-PCOS patients overweight/obese intervention) whereas patients who didn't get the intervention showed an increase in their calorie intake. These are shown in table 10.

Food Frequency Questionnaire

After the 6 months duration, it was noted that PCOS patients had a significant change in the number of portions of all food types where number of portions improved after the intervention for all food types. On the other hand, non-PCOS patients had some food types that didn't change significantly with the intervention such as potato, squash, organ meat, luncheon meat, and Arabic sweets. Changes in portions of all food types after 6 months are shown in table 18. Similarly, Patients who were followed for 12 months showed also a significant improvement in all food types after getting the intervention compared to those who didn't get the intervention where all high calorie food decreased in number of portions while low calorie food increased in their number of portions, as shown in table 21 (Refer to Appendix 12-14).

5.3 Comparison between obese and lean patients

Table 18 shows comparison in weight between obese and lean subgroups after the 6 months follow up. It was noted that PCOS patients who were obese lost more weight than lean patients when receiving the intervention (-8.2 and -1.5 kg respectively), and as a result 90% of those obese patients met the 5% loss in body weight compared to only 16.3% of lean PCOS patients. Note that obese non-PCOS

patients who got the intervention lost more weight than PCOS ones (-11.6 kg compared to -8.2 kg, making -13.3% compared to -10.03% body weight loss).

Table 18: Average change in weight after 6 months between the 8 study groups

	PCOS				Non-PCOS				p-value
	Obese		Lean		Obese		Lean		
	Intervention (A)	Control (B)	Intervention (C)	Control (D)	Intervention (E)	Control (F)	Intervention (G)	Control (H)	
Initial Weight (kg)	81.7(±16.4)	86.4(±12.7)	53.5(±3.4)	56.8(±3.9)	87.1(±8.2)	82.6(±9.1)	54.4(±16.5)	51.0(±2.0)	*0.001
Weight change (kg)	-8.2(±7.1)	4.96(±3.9)	-1.5(±2.1)	3.13(±3.45)	-11.6(±3.6)	1.01(±0.3)	-0.81(±3.4)	0.12(±1.0)	**<0.001
Weight change (%)	-10.03(±6.7)	5.74(±4.8)	-2.8(±3.6)	5.5(±6.4)	-13.3(±2.3)	1.22(±0.5)	-1.48(±3.9)	0.23(±2.0)	**<0.001
Percentage of the group that achieved minimum of 5% weight loss	90%	0%	16.3%	0%	100%	0%	16.7%	0%	**<0.001

5.4 Changes after 12 months

Table 19 12-months changes between intervention and control PCOS patients

Factor		Intervention	Control	p-value
Clinical Hyperandrogenism	Hirsutism score	-3.5 (±0.8)	0.4 (±0.2)	**<0.001
	Time to ovulation	49.5 (±2.5)	38.5 (±10.8)	**<0.001
	Number of cycles	3.0 (±0.1)	2.0 (±0.3)	*0.032
Body composition	Waist circumference	-5.0 (±6.1)	7.0 (±1.0)	**<0.001
	Hip circumference	-6.0 (±6.1)	7.5 (±0.5)	**<0.001
	Waist/Hip ratio	0.005 (±0.01)	0.0005 (±0.02)	0.470
	% Body fat	-0.05 (±0.5)	1.6 (±1.6)	**<0.001
	% EBF	-3.0 (±3.0)	1.1 (±1.1)	**<0.001
	% BM	0.5 (±0.2)	0.6 (±0.6)	0.522
	BMI	-5.1 (±3.4)	2.0 (±0.3)	**<0.001
	BMR	14.5 (±18.0)	-2.0 (±2.0)	**<0.001
	Change in weight (kg)	-10.5 (±10.8)	6.0 (±1.0)	**<0.001
	% change in weight	-11.0 (±11.3)	9.1 (±0.4)	**<0.001
Biomedical markers	CRP (mg/L)	-1.1 (±0)	0.3 (±0.1)	**<0.001
	FBS (mg/dl)	-12.5 (±3.6)	0.5 (±6.6)	**<0.001
	LDL(mg/dl)	-18.5 (±3.6)	2.0 (±12.3)	**<0.001
	HDL (mg/dl)	0.0 (±1.0)	2.5 (±1.5)	**<0.001
	TG (mg/dl)	-19.0 (±8.2)	3.5 (±4.6)	**<0.001
	Testosterone (ng/dL)	-3.05 (±0.3)	-2.05 (±3.1)	0.196
	Insulin (µU/mL)	-2.3 (± 0.3)	-0.4 (±0.7)	*0.004
	Vitamin D (ng/mL)	2.3 (±0.0)	0.3 (±0.2)	**<0.001
Scores of psychological assessment	BDI-II	-2.0 (±0)	1.0 (± 1.0)	**<0.001
	GAD-7	-2.0 (±0)	0.5 (±0.5)	**<0.001
	Physical activity	4.2 (±0.2)	0.0 (±0)	**<0.001

5.4.1 Anthropometric measurements

A significant difference occurred in weight change between interventions and controls where intervention patients lost about 10.5 kg making about 11% loss in their body weights while controls gained about 6 kg after the 12 months making about 9.1% gain in their body weights. Most body composition parameters had a significant improvement in the intervention group more than the control group except for waist/hip ratio and % body mass where a null change occurred similarly in both groups. The intervention patients had a 5 cm decrease in their waist circumference within 12 months period (compared to 7 cm increase with controls), 6 cm decrease in their hip circumference (7.5 increase with controls), 0.05% decrease in body fat (1.6% increase with controls), 3% decrease in EBF (1.1% increase with controls), 5.1 decrease in their BMI (2 kg/m2 increase with controls), and 14.5 units improvement in BMR (decrease in 2 units with controls)(Refer to table 19).

5.4.2 Gynecology of 12 months follow-up

Table 16 summarizes all factors studied for PCOS patients who were followed up to a period of 12 months. Hirsutism score decreased significantly by 3.5 units for intervention group compared to an increase of 0.4 units in the control group. Similarly, time to ovulation increased more in the intervention group (by 49.5 days) than the control group (increased by 38.5 days), and number of cycles also increased more in the intervention group than the control one (increased by 3 cycles and 2 cycles respectively).

5.4.3 Biomedical markers

All biomarkers except testosterone changed significantly between intervention and control PCOS patients who were followed for 12 months. CRP decreased by 1.1

mg/L in the intervention group compared to an increase by 0.3 mg/L in the control group. Similarly FBS decreased by 12.5 mg/dl in the intervention group compared to 0.5 mg/dl increase in control group. LDL decreased by 18.5 mg/dl compared to 2mg/dl increase with controls, TG decreased by 19 mg/dl compared to 3.5 mg/dl increase with controls, but HDL didn't change with intervention patients and increased by 2.5 mg/dl with controls. Testosterone decreased by 3.05 ng/dl compared to 2.05 ng/dl decrease with controls (but this difference was not significant), insulin decreased by 2.3 μU/mL compared to 0.4 μU/mL decrease for controls, and finally vitamin D improved by 2.3 ng/ml compared to only 0.3 ng/ml improvement with controls.

5.4.4 Psychological status (n=30)

Also scores of psychological status and physical activity changed significantly between intervention and control patients after 12 months follow up. The 2 psychological status scores (BDI-II and GAD-7) decreased by 2 units for intervention patients after 12 months, compared to a small increase in the control group (by 1 and 0.5 units respectively).

5.4.5 Physical activity

Physical activity improved by 4.2 units with intervention patients and didn't change for controls ($p<0.001$ for the 3 scores).

5.4.6 FFQ showed significant results

FFQ has shown statistically significant improvements in all food items. Results are shown in table 22 (Appendix 13).

Chapter 6
Discussion

A number of studies (please refer to appendix 7) have focused on the impact of diets adopted by women suffering from PCOS on the medical intervention periods and the weight loss; however, few have addressed the effect of nutritional guidelines and counseling on PCOS overweight or obese versus lean women and compared these results with non-PCOS women in their reproductive stages of life. This study demonstrates that nutritional guidelines and weight management provided to overweight and lean PCOS patients result in significant weight loss, improvement in reproductive capacity, biochemical and nutritional levels or states in women with PCOS.

The aim of the study was to assess the efficacy and effectiveness of public health nutrition intervention designed to create, enhance, and sustain healthy lifestyles and LSM among PCOS patients attending the Obstetrics and Gynecology Clinic at the American University of Beirut Medical Centre (AUB-MC) in Lebanon. This aim was based on the observation that some women with PCOS suffer from obesity, which has been shown that the disorder in some cases was developed due to weight gain (Moran *et al*., 2006).

To translate our aim into a quantitative well developed and controlled research study, the research was seeking to achieve a primary weight loss (from 5-10% of initial body weight) among overweight or obese PCOS, and weight maintenance among lean PCOS patient as well as measure the impact of such a dietary plan on the biochemical, psychological, and nutritional status . As such, a weight loss ranging between 5 to 10 percent of the initial body weight considered optimal to see improvements in both the mild symptoms of PCOS, the risk factors of metabolic syndrome and to tackle the insulin problem (Norman *et al*.,2004).

Turner-McGrievy *et al*., 2014 published a randomized pilot to assess the effect of weight loss on women with PCOS comparing vegan to low-calorie diet. Vegan women lost more weight at 3 months but both groups had no difference at 6 months intervention. This conclusion shows that low-calorie diet over a longer term (6 months) has a similar effect to any exclusion diet.

During the study, women involved had high adherence to the guidelines and this is due to the regular telephonic communication with them. The peer society pressure for the married women to get pregnant played a major role to their compliance in order to reach the favorable end results of getting pregnant. The applied nutritional guidelines proposed have shown successful results in PCOS treatment. Our employed guidelines involved dietetic intervention lacking psychological focuses on the implementation of healthy eating principles in addition to physical activity promotion as a combined treatment. The results of the study offer good proof that the use of these guidelines over a long-term upon diagnosis can help improve the symptoms of PCOS. They also provided strong evidence on the long term adoption improving the anthropometric and reproductive profile.

The metabolic syndrome parameters are still not widely used upon diagnosis for PCOS. In Lebanon, PCOS women showed prevalence of metabolic syndrome seen upon the laboratory tests conducted in this study. Women with PCOS have presented with underlying metabolic syndrome traits.

As such, this study discusses several interrelated topics in light of the pilot study followed by the scale up intervention conducted over a period of 6 months each. Below, we present the major topics that form the scope of the research study:

6.1 Baseline characteristics
6.2 Anthropometric measurements
6.3 Gynecology characteristics
6.4 Biomedical markers
6.5 Psychological status and physical activity
6.6 Nutritional assessment: 24-hour recall and FFQ
6.7 Twelve months follow-up for a subpopulation
6.8 Comparison PCOS versus non-PCOS women
6.9 Strengths and limitations

6.1 Scale up baseline characteristics:

During the pilot study (n=76) and scale up intervention (n=588) women attending the Obstetrics and Gynecology clinics at the AUBMC were recruited and followed up Most patients recruited fell in the age groups 2 and 3, i.e. age ranges between 20 to

25 and 25 to 30 years respectively. The age ranges show that most women suffering infertility and reproductive concerns fall in these two groups. Furthermore, it is important to note that most of the recruited population is of good educational standard (Bachelor and master levels). As a good educational background is important to raise awareness within the PCOS community regarding the disorder and the importance of adopting and adhering to lifestyle modification, this is of extreme significance to the aim of the research.

6.2 Scale up anthropometric measurements:

During the pilot study and scale up intervention, women suffering PCOS managed their weight by losing 7.7 Kg (±5.5) and 8.2 Kg (±7.1) respectively during pilot and scale up phases among overweight and obese intervention groups. On the other hand, the controls groups had no improvement in their weight status but portrayed an increase in their weight. As such, the percentage change in body weight during scale up intervention for PCOS women in comparison to PCOS women who did not receive any intervention lies within the set expectations.

The difference between the two groups is statistically significant. The patients met the primary goal of 5% loss in body weight compared to control patients who gained weight. These results are higher than the pilot study results where % change in body weight was -10.03% (±6.7) for PCOS overweight/obese interventions and 5.74 %(±4.8) for controls. The difference in results between pilot and scale up is due to recruiting a higher population size, which gives us a higher statistical power and accuracy of details.

In light of our findings, a previous study reported the effect of adopting a weight loss plan by employing the DASH diet employing healthy food advices in overweight and obese PCOS women (Asemi *et al*., 2014). The results of Asemi *et al*., (2014) showed a decrease in body weight of while this study results showed a greater reduction average body weight of interventions groups. The present data showed that a consumption of the proposed nutritional guidelines led to a significant reduction in weight in comparison to other studies.

In our research conducted on PCOS women within the intervention group, we noted that the study group managed to achieve weight loss of 5%-10% of their initial body

weight after 6 months of intervention. This is due to the compliance of the intervention groups to the professional nutritional counseling and follow up provided by the researcher. These results confirm the potential of the intervention for a period of 6 months in comparison to other studies.

Furthermore, the study demonstrates that a 6 -months nutrition intervention results in significant ($P<0.001$) weight loss in PCOS women, with no exclusion of any food groups or elements. By comparing these results to Turner-McGrievy *et al*.(2014) who employed low glycemic index vegan diet, this study proposed more improvement in term of weight loss (-9.5 Kg) with detriment to the research subjects over 6 months. We highlight the fact that since no food groups are being excluded from the diet, it is expected to facilitate for the research subjects in general to adhere to the diet. This raises the probability of adopting such dietary plans as part of the research subjects' lifestyles in the long run.

Furthermore, a study by Phy *et al*. (2015) employed a shorter period of intervention while measuring the weight loss in light of dietary management period lasting 8 weeks. The mentioned study adopted a low carbohydrates and a low dairy diet, which resulted in a weight loss of 8.6 Kg (± 2.3). Comparing these results to ours, the Phy *et al*. (2015) study has shown a more effective weight loss over a shorter period. The limitations in Phy's study is the dietary restriction used (lowering carbohydrates and dairy product) which may further decrease many minerals such as calcium in the first place. The dietary restriction can't be employed on the long term as a lifestyle modification for PCOS women. The dietary intervention is for research purposes and rather can't be used a nutritional guidelines for PCOS women on the long run.

Yet, as discussed earlier, the objective is to enable women suffering from PCOS to maintain nutritional balance for the longest periods possible as well as help them integrate into a healthier lifestyle especially those susceptible to weight gain. As such, the proposed diet along with the intervention time, do not fall short with the results delivered in light of those provided by Phy (2015).

In light of the above, Moran *et al*. in 2006 studied weight loss in PCOS women over 8 weeks period of time by promoting meal replacement and showed an average weight loss which falls below the results provided by our research study. The limitations of Moran *et al*.'s study in 2006 are the short term employment for this

study. Short term assessment cannot confirm the effect of such a nutritional intervention on the long term use by PCOS women. In addition, many PCOS women when being studied, meal replacement can cause a financial concerns for them, some can afford while others don't. The sample size used is too small (n=43).Yet again, providing PCOS women with meals is not potentially effective on the long run, which highlights the importance of incorporating a lifestyle modification that is long term, well planned, and affordable to all PCOS cannot be achieved by simple meal replacement and rather a combination of weight loss plan, nutritional education and guidelines to improve their lifestyle.

In our study, a significant decrease was seen in waist circumference ($P<0.001$) among the overweight or obese and lean intervention groups in comparison to the control groups who gained centimeters. Our findings were relatively lower in comparison to the low starch/low diary 8 weeks diet (Phy *et al*., 2015) which showed a significant decrease in waist circumference ($P < 0.0001$). As waist circumference is an indicator of abdominal obesity and linked to insulin resistance, a change in the anthropometric indexes such as waist circumference is interpreted in the case of the low starch/low diary weight loss group, due to the elimination of carbohydrates and simple sugars from the diet (Phy *et al*., 2015). This justifies that a shorter period of dietary intervention (8 weeks) can decrease abdominal obesity and waist circumference rather than longer period of time (6 months) in the study. Low carbohydrates dietary intervention has shown to decrease abdominal obesity and this is clearly shown in the results of Phy *et al*., (2015). Macronutrient limitation as part of research intervention can be employed over a short period of time (8 weeks) given significant results. Abiding for macronutrient restriction on the long term can be hard to PCOS women.

This scale-up study presents evidence that a non-elimination diet employing hypo-caloric dietary management can produce relatively similar results to the type of diets over shorter period. In the control groups, PCOS patients gained fewer centimeters in waist circumference due to their non-adherence to any nutritional intervention and thus progression to weight gain manifested by a change in anthropometric measurements.

Furthermore, in the scale-up intervention a decrease in percentage body fat was seen among interventions versus controls with P <0.001. These results were higher than the pilot study results (interventions versus -controls) (with P= 0.064). The difference in results is due to the recruiting of a higher population size in the scale up intervention (n=588) in comparison to the pilot study (n=76). As the decrease in body fat is essential to induce ovulation and improve the reproductive hormonal profile in PCOS women confirming improvement in their reproductive status.

Furthermore, a higher BMI decrease in the scale up study than pilot study for interventions groups. While controls had an increase in weight and thus BMI over 6 months period of time. The study results are higher than those reported in Asemi *et al*., (2014). The reason of the higher decrease in the study is the employment of a higher population size (n=147) in comparison to Asemi *et al*., (2014) study (n=24) and the longer period of dietary intervention (6 months versus 8 weeks). This suggests the need of a longer period of nutritional intervention such as 6 months to properly quantify and assess the improvements and impacts on the BMI indexes.

As such, we conclude that in relation to BMI, this study clearly reflects that over a 6-months period of dietary intervention improvement in the composition of PCOS patients and decrease the progression towards obesity in susceptible PCOS patients can be noted.

6.3 Scale up gynecology results:

Hirsutism score is utilized to assess the hair pattern distribution in PCOS women. According to the scale-up, study results both PCOS and non-PCOS women in the intervention groups had slight decrease in the hirsutism score with no statistical significance. The scale up results are similar to the pilot study results for interventions and controls with P =0.13 for PCOS groups in comparison to controls in scale-up for PCOS.

The results of this study are not in agreement with Naderpoor *et al*., (2015) study where hirsutism score decreased by 2.4 employing a sample size of 114 but not following weight loss through nutritional guidelines and counseling and rather metformin (insulin-sensitizing hormone) treatment. This concludes that metformin can induce a higher decrease in clinical hyperandrogenism in PCOS women than

nutritional guidelines. This conclusion cannot be followed on all PCOS women, as metformin is not prescribed to all PCOS women where no insulin resistance is diagnosed. Thus, the proposed nutritional guidelines for weight management for PCOS exhibit potential first line treatment in the long run and can be used for all the PCOS population and not just a selection.

In addition, the study demonstrated that weight loss using nutritional guidelines improves pregnancy rate among women trying to conceive and live birth rates in interventions groups with infertility. Seventy percent of the women trying to conceive (n=165) were able to conceive spontaneously noting that this study only included patient with PCOS etiology only for infertility. This suggests that the guidelines proposed may be used as well for PCOS women aiming to conceive. Hence, adopting a balanced dietary plan with no exclusions to food groups can result in positive outcomes in patients trying to conceive.

The results of the scale up study are in parallel with the findings provided by Kort *et al*., (2014). This demonstrates that in this case of a 10% weight loss of initial body weight ovulatory pattern in PCOS women can be restored following these guidelines. Intervention groups (overweight/obese and lean) had less time for due to the weight loss and increased physical activity while controls have a longer time for ovulation. Other findings such as those provided by Lass *et al*. (2011) showed similar results over a longer period (1 year) where amenorrhea significantly decreased ($P< 0.001$) in the weight loss group.

Before ending with a conclusion to this point, the scale up results are biased with the fact that ovulation induction medication are part of the usual care of PCOS pharmaceutical treatment. Hence, weight loss dietary intervention promotes ovulation induction agents in PCOS women and reproductive profile improvements cannot be achieved without weight loss in overweight/obese PCOS women.

As such, the number of cycles increased from baseline and after 6 months for interventions and slightly increased in controls with $P <0.001$ and this is due to ovulation induction drugs. This study revealed that the proposed guidelines and consequent weight management in intervention groups with PCOS lead to significant weight loss and thus increase in menstrual episodes. These findings are aligned with the finds provided by other studies such as Orstein *et al* ., 2011 who proved

improvement in menses regularity in overweight/obese women with PCOS after weight loss over 12 weeks period of time.

6.4 Scale up biochemical markers:

The percentage weight loss of 10 % in the overweight/obese intervention group showed significant changes in some of the biochemical markers (total cholesterol FBS, CRP, LDL, and testosterone) with P values < 0.001. These results do not fall in parallel with the pilot study results where non-statistical significant changes were noted. The C-reactive protein marker showed no statistical significance change in pilot study and this is due to the lower percentage of weight loss of initial body weight. The results are opposite to the findings of Moran *et al*., (2007) who found that CRP levels are not significantly changing by weight loss in PCOS women in a shorter period of time dietary intervention (8 weeks). In light of the different findings (ours vs Moran), our findings demonstrated the need for a longer period of testing (6-12 months) in order to detect an improvement in CRP levels change.

This scale up study proves that weight loss can cause significant (P<0.001)changes induced by weight to CRP and thus adds to the literature a new finding to be further looked at in the future in treating PCOS women who are at risk of metabolic syndrome.

On another note, the study over the 6 months period noted improvements in lipid profile of concerned patients under the intervention group, which was reflected by a decrease in LDL and total cholesterol in comparison to control groups with a P values (0.001 and 0.001) respectively.

Previous studies tackling the same aspect demonstrated significant decrease in total cholesterol and LDL after 1 month intervention employing a high protein diet (protein 40%)(Stamets *et al* .,2004). Yet, a high protein (protein > 30 %) diet cannot be used over a long period of time due to the side effects that can cause to the body and excess load on kidneys, and thus cannot be incorporated within a lifestyle modification plan as PCOS women need a “attainable” lifestyle modification plan which will help improve and maintain the quality of life over the long run.

In light of the above, the nutritional intervention proposed and its 10 % weight loss showed a statistically significant difference in FBS reduction between intervention

and controls with a P value< 0.001. During scale up intervention FBS had a higher decrease in FBS than the pilot study intervention. The greater decrease in scale up is due to the high population size. Phy *et al*., (2015) showed that low starch/low dairy diet for 8 weeks can significantly decrease fasting insulin and this is justified by the low starch intake. Decreasing one food group showed efficient results in the metabolic parameters such as FBS and insulin resistance (Phy *et al*., 2015) but fitting this type of diet into a lifestyle can be hard especially for PCOS women who face episodes of carbohydrates cravings. As such, this adds up to the collected evidence that in order to promote a healthy well-balanced lifestyle, PCOS women must be provided with attainable dietary plans that are both efficient and effective on different metabolic levels.

Hirsutism is evidently linked to hyperandrogenism, and results propose that total testosterone is a critical parameter in PCOS. On the endocrine level, testosterone decreased for intervention groups and increased for controls. This difference in change is statistically significant with a P = 0.001.

According to a study conducted by Lass *et al*., in 2011 testosterone and hyperinsulinemia are positively correlated. The change in weight seen in this study among intervention groups showed that increased weight loss can decrease total testosterone level among intervention PCOS women with a significant difference (p=0.001)in comparison to control groups who gained weight and thus testosterone. This suggests that weight loss is attributed to a decrease in total testosterone and thus any further endocrine disorders among PCOS women. As previously seen in the pilot study, the change in weight seen in overweight/obese intervention group was not seen to be related to the parallel change in insulin and total testosterone level. The pilot study results support the hypothesis that obesity and PCOS are linked to insulin resistance. This was further assessed in the scale-up confirming that weight loss in the intervention groups is seen to be related to insulin resistance and total testosterone level. The high population size used in the scale up intervention in comparison to the pilot study allowed us to reveal important correlation to be considered in the future. A further discussion is done about the compliance over a longer period of time and its effect on the metabolic, endocrine and anthropometric measurement comparing 6 months versus 12 months intervention study.

6.5 Scale up psychological status:

The pilot study showed that a moderate caloric-restricted diet in overweight and obese PCOS women improved depression and anxiety scores significantly (P = <0.001 for BDI-II and P< 0.001 for GAD-7) after providing them with the nutritional counseling. During the scale up study, we demonstrated that the nutritional intervention with higher percentage of weight loss has similar effect with statistical significance between intervention and control groups in the PCOS groups with P<0.001. Other researchers suggest that exercise and psychological results are independent(Byrne *et al* ., 1993) (La Fontaine *et al* .,1992).We demonstrated that the addition of exercise to the nutritional intervention as part of lifestyle modification results in improvements in the psychological assessments among PCOS women facing depression and anxiety, which is seen in the results of BDI-II and GAD-7. At the end of the intervention, the intervention groups appeared less psychologically disturbed that could point the statistical significance. In addition, the scale-up group's results are in parallel with the pilot study results. As such, we can conclude that physical activity(defined by 30 min daily medium intensity exercise ; light jog, swimming, cycling, etc..) in combination with nutritional intervention leading for weight loss restored the self-esteem and body image among PCOS women and decreased their anxiety related to reproductive concerns and this is clearly seen among women specially trying to conceive. A group of forty-nine obese PCOS women enrolled in a study assessing the effect of diet alone, combined with exercise or diet and combined aerobic –resistance exercise on their depression level. In this study, patients reported improvement in their in depression and demonstrated that dietary restriction combined with exercise have a significant improvement on the depression scores on overweight and obese PCOS women (P<0.001) (Thomson *et al*., 2010). These results are in line with this study findings. For example, in both studies PCOS overweight and obese women followed a caloric restriction diet combined with physical activity. Overweight and obese PCOS women following low-protein high-carbohydrate (LPHC) diet versus a high-protein low-carbohydrate (HPLC) diet reported a significant reduction in depression score among The HPLC diet group with no change for the LPHC diet group(Galletly *et al*.,2004).The findings of this study are in agreement with the scale-up study results. The type of diet

followed by the overweight and obese women in PCOS in the proposed nutrition guidelines is hypo-caloric while Galletly *et al*., (2004) study compared two different diets on depression score. The limitation of the study was to assess the effect of different type of diets on the psychological profile in PCOS women.

6.6 Scale up physical activity results

PCOS women reported improved psychological states in parallel with increased physical activity along with improvement on the metabolic level (fasting insulin and FBS). The key factor for psychological status in the intervention groups is the addition to the physical activity status, this is clearly seen during the pilot study, and the scale up intervention where increased PA levels 3.0(±1.5) for PCOS overweight/obese intervention at the end of the study period is strongly associated with improved metabolic, anthropometric and psychological status (especially in the PCOS intervention groups)

6.7 Scale up nutritional assessment: 24-hour recall and FFQ results

The objective of the study was to assess the effectiveness of the nutritional counseling on the improvement of PCOS women well-being and design educational sessions tailored to PCOS women raising their nutritional awareness pertaining healthy food options and developing the latter into a lifestyle. Researches showed that PCOS progression is linked to obesity (Sedighi *et al*.,2014 , Moran *et al*., 2015). Therefore, the caloric restriction of obese women leads to weight loss. The food intake of PCOS women was assessed at baseline and after the intervention for both overweight/obese and lean PCOS women. The lean PCOS women showed lower intake in comparison to overweight and obese PCOS women. The nutritional guidelines and counseling proposed healthy food options including complex carbohydrates , fat (PUFA and MUFA), fruits and vegetables consumption employing caloric range between 1200 and 1500 calories for weight loss and 1800 calories for weight maintenance. The major finding was that dietary intervention showed significant effect on weight loss and fasting insulin level. As such, the reduction in insulin level is thought to improve the endocrine abnormalities in PCOS.

Furthermore, adherence to the nutritional guidelines (Please refer to appendix 8 and appendix 9) in both intervention groups for a period of 6 months affected FBS and fasting insulin level. The food intake of the patients was assessed using food frequency questionnaire adapted to the Lebanese population and two 24 hour recalls reporting weekday intake and weekend intake. When comparing the FFQ between the pilot study and scale up intervention, many results have changed. An increased number of food options showed significant changes before and after the intervention specifically in the intervention groups. The change in intake between intervention groups and controls was statistically significant among all food items with a P <0.001 except for fresh fish, orange and kiwi with P=0.003, P= 0.002 and P=0.003 respectively and this is due to their widespread use among Lebanese women as detected in the FFQ. The FFQ also showed high carbohydrates intake among all PCOS women but the nutritional assessment by FFQ showed their adherence and compliance to the guidelines provided during counseling. As such, this is the first study assessing food intake among PCOS women in the Middle East region, and their changes after a nutritional dietary intervention.

6.8 Twelve months follow-up

A subpopulation from the pilot phase was selected to continue an extra 6 months follow-up with a total of 12 months intervention and control phase(N=30). The results showed an improvement in the hirsutism score with an extra decrease among interventions during the 6-12 months phase and no change among controls. This change is linked to decrease in testosterone. Hirsutism pattern is a clinical justification of the biochemical change in the testosterone level. In comparison to the pilot study the results are similar in the hirsutism score but on the biochemical level testosterone decreased more. Lass *et al*., 2011 showed a less decrease in testosterone level L in comparison to the study. The reason of their outcome is the population recruited consisting of adolescents with PCOS. Adolescents' compliance can be less in comparison to adults who have high compliance due to their reproductive concerns. Anthropometric measurements decreased in extra values during the extra phase of interventions. Waist circumference decreased by an extra of 4 cm (±4.1) and this change is statistically significant with P< 0.001 in

comparison to controls who gained 5.5 cm (±3.6). The decrease in waist circumference is an improvement in the abdominal obesity which exposes the PCOS women to anovulation (Aubuchon *et al*., 2012). The number of cycles changed as well and an extra number of cycles were seen during the additional phase. This demonstrated that a lifestyle modification among PCOS women is potential to help them restore their ovulation. Weight decreased by an additional amount in the extra phase. During the second phase (pilot study) a higher weight loss reduction was seen than the first phase .Controls showed an increase in the total body weight and the change was statistically significant between control and interventions and between 6 and 12 months with a $P< 0.001$. The aim of this study was met during the additional phase where around 7.8 % of weight was reduced from initial body weight.

On the biochemical level, C-reactive protein as inflammatory marker showed a slight decrease in the additional 6 months phase and we report that this reduction is statistically significant between interventions and controls with a $P< 0.001$. We conclude that the weight loss in the intervention groups has decreased CRP among PCOS women and the study findings are not in line with Moran *et al*., 2007. This conclusion is due to the large sample size population used during this study. Women with PCOS are at high risk of metabolic syndrome and thus weight loss can improve the metabolic considerations by decreasing the inflammatory marker CRP. During the pilot study we suggested an extra amount of weight loss to assess the effect of the increased reduction on the metabolic syndrome parameters and the outcome of the 12 months follow-up has justified the need of a longer period of time for intervention. Furthermore, the metabolic effects of PCOS with their biochemical presentations have shown many improvements in this extra phase with reduction in FBS, LDL and TG with a P values <0.001. The lipid parameters showed improvements after an extra 7 Kg weight loss. The results demonstrated that lifestyle modification through continuous weight loss for obese PCOS women and weight maintenance for lean PCOS women can give significant results. Fasting insulin and total testosterone showed a reduction that was not statistically significant and the results are not in parallel to Phy *et al*., (2015) who showed that reducing starch and dairy from the diet can reduce significantly fasting insulin.

Therefore, insulin is affected by the carbohydrate intake in PCOS patients. The number of cycles has increased from 2 to 3 cycles among interventions PCOS women ($P< 0.001$) and this is linked to a decrease in weight that has improved ovulatory function and restored menses.

For PCOS women weight loss/ maintenance plays a key role in restoring self-image and esteem. It is noted that the depression and anxiety score showed improvements in BDI-II and GAD-7) with $P<0.001$ for both scores, after this second phase of weight loss. Noting an improvement in their physical activity (+1.2 h/w (±0.4)) duration was a result of their educational sessions about physical activity as part of lifestyle modification. We conclude then that weight loss combined with physical activity possesses a positive impact on PCOS women who presented with depression and anxiety state.

As a conclusion, a twelve months nutritional education employing a range of 1200-1500 calories per day for weight reduction, and 1800 calories per day for weight maintenance for PCOS women resulted in weight loss for the intervention groups and weight maintenance for lean intervention groups. On the other hand control groups showed an increase in their total weight.

The employed nutritional guidelines outcomes showed a positive deviation to a healthy lifestyle among PCOS women. This suggests the need for nutritional intervention for all PCOS women to prevent any further complications, decrease the present metabolic presentations and make it a lifestyle and not just a short period of intervention.

6.9 Comparison PCOS versus non-PCOS

6.9.1 Anthropometric measurements

In this study, non-PCOS women were weight and age matched to the PCOS women in order not to bias the study results. Only very few studies have investigated the effect of weight between PCOS and non-PCOS recruiting overweight/obese and lean women.

6.9.2 Gynecology

When comparing women with PCOS and women without PCOS we observe non-equivalent weight loss where non-PCOS women lost more and this is due to the less insulin resistance among non-PCOS women (Moran *et al.,* 2007). The weight reduction is needed to be greater among overweight/obese PCOS women to observe metabolic improvements among them. The weight loss manifested by a reduction in waist circumference was an improvement for abdominal obesity especially for those who are insulin resistant. Overweight/obese women with PCOS had less reduction in waist circumference in comparison to overweight/obese non-PCOS women (Moran *et al*.,2007).The excess body weight reduction was greater in non-PCOS women than in PCOS women justifying that non-PCOS women are able to lose more weight than PCOS women reflecting metabolic considerations at the molecular level of PCOS women. The limitations of Moran *et al*., (2007) study were the low population size recruited and the low duration of time employed for this study (8 weeks).

Women with PCOS face menstrual irregularities while women without PCOS don't experience any. Thus weight loss is required for PCOS women in order to regulate their menses and less likely for non-PCOS (Moran *et al*., 2007). The difference between PCOS and non PCOS is statistically significant with $P<0.001$ in number of cycles. Women with PCOS had 4.3 as number of cycles while women without PCOS had higher number of cycle and this is due to their regularity of menses (5.6 cycles) .This doesn't abandon the fact that overweight/obese women without PCOS should lose weight as prevention of progression to further reproductive abnormalities such as PCOS (Franks *et al*., 2002).

6.9.3 Biochemical markers

The change in IR in PCOS women was contingent to the weight loss seen in the abdominal area. In the same work we did we report that a greater reduction in IR was found among non PCOS women associated to greater weight loss. This confirms that women with PCOS have significantly higher insulin resistance than non-PCOS women and these results are in line with Moran *et al*., study in 2007. Changes in weight are associated with insulin resistance where high insulin

resistant women are less likely to lose more weight than normal insulin level. Women with less insulin resistance require less reduction in weight to benefit metabolically (Moran *et al*., 2007). In weight loss surgeries, women with less IR at baseline demonstrated more reduction in CRP after weight loss (Moran *et al*., 2007). In the study, weight loss of 8.2 Kg in overweight/obese with PCOS is associated with 1.7 mg/L CRP reduction while overweight/obese non-PCOS women experienced less CRP reduction due to the lower baseline value between both PCOS and non-PCOS. Weight loss exerts more beneficial effect on PCOS women due to their higher inflammatory states based on higher CRP level compared to non-PCOS. The conclusion to this point is limited due to the lack of further investigation using extra inflammatory marker such as IL-6 and TNF –a(Moran *et al*,2007) .This confirms that weight loss is more beneficial for insulin resistant women regardless of PCOS status. This is a strong reason for the need of guidelines to weight loss or weight maintenance in order to manage weight, improve insulin resistance and thus CRP level. Furthermore, weight management benefits conferred by the proposed guidelines can prevent or treat from one side high inflammatory state and type 2 diabetes among PCOS women. The FBS reduction observed among overweight/obese PCOS women was less than the non-PCOS women and this is associated to the greater insulin resistance among PCOS women. The weight loss effect showed statistical significance between PCOS and non PCOS overweight/obese women on the lipid profile. The results of this study are similar to other studies (Moran *et al*., 2007). Women without PCOS showed less decrease in LDL level than PCOS women following weight loss. This is justified by the fact that PCOS women are under statin medications which justifies the increased reduction in LDL, total cholesterol and TG. To conclude this point, weight loss is needed more in PCOS women to exert beneficial effect on the lipid profile as risk factor to metabolic syndrome.

Metabolic syndrome parameters

Diastolic and systolic pressure showed greater decrease among PCOS women and slight increase among controls) and this is exerted by the weight loss effect induced by the proposed guidelines. Decreasing the diastolic and systolic pressures

are one the major keys to metabolic syndrome and thus preventing any further cardiovascular disorders such as hypertension.
Weight reduction is known to affect positively the psychological abnormalities among women especially PCOS women (Conte *et al*., 2015).

6.9.4 Psychological status

This is one of the very few studies to have investigated the effect of weight loss on PCOS and non –PCOS women following weight loss or maintenance. The depression score decreased slightly more in non-PCOS interventions women. In this study, women showed to have higher score of psychological disturbances assessed by BDI-II and GAD-7 before the treatment. The BDI-II score decreased between both interventions PCOS and non-PCOS are slightly similar. The similarity in reduction between both groups constitutes a great point confirming that both type of women can benefit equivalently from the proposed guidelines and thus weight loss/maintenance. The control groups for both PCOS and non-PCOS have shown slight increase in BDI-II score. The GAD-7 score reduction was greater in non-PCOS intervention groups than PCOS intervention group's .This demonstrates that non-PCOS women benefit the most at this level from PCOS women. This can draw a conclusion that the anxiety level among PCOS is high enough to be affected by weight loss / maintenance due to the different hormonal treatment taken by PCOS women.

6.9.5 Physical activity

The physical activity improvement was greater among PCOS women than non-PCOS) and this is oppositely seen in weight reduction where increased physical activity did not affect increased weight reduction among PCOS women. This should be further investigated with controlled environment on both PCOS and non-PCOS where equal physical activity should be practiced to assess the exerted benefits on the different groups of women. To conclude weight reduction is affected mainly by insulin level even when combined with physical activity.

6.9.6 Food Frequency Questionnaire

The food frequency questionnaire showed significant change and improvement in food choice with $P<0.001$ and this is justified by the reduction in caloric intake calculated in the 24 hour recall. Note that this is the first study assessing the food intake of POCS women using this FFQ adapted to the Lebanese population. When comparing these results with pilot study results the single items analysis showed similar results with an increase in the fruits and vegetables intake per week and a reduction of simple carbohydrates intake among all intervention groups. Significant results have been seen with an increased consumption of green salad (5 serving sizes) among intervention groups and decrease in white bread intake, pasta and rice. The intervention groups PCOS and non-PCOS were receiving educational sessions about health food consumption such as increasing fruits and vegetables intake, lean cut proteins and limited number of healthy fat intake, decrease full fat dairy products and excess caloric intake. This strategy exerts beneficial effects such as preventing any deficiencies among women in minerals and vitamins that are needed for their reproductive health. The novelty of this study is the healthy lifestyle education such as consuming healthy options, decrease excess caloric intake, increase moderately physical activity level and not just for the purpose of interventional study instead for a lifestyle modification over lifetime. The conclusion drawn is similar to the pilot study where intervention groups showed compliance to the nutritional guidelines. In conclusion, these guidelines present a powerful tool to be used in the future for PCOS and non-PCOS women in order to give healthy habits.

Six months nutritional intervention employing nutritional guidelines exerts greater beneficial effect on non-PCOS women rather than PCOS women. This is due to the comparatively higher weight reduction among non-PCOS women. Additionally a 12-months nutritional intervention has shown cumulative improvements on the endocrine and metabolic levels for PCOS women, concluding that lifestyle modification over a one-year period is required to lose weight for overweight/obese subjects with PCOS and thereby exert improvements in nutritional, biochemical, psychological and reproductive status.

Chapter 7
Author's critical analysis

7.1 Author's critical analysis on the intervention

This study is not only concerned with nutritional status in PCOS but sits within the broad finding of public health nutrition. Because of this data presented are very broad. The strengths of nutritional support intervention overweight pharmaceutical interventions as seen by the findings.

The overall results of this project validate the author discussion to use non-elimination diet to support the nutritional status of the patients. The author recommends studies of this kind employing non-elimination diet.

During this study patient drop-out was in low rate. Based on the regular phone calls with the patients women followed the guidelines but some faced some financial constraints for those coming from low socio-economic background. Confidentially is commonly seen in Lebanon among women attending the obstetrics and gynecology clinics for menses irregularity and infertility. Women with absence of menses and infertility were facing social fear from inability to fit the cultural norms. Absence of menses can be due to pregnancy via non-marital sexual relation, and infertility would restraint them from getting kids and building a family after marriage. Abdominal ultrasound for the PCOS diagnosis is available at the clinics but due to some financial restraints to low socio-economic women scans were done rarely. Women diagnosed with PCOS during this study were assessed using an abdominal ultrasound for the ovaries to confirm the syndrome. For these type of women if ultrasound was done at early stage of PCOS phase the treatment can be easier and they would have not gained weight. On the other side, obese women with undiagnosed PCOS could have treated obesity at early stage and decrease the complications faced. This justifies the lack of knowledge about the important of nutritional support and obesity management for women in general and PCOS in specific. Wrong dietary practices and CAM use followed by PCOS women were seen during this study. Women used to follow strict and elimination diets brought from non-qualified sources to lose weight in a short period of time such as a week or two but this end up in regaining the weight and in excess which caused a sharp increase in obesity. Women failing to conceive or women with irregular menses suffer from disturbed mental health issues like depression, anxiety and irritability

leading to excess eating and obesity. The regular follow-ups and phone calls done during the study have a great impact on the patients following the intervention to increase the support and encourage them on deviating into healthy lifestyle. The lean intervention group had a great maintenance on their weight and a better insight over their dietary practices with the follow-ups tools. This adds to the literature that regular support by phone calls and close follow-up sessions give a better result for PCOS women.

During the first stages of planning for this study a literature review was conducted in order to propose nutritional guidelines including a food plan compromising all the nutritional elements such as carbohydrates, proteins, Fats (MUFA and PUFA). The carbohydrates chosen and advised were the complex carbohydrates including multi-cereals bread and fruits in order to assure the intake of vitamins and minerals needed to support the function of many organs and system in the human body mainly the reproductive system for females. Advising on consuming complex carbohydrates had tremendous effect on the fasting blood glucose and insulin resistance and this was observed in the biochemical markers tested and the changes seen before and after 6 months of intervention. I think we could have tried a sub-population using no carbohydrates in the research stages to compare the results between the proposed guidelines and non-carbohydrates diet on the metabolic profile especially fasting blood glucose and fasting insulin. In the research conducted by Douglas *et al* ., 2006 confirming that a reduction in the dietary carbohydrates reduces fasting insulin among PCOS women and thus can improve the reproductive outcomes. The employed nutritional guidelines and counseling haven't proposed any reduction in the dietary carbohydrates on the contrary, the guidelines motivated on the consumption of a normal dietary regime including all food elements with a reduction in caloric intake.

Many research projects have studied the different type of diets on PCOS features to better understand the metabolic aspect of weight loss (Chavaro *et al*., 2007; Douglas *et al*., 2006; Mehrabani *et al*., 2012). These studies have focused only on selected diets studying selected profile such as metabolic, reproductive, anthropometric or psychological. The focus of our research was the reproductive outcome especially the changes seen in the hormonal level employing parameters

such testosterone, the ovulation date used in the pilot study, the regularity of menses and the fertility rate for married women in the scale up phase. I think extra reproductive parameters could have been used such thyroid stimulating hormone, luteinizing hormone and follicle stimulating hormone to elaborate more on the role of the nutritional guidelines affecting the whole reproductive profile of women. The advantages of this research are the impact on the Lebanese women reproductive profile of ability to conceive and thus giving a therapy to follow. Moreover, the nutritional guidelines effects showed that the reduced weight in the intervention groups is excess body fat and not lean mass or water. Decreasing body fat is essential for the abdominal obesity affecting the ovulation in PCOS women.

On the other hand, studying the hormonal level changes among PCOS women in this study was essential to map the effect of weight loss on testosterone. Some hormonal profiles tests should have been added to this study such as Follicule stimulation hormone, Luteinizing hormone and Dehydroepiandrosterone sulfate. The gained outcomes could have been more elaborated and the effect of weight loss could have been widely studied if these hormones were used as parameters in this study.

In addition, one of the advantages of this study is the proposed life-time nutritional guidelines for women with PCOS in the Lebanese population facing weight changes and weight loss challenges. Weight gain mainly is a factor for PCOS and the infertility complications in addition to other metabolic disorders such as type 2 diabetes and metabolic syndrome. Our considerable challenge that was faced involved patients that were seeking CAM treatment for weight loss without avail. Those patients who had originally sought rapid results found difficulty adhering to long term nutritional treatment. At the end of the study successful results have been achieved.

7.2 Limitations and strengths:

Few limitations set constraints on the study at hand. There was no major problem anticipated in this study. The study methodology was planned to prospectively follow the patients using weight management guidelines. The pilot study and scale up phase followed the same methodology. During the pilot study few parameters were

adjusted in the scale-up technically to obtain better outcomes such as the indication of ovulation for reproductive profile analysis. On the other hand, the nutritional guidelines used were prepared to fit the overweight/obese group and the lean group. Another guideline could have been prepared during this study which is the high protein diet. But due to the lifestyle change purposes of this study a hypocaloric guidelines were prepared with no dietary restriction. The study parameters were measured three times (at baseline, after 3 and 6 months from baseline). During weight management the nutritional follow-up should have been done every 2-3 weeks but due to the long distances travelled by many patients a phone call follow-up was done to track with the patients the study guidelines. During the scale-up testing, body temperature measurement was eliminated and period onset, number of cycles, pregnancy test and regularity of menses was observed to assess the reproductive outcomes. One of the study limitations is the difficulty to follow with the patients due their high number, but the researcher with the nurses in the Obstetrics and Gynecology department at AUBMC managed to keep calling the patients to remind them of their follow-up appointments. The lack of fund is an additional weakness of this study where extra funds were needed to further analyze the changes in TSH, AMH, and DHEAS to map the hormonal changes correlated with total testosterone after weight loss. It limited the work where ultrasound was needed to detect the change is cysts size and number among PCOS women before and after the intervention. Furthermore, the uses of hormonal treatment for some PCOS women caused them to hardly adhere to the guidelines but during the counseling sessions patients were educated about the importance of adherence to the dietary plans set.

On the other hand, the study also had several strengths and future prospective applications. For instance, this is the first study that assessed the effect of weight loss among PCOS women on metabolic, reproductive, anthropometric, nutritional and psychological levels. This study is a randomized control trials with structural follow-up to increase the adherence and compliance to the proposed guidelines. In addition, it is one of the few dietary interventions employing the high sample size for PCOS women. Low patient dropout (n=8) during the intervention was seen due to the high interest of women to regain their body image and restore their reproductive profile and conditions. This is due to the cultural background of the society where

reproductive concerns play a major role in marriage and life cycle. Attrition rate was low(1.3) in this study because women were seeking help to benefit in their treatment and this is why in the future PCOS women should start nutritional interventions after diagnosis directly. There was no financial barrier to PCOS women in this study although hormonal treatment and hospital visits were expensive in Lebanon but patients were offered this intervention free of charge along with the blood tests conducted regularly. The body composition machine was used to assess the body composition in term of fat and muscle percentage for the PCOS patients. In-body or CT imaging is more accurate to tackle body fat changes especially visceral fat. As for the biochemical analysis, the laboratory setting was available at the hospital for blood withdrawal and this is strength for the study.

As such, based on the findings provided in this research paper along with the comparisons provided against existing literature, we conclude that dietary intervention and its proper integration into active healthy lifestyle offers prospective applications in the field treatment of women suffering from PCOS, which must be further investigated and refined to tailor it to the needs of the PCOS community.

Confounding factors

Random assignment, as a result of confounding by indication, does not affect randomized trials (Johnston, 2001). Since the control characteristics (inclusion criteria) of the various groups (overweight/obese Interventions and controls, lean interventions and controls) and subgroups (intervention versus Control) were the same, the probability of confounding was smaller in this study. By expanding the types and values of analogies performed in the analysis, devaluation in the possibility for the occurrence and confounding factors was obtained, such that these confounding variables can be classified by their origin. The choice of measurement instrument (operational confound), situational characteristics (procedural confound), or inter-individual differences (person confound). By augmenting the figures of comparisons executed in the proximate analysis, a contraction in the probability of happening and results of operational confounding factors were realized in this study. Equivalently, to guarantee the strength and validity of the findings and a reduction in the potential for operational confound, the

data from anthropometric, 24 hour recall , and general assessment measurements were repeated twice and attested by another medical staff. To diminish situations of confounding, either before the study implementation or after the analysis, a peer review in the advancement of the theoretical scheme and at the end of the pilot intervention was used. The peer review likewise, depended on cumulative ability within medical colleagues in the care and support program for PCOS, to recognize possible shortcomings in the study design and analysis, containing methods where the data may depend on confounding. To lessen the incident of self-selection by patients or prejudice by the study planner, the people under study were randomly split after brief instructions, using the Randomized Control Trial (RCT) design. This methodology divides the members into groups (overweight/obese and lean) and subgroups (interventions versus control) significant to the criteria that match the research question.

7.3 Conclusion

Implementing nutritional counseling upon the PCOS diagnosis is expected to improve lifestyle of PCOS women by the onset of ovulation induction and hormonal therapy. Weight losses, resumption of reproductive features and improvement in nutritional and biochemical profile have met the study's objectives. The desired results succeeded in making the nutritional framework proposed as part of the PCOS treatment and to determine its role in the gynecological framework in Lebanon.

Up to our knowledge this is the first study to demonstrate that the nutritional guidelines can lead to weight loss in overweight/ obese women and maintain weight in lean PCOS women. This study results confirm that if nutritional guidelines and counseling along with structured PA regimen are given upon diagnosis with PCOS their weight and metabolic features can be managed and lead to an improved lifestyle among Lebanese women with PCOS. The proposed nutritional guidelines for PCOS women showed superiority to other elimination diets and pharmaceutical interventions in terms of cost effectiveness and sustainability. Such lifestyle interventions succeed for women with PCOS and thus can positively impact their families and especially daughters given the heritable aspect of PCOS. These

guidelines are considered an excellent educational tools for women with PCOS which no short term elimination diet or medication could hope to do.

7.4 Future plans

A 2005 research study has assessed the effect of B Vitamins and folic acid supplementation and its effect on serum levels of homocysteine in PCOS women (Kilicdag *et al*., 2005). They concluded that B-group vitamins and folic acid offset the increase in homocysteine levels in PCOS women using metformin (Kilicdag *et al*., 2005). Therefore, dietary supplementation and functional food should be further studied to ascertain their effects on PCOS women who do not follow the proposed nutritional guidelines for both overweight/obese and lean women.

The following plans are proposed to be done in the future work:

1- Effect of vitamins B 6, B 12 and folic acid supplementation on homocysteine level and fertility outcomes.
2- Include a structured physical activity plan for PCOS to be tested further and be added to the nutritional guidelines.
3- The nutritional guidelines for overweight/obese and lean PCOS women be shared for Lebanese women and applied in the health care institutions.
4- Workshops and sessions planning for PCOS women following the gynecologists treatment at the health institutions in Lebanon including sessions on healthy cooking, importance of weight loss for overweight and obese, weight maintenance for lean , group support, psychological support(such as psychotherapist strategies to each PCOS women). Family members (such as father, mother, husband and siblings) should be included in these sessions to enlighten them about the conditions and importance of support to achieve the desired outcomes.
5- Expand the nutritional intervention to rural areas in the south, north, mountains and Bekaa (Lebanon valley) areas.
6- Conduct mobile clinics and awareness campaigns to educate the patients about the importance of nutritional care for gynecologist, health care providers and PCOS women.

7- Plan TV and radio interviews to address the nutritional guidelines support and team up with health bloggers and digital influencers.
8- Plan a recipes and cooking book and e-book for PCOS to help them find the right meals to eat during their lifestyle modification. Appear on cooking programs in the Lebanon and the Middle East region to expand this project and reach the highest population.
9- Workshops for endocrinologists, gynecologists , nurses , health care staff to educate them about the importance of nutritional guidelines support for all PCOS women to incorporate along with their medical care

Chapter 9

References and appendices

9.1 References:

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9.2 Appendices

Appendix 1: Patient consent form (available in Arabic language also)

Patient information sheet is duplicated because AUBMC uses this practice form as consent to patients.

Consent to participate in a research study

Study title: "Public health nutrition intervention to enhance healthy eating and lifestyle modification among Lebanese patients with polycystic ovarian syndrome."

Principal Investigator: Dr. GhinaGhazeeri
Co-investigator: Caroline Hamadi
Address: American University Hospital
Cairo Street
Beirut, Lebanon
Phone: (01) 350 000 ext 5618

Setting:AUBMC , in the Women's Health Center private clinics , and Obstetrics and Gynecology outpatient clinics.

You are being asked to participate in a clinical research study conducted at the American University of Beirut. Please take time to read the following information carefully before you decide whether you want to take part in this study or not. Feel free to ask your doctor if you need more information or clarification about what is stated in this form and the study as a whole.

Why is this study being done?

Polycystic Ovary Syndrome (PCOS) is one of the common disease which affect women in their reproductive age. Obesity co-exists with PCOS and nutritional guidelines for healthy eating is an essential issue to be discussed with PCOS women. Other metabolic disease are accompanied with PCOS women which causing PCOS to be a public health disease .Thus research towards nutritional treatment is potential to improve the abnormalities .Weight loss was proposed as a major treatment for obese PCOS women .Other nutritional guidelines were also

noted as part of the metabolic abnormalities treatment. Specific nutrients in the daily diet were found to help improve the health status of PCOS patients whether lean or obese.

The aim of the study is to address nutritional education to lean, overweight and obese women with PCOS as a step towards lifestyle modification and dietary behavioral changes. In our public health intervention, we aim to provide educational tools about healthy food choices, healthy eating pattern and promoting physical activity as an influence for these PCOS patients to control their weight.

This study is very important in order to decrease PCOS complications.

This study is in the purpose of a PhD degree for the research assistant of Dr Ghina GHazeeri,Caroline Hamadi and data will be used to complete her PhD degree at the University of Westminster, London, United Kingdom.

This study will take place at AUBMC in the Women's Health Center private clinics and Obstetrics and Gynecology outpatient clinics.

A consent form will be distributed only at these units.

Up to 294 PCOS women will be recruited in the whole study. In the first year 76 patients will be recruited for the pilot study.

What will happen if you take part in this study?

Potential candidates for the study will be approached by the research assistant who will explain the study and its objectives and then will ask eligible women to participate and sign a consent form approved by the institutional review board (IRB).

After the Informed consent has been signed, an assessment form will be filled and candidates will fill some questionnaires; food frequency questionnaire, 24- hour recall, physical activity, reproductive health, psychological(anxiety and depression) and socio-demographics questions. Then a nutritional assessment with body analysis(by analyzing the percentage body fat, percentage body muscle ,basal metabolic rate using ELG 3rdgeneration impedance machine with no invasive method), weight, height and BMI measurements will take place. You will be asked to withdraw 3 ml of blood sample. You will be subject to routine checkup for women with polycystic ovary syndrome regarding medical history, physical examination and

hematologic laboratory tests. You will then randomly grouped according to your weight (lean versus overweigh/obese) into 1 of 4 groups.
One group (lean): one subgroup will receive their gynecologist treatment, second subgroup will receive an extra nutritional education sessions on their visits. Second group (Overweight/obese): one subgroup will receive the gynecologist treatment and a second subgroup will receive nutritional education sessions on their visits and weight loss therapy by diet management.
The study involves a total of 3 visits (At the beginning of the study, at 3 and 6 months) .Each visit will take a maximum 1 hour of your time .At each visit you will be subject to the different questionnaires and assessments and be subject to 3 ml of blood sample withdrawal for laboratory analysis performed at AUB-MC in each visit. Therefore there will be a total of 3 blood samples taken over a period of 6 months during which you will be subject to our nutritional intervention and your results will be compared for changes. You should be fasting before taking your blood sample at each visit.

How long will I be in the study?

You will be in the study for a 6 months period.
The investigator may decide to take you off the study if she feels that is in your best interest .If you will not follow the guidelines of the study you will be asked to stop in the study.
You can choose at any time stop being part of the study and if you decide so you should inform the researcher.
Significant new findings in this study will be conveyed to you.

What side effects or risk could result from being in this study?

Blood withdrawal may cause a discomfort or blue discoloration at the site of blood withdrawal. Other risk such as the feeling of being forced to lose weight will cause an additional risk.
Dr Ghina Ghazeeri will be contacted in case any occurrence of risk in the study.

What are the benefits of participating in this study?

You will receive your blood results and be gaining nutritional information regarding your medical status. You will be able to modify your lifestyle and increase your daily physical activity.
We hope to set up the nutritional guidelines of PCOS patients in this study and help your condition in the future.

How is confidentiality assured?

If you agree to participate in this research study, the information will be kept confidential. Your name or other personal identifiers will not be used during the study. All information about you from this research project will be kept in a locked office . Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your medical records which may be audited without violating confidentiality, your name will not be used.
Would you like to be informed of new studies conducted in the future?
Yes ☐ No ☐

You have not given up any of your legal rights by signing this form. There will be confidentiality of subjects' records.

A refusal to participate will involve no loss of benefits.

Investigator's Statement:

I have reviewed, in detail, the informed consent document for this research study with _____ (name of patient, legal representative, or parent/guardian) the purpose of the study and its risks and benefits. I have answered to all the patient's questions clearly. I will inform the participant in case of any changes to the research study.

_____ _____ _____

Name of Investigator or designee **Signature**

_____

Date & Time

Patient's Participation:

I have read and understood all aspects of the research study and all my questions have been answered. I voluntarily agree to be a part of this research study and I know that I can contact Dr. GhinaGhazeeri at 5618 or any of his/her designee involved in the study in case of any questions. If I feel that my questions have not been answered, I can contact the Institutional Review Board for human rights at 5445 . I understand that I am free to withdraw this consent and discontinue participation in this project at any time, even after signing this form, and it will not affect my care or benefits.I know that I will receive a copy of this signed informed consent.

_____ _____

Name of Patient or Legal Representative or Parent/Guardian

Signature

_____

Date & Time

Witness's Name

Witness's Signature

(if patient, representative or parent do not read)

Date & Time

Appendix 2: Patient information sheet

Public health nutrition intervention to enhance healthy eating and lifestyle modification among Lebanese patients with polycystic ovarian syndrome.

Principal Investigator: Dr. Ghina Ghazeeri

Co-Investigator: Caroline Hamadi

We would like to invite you to participate in this research study. Please read the following details regarding the study and your involvement carefully before you decide to take part. If you require further clarification or have additional questions contact us.

What is the purpose of the study?

Polycystic ovarian syndrome (PCOS) is a common endocrine syndrome and the most common cause of anovulatory infertility affecting women in the reproductive age. Diet management , increasing healthy food choice and physical activity promotion may improve the metabolic , clinical and reproductive outcomes by educating patients with PCOS on healthy food and promote physical activity. Therefore the aim of this study is to enhance healthy eating and lifestyle modification among polycystic ovarian syndrome patients attending the obstetrics gynecology clinic at the American University of Beirut Medical Center(AUBMC) Beirut , Lebanon.

This study is in the purpose of a PhD degree for the research assistant of Dr Ghina Ghazeeri,Caroline Hamadi and data will be used to complete her PhD degree at the University of Westminster , London , United Kingdom.

Can I take part in the study?

Yes if you are:

- Patients aged 18 years or above and less than 49 years
- Diagnosed with PCOS according to the Rotterdam ESHRE / ASRM sponsored PCOS consensus workshop group, 2004.

What is expected from me?

You will be asked to sign a “consent form” and a copy of the “information sheet” will be provided. Physical, biochemical , psychological, nutritional and anthropometric assessments will be taken and you will be subject certain questionnaires(food frequency questionnaires, physical activity questionnaire , 24-hour recall).You will be then subject to nutritional education/counseling and follow the guidelines proposed (intervention groups only) and structural weight loss program for obese patients.Obese patients will be subject to monthly follow-up with their diet and weight change .During the intervention the patients focus should be on the outcomes of the lifestyle modification as positive issue to their health status. You will be grouped according to your weight and the treatment you are following.

What types of measurements / tests will be performed on me?

On the first day of the study following measurements will be taken:

- Body composition (Weight ,Height and BMI,percentage body fat , percentage body muscle , percentage body water)
- Nutritional assessment: Food frequency questionnaire and 24 hour recall, physical activity questionnaire
- Pschychologicalquestionnaires : GAD-7 and BDI-II.
- Medical assessment : reproductive questions , hirsutism score and hypertension
- Biochemical analysis: FBS, HDL ,LDL ,TG total cholesterol , CRP, serum testosterone and Vitamin D.

All the above mentioned measurements will be repeated at start, the end of 3 months , 6 months of participation.

Are there any risks of taking part in the study?

Blood withdrawal may cause a discomfort or blue discoloration at the site of blood withdrawal. Other risk such as the feeling of being forced to lose weight will cause an additional risk.

DrGhinaGhazeeri will be contacted in case any occurrence of risk in the study.

How will privacy be kept during the study?

If you agree to participate in this research study, the information will be kept confidential.Your name or other personal identifiers will not be used during the study.All information about you from this research project will be kept in a locked office . Unless required by law, only the study doctor and designee, the ethics committee and inspectors from governmental agencies will have direct access to your medical records which may be audited without violating confidentiality, your name will not be used.

What are the benefits of taking part in the study?

You will receive your blood results and be gaining nutritional information regarding your medical status. You will be able to modify your lifestyle and increase your daily physical activity.

We hope to set up the nutritional guidelines of PCOS patients in this study and help your condition in the future.

Significant new findings in this study will be conveyed to you

Refusing to participate will involve no loss of benefits.

What is the process to withdraw from the study?

If you decide not to be a part of the study, you are free to withdraw at any time. Your participation in the study is completely voluntary. On withdrawal all your records will be erased. Subject's participation may be ended by the investigator.

How will the results be used after the study?

The results would be taken and written by the investigator and will be used for research outcomes (but individuals will not be identified). The data will be used as part of the PhD degree of Caroline Hamadi in University of Westminster,London , United Kingdom .The research outcome will be available to you. If you wish to read a copy of the final report please contact the researcher.

What should I do next to take part in the study?

Please fill in the “consent form” and contact me to arrange for an appointment.

Please note that the decision to participate in this study should be your own and choosing not to take part will not disadvantage you in any way.

For any further questions or information please contact me:

DrGhinaGhazeeri and Caroline Hamadi:

Gg02@aub.edu.lb

c.hamadi@my.westminster.ac.uk

Or call on00961- 01350000 extension 5618

Appendix 3 : Assessment form used

Assessment form

A.Personal information

Date of visit: Type of visit : 1 ☐ 2 ☐ 3 ☐

Patient number:

Age bins : 18-20 ☐ 20-25 ☐ 25-30 ☐ 30-35 ☐ 35-40 ☐ 40-45 ☐ 45-50 ☐ 50-55

Marital status: Married ☐ Single ☐ Divorced ☐ Widowed ☐

Insurance : None ☐ NSS ☐ HI ☐ Private ☐ Other

B.Personal/Family history:

Medical history

Do you have any history of PCOS? Yes No

Do you suffer from any present health condition ? Yes(specify)

nO

Surgical history

Medications (name, dose and frequency)

Family history of disease

Personal history of disease

C.Social assessment

Alcohol intake: No ☐ Yes ☐ Specify

Smoking : No ☐ Yes ☐ Specify

D.Obstetrical and gynecological history:

Gravida Para Abortion Ectopic

Mode of delivery

Age at menarche: years

Current symptoms of PCOS:

Duration of PCOS?

Period: Regular Irregular: specify

Are sexually active?

Are you pregnant?

Acne:

Hirsutism score:

Infertility ? Yes No

Thinning of hair sculp? Yes No

Current treatment of PCOS?

Oral contraceptives Metformin Anti-androgen

Fertility agent Lipid lowering drugs

Others

E.Hypertension measurement:

Systolic BP

Diastolic BP:

F.Nutritonal assessment :

Have you had any weight loss/weight gain in the last 3 months?

Weight : kg Height: cm

Waist circumference: cm Hip circumference : cm

Wait/Hip ratio:

Percentage body fat : %

Percentage excess body fat : %

Percentage body muscle : %

BMI(Body mass index):

BMR(Basal metabolic rate):

G.Biochemical analysis :

C-reactive protein:

Fasting blood sugar (FBS) :

Lipid profile : LDL-C:

HDL-C:

Triglyceride:

Serum testosterone :

Insulin:

Vitamin D(25)OH :

H.Socio-demographic section:

What is the highest education level you have completed ?

How many people are currently living in your household including yourself?

Please describe the home where you live: owned,rented, friends,family :

How do you pay your healthcare ?Goverment, private insurance, self-pay ?

Do you have a job? No Yes(specify)

What is your total combined family income for the past 12 months,from all sources and wages?

<500 $ 500-1000$ 1000-2000$

>2000$

E. Psychological assessment

1.BDI-II

2.GAD-7

Appendix 4 : Physical activity Questionnaire

	Now I will ask you questions relating to your daily activity as this will help us to determine how active you are. Please answer these questions with respect to your activities over last ONE MONTH.			
	Work related activity			
1.1	How many days in a week do you work?			☐ [In completed days]
1.2	On an average, how many hours per day do you spend at work?			☐☐.☐ [In completed half hours]
1.3	**Of the hours you spend at work, how many hours do you spend in (completed half hours):**			
	(a) **Standing:** Activities such as talk, lab work, supervise, mild cleaning, cattle grazing done standing.	(b) **Sitting:** Activities such as typing, computer work, cleaning grains, eating lunch, driving, ironing, done sitting etc	(c) **Walking**: walking around, strolling	(d) **On activities more strenuous than walking**: Fetch water/ fuel, fooder. weeding, chop wood, ploughing, pounding rice, walking with a load.
	☐☐.☐ [hours]	☐☐.☐ [hours]	☐☐.☐ [hours]	☐☐.☐ [hours]
1.4	**If you spend any time at work on activities more strenuous than walking, please list the activities that you do most in terms of time:**			
	(a)			
	(b)			
	(c)			
	(d)			
1.5	On an average, how many hours do you sleep in a day? ☐☐.☐ [Completed half hours]			
	Apart from work, how do you spend your time (over the last month): Frequency options: [1=Daily; 2=Once a week; 3=2-4 times/week; 4=5-6 times/week; 5=2-3 times/month; 6=Once a month]			
2.1	**Sports / games / exercise (for eg. walking, badminton, jogging, cricket……etc)**			
	(a) Name of activity		(b) Duration	(c) Frequency
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
2.2	**Hobbies involving manual labour (for eg. Carpentry, gardening ……..etc.)**			
	(a) Name of activity		(b) Duration	(c) Frequency
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐
	_____		☐☐☐ [mts]	☐

	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
2.3	**Household activities (for eg. sweeping, collecting fuel/fodder/water, animal care, cooking, washing child care……. etc.)**		
	(a) Name of activity	(b) Duration	(c) Frequency
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	Apart from work, how do you spend your time (over the last month): Frequency options: [1=Daily; 2=Once a week; 3=2-4 times/week; 4=5-6 times/week; 5=2-3 times/month; 6=Once a month]		
2.4	**Sedentary activities for e.g. Reading, watching TV, prayer, carom, computer games, travelling……. etc.)**		
	(a) Name of activity	(b) Duration	(c) Frequency
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	_____	☐☐☐ [mts]	☐
	Other activities	(a) Duration	(b) Frequency
2.5	Eating (breakfast, dinner)	☐☐☐ [mts]	☐
2.6	Brushing, shaving & bathing	☐☐☐ [mts]	☐
2.7	Dressing	☐☐☐ [mts]	☐
2.8	Socialising (talking outside working hours)	☐☐☐ [mts]	☐
2.9	Travelling to and fro from work	☐☐☐ [mts]	☐
2.10	How do you travel to and fro from work?	_____	

Appendix 5: Psychological assessment forms

Appendix 14
Beck Depression Inventory

Roche

V 0477

Beck Depression Inventory

CRTN: _____ CRF number: _____

Baseline

Page 15 patient inits: _____

11. Agitation

0 I am no more restless or wound up than usual.
1 I feel more restless or wound up than usual.
2 I am so restless or agitated that it's hard to stay still.
3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.
1 I am less interested in other people or things than before.
2 I have lost most of my interest in other people or things.
3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.
1 I find it more difficult to make decisions than usual.
2 I have much greater difficulty in making decisions than I used to.
3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.
1 I don't consider myself as worthwhile and useful as I used to.
2 I feel more worthless as compared to other people.
3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.
1 I have less energy than I used to have.
2 I don't have enough energy to do very much.
3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.
1a I sleep somewhat more than usual.
1b I sleep somewhat less than usual.
2a I sleep a lot more than usual.
2b I sleep a lot less than usual.
3a I sleep most of the day.
3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.
1 I am more irritable than usual.
2 I am much more irritable than usual.
3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.
1a My appetite is somewhat less than usual.
1b My appetite is somewhat greater than usual.
2a My appetite is much less than before.
2b My appetite is much greater than usual.
3a I have no appetite at all.
3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.
1 I can't concentrate as well as usual.
2 It's hard to keep my mind on anything for very long.
3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.
1 I get more tired or fatigued more easily than usual.
2 I am too tired or fatigued to do a lot of the things I used to do.
3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I am much less interested in sex now.
3 I have lost interest in sex completely.

3456789101112 ABCDE

Appendix 15
Mood/Depression Assessment Questionnaire

Mood/Depression Assessment Questionnaire

1. Since your last visit have you felt depressed, sad or blue much of the time?
   yes ☐
   no ☐

2. Since your last visit have you often felt helpless about the future?
   yes ☐
   no ☐

3. Since your last visit have you had little interest or pleasure in doing things?
   yes ☐
   no ☐

4. Since your last visit have you had trouble sleeping many nights?
   yes ☐
   no ☐

Are two (2) or more of the above questions marked YES while undergoing treatment in this protocol?

yes ☐ → *complete a Beck Depression Inventory. If score is 30 or less, patient may continue in the study. If score is ≥ 31, patient will need to complete all final assessments and be dropped from the study. The investigator may recommend that the patient be referred for a professional psychiatric assessment.*

no ☐

Appendix 6: Prevalence of PCOS according to the different criteria

Population	NIH criteria	Rotterdam criteria	AE-PCOS criteria
728 Australian women	8.7%	17.8%	12.0%
820 Iranian women	7%	15.2%	7.92%
929 Iranian women	7.1%	14.6%	11.7%
392 Turkish women	6.1%	19.9%	15.3%

Appendix 7: Effects of LSM and weight loss programs on metabolic correlates of PCOS (1994-2013)

Table 20 Literature review on the effects of LSN and weight loss programs on metabolic correlates of PCOS (1994- 2013)

Studies	Intervention	Variables	Outcome	Limitations/comments
Guzick *et al*., 1994	12 obese, hyperandrogenic, anovulatory women randomized to 12-week weight loss program or 12-week "waiting list" in control group	• Ovulation • Fasting insulin and glucose measurement • *Sex hormone-binding globulin* (SHBG) • Total and non-SHBG bound testosterone (*non-SHBG-T*) concentrations • Luteinizing hormone (LH) pulse frequency, amplitude, and concentration • *Follicle stimulating hormone (*FSH) concentration	• Women in the treatment group lost an average of 16.2 kg • They showed a significant increase in SHBG, decline in non-SHBG-T, and decline (though non-significant) in fasting insulin • Four of six subjects in treatment group resumed ovulation • No changes were evident in LH pulse frequency or amplitude or in mean LH and FSH concentrations	Weight loss in obese, hyperandrogenic, anovulatory women appears to reduce insulin and non-SHBG-T concentrations despite the absence of a change in gonadotropin secretion and may lead to resumption of ovulation
Holte *et al*., 1995	13 obese insulin-resistant women with PCOS on weight loss program	• Insulin sensitivity • Basal sex steroid hormones	• Mean weight loss was 12.4 kg • Reduction from a BMI of 32.2 kg/m2 to 27.6 kg/m2	Insulin resistance in obese women with PCOS was reduced by weight loss to similar levels as

		• Gonadotropins and free fatty acids (FFA) • Skin folds and waist hip ratio	• Insulin sensitivity index improved on average 132% and plasma FFA by 32% • Serum SHBG levels increased by 35% • The sum of truncal-abdominal skin-folds were reduced by 28%	BMI-matched control subjects, suggesting that insulin resistance in PCOS is not a feature of PCOS per se
Clark *et al*., 1995	Subjects underwent weekly program of behavioral modification for 6 months	• Weight • Pregnanediol as a marker for the return of ovulation • Pregnancy • Waist/Hip ratios • Serum testosterone, insulin concentrations, SHBG concentrations, fasting glucose, and LH concentrations • Psychometric assessment	• Weight loss 6.3 ± 4.2 kg • 12 of the 13 women in the study group were ovulating spontaneously • Of the 13 women who completed the program, only two had not conceived 12 months after completion of the program • Waist/Hip ratio >0.8. Weight loss did not significantly alter the ratios over 6 months • Drop in serum testosterone and insulin concentrations, rise in SHBG concentrations, and no change in the fasting glucose or LH concentrations • Significant improvement in all the psychological parameters measured	Weight loss with a resultant improvement in ovulation, pregnancy outcome, self-esteem and endocrine parameters is the first therapeutic option for women who are infertile and overweight The subjects underwent the program but the results didn't show the sustainability of these patient once the behavioral modification

				program has been stopped
Clark *et al* ., 1998	Obese infertile women underwent a weekly program aimed at lifestyle changes in relation to exercise and diet for 6 months	• Weight • Ovulation • Pregnancy and live birth • Miscarriage rate • Psychometric measurements	• Weight loss an average of 10.2 kg/m2 • 60 of the 67 anovulatory subjects resumed spontaneous ovulation • 52 achieved pregnancy (18 spontaneously) and 45 a live birth • The miscarriage rate was 18%, compared to 75% for the same women prior to the program • Psychometric measurements improved	These results continue to support the view that all who treat infertility should consider weight loss to be a prerequisite for obese women prior to any assisted reproduction program
Crosignani *et al*., 2003	33 anovulatory overweight patients with PCOS were recommended a 1200 kcal/day diet and physical exercise	• Anthropometric indices • Ovarian imaging parameters were assessed at baseline and after weight loss of 5 and 10%	• 76% lost at least 5% of their body weight • Anthropometric indices were significantly reduced • Significant reduction in ovarian volume and in the number of microfollicles per ovary • 18 had a resumption of regular cycles and 15 experienced spontaneous ovulation	Weight loss is an effective treatment and should be considered the first-line approach for infertile overweight PCOS patients

			• 10 spontaneous pregnancies occurred in patients who lost at least 5% of their weight	
Hoeger *et al*., 2004	38 overweight or obese women with PCOS were randomized to one of four 48-week interventions: metformin 850 mg two times per day, LSM plus metformin 850 mg two times per day, LSM plus placebo, or placebo alone	• Dropout and compliance with a long-term LSM • Weight loss • Preliminary estimates of treatment effect on ovulation (measured by weekly urinary pregnanediolglucuronide), and on total total and free androgen index	• Dropout rate was 39% • Modest weight reduction was found in all treatment groups, with the most significant reduction occurring with the combination of metformin and lifestyle intervention • Significant androgen reduction occurred in the combination group only • Women who lost weight, with or without metformin, were estimated to be 9 times more likely to become regularly ovulatory • Women in the metformin group who lost weight were esti- mated to be 16 times more likely to become regularly ovulatory	Weight reduction might play the most significant role in restoration of ovulation in obese women with PCOS
Stamets *et al*., 2004	Examined the effects of a 1 month dietary intervention on the PCOS phenotype. Patients were randomized to one of	• Change in body weight • Biometric, hormonal, lipid and lipoprotein, and markers of glucose	• Both the HP (_3.7 ±1.9 kg) and HC (_4.4 ±1.5 kg) diets resulted in significant weight loss • No differences between diets on a variety of measures	It is important to try decreasing androgen level by diet management. Weight loss has shown that

	two energy-restricted diets; high protein (HP: 30% protein, 40% carbohydrate, and 30% fat) or high carbohydrate (HC: 15% protein, 55% carbohydrate, and 30% fat). The fat content was held constant in both diets	homeostasis and energy metabolism	including circulating androgens, measures of glucose metabolism, and leptin. • Decline in circulating androgens, fasting and area under the curve (AUC) insulins on a 3-hour oral glucose tolerance test, and fasting and AUC leptin levels • There was a high prevalence of menstrual bleeding during the trial	decreasing weight can affect androgen level and thus affect any related PCOS complications
Escobar – Morreale *et al*., 2005	17 premenopausal women studied prospectively for the effect of bariatric surgery on PCOS	• Hyperandrogenism • Menstrual function • Insulin resistance	• Weight loss (41 ± 9 kg after 12 ± 5 months) was paralleled by decreases in the hirsutism score and free testosterone androstenedione and dehydroepiandrosterone sulfate • Amelioration of insulin resistance • Restoration of regular menstrual cycles and/or ovulation in all patients	Bariatric surgery is a choice of weight loss but not all PCOS are qualified candidate for this option. Healthy food choice and physical activity promotion is essential in PCOS patients
Mavropoulus *et al*., 2005	6-months effects of a low-carbohydrate-ketogenic diet (LCKD) on overweight and	• Weight • Free testosterone • LH/FSH ratio • Fasting insulin	• Significant reductions from baseline to 24 weeks in body weight (-12%), percent free testosterone (-22%), LH/FSH	Further research is needed to determine if the

	obese PCOS women. Patients were instructed to limit their carbohydrate intake to 20g or less per day for 24 weeks	• Insulin glucose • Testosterone • Triglyceride • Body hair • Pregnancy	ratio (-36%), and fasting insulin (-54%). • Non-significant decrease in insulin, glucose, testosterone, triglyceride, and perceived body hair • Two women became pregnant	benefits were from weight loss or from carbohydrate restriction specifically
Tang *et al*., 2006	Obese, oligo-/amenorrhoeic women with PCOS received metformin (850 mg) twice daily compared with placebo group over 6 months. All received the same advice from a dietitian	• Change in menstrual cycle • Changes in the insulin sensitivity and lipid profile • Change in arthropometric measurements	• Both groups showed significant improvements in menstrual frequency and weight loss • No significant changes in insulin sensitivity or lipid profiles in either of the groups • Those who received metformin achieved a significant reduction in waist circumference and free androgen index	Metformin did not improve weight loss or menstrual frequency in obese patients with PCOS Weight loss alone through lifestyle changes improves menstrual frequency
Moran *et al*., 2006	Short-term(8 weeks) and longer-term (6 months) carbohydrate- or fat-restriction strategies in overweight women with PCOS	• Weight maintenance • Improvements in reproductive and metabolic variables	• Significant reductions in weight, waist circumference, body fat, insulin, total testosterone, and free androgen index • Improvements in menstrual cyclicity occurred for 16 (57.1%) of 28 subjects	Mealreplacements are an effective strategy for the short-term management of PCOS. Moderate fat or carbohydrate restriction was equally effective

Qublan *et al*., 2007	64 women randomly divided into 2 groups for 6 months: Group 1 was assigned to take 1200-1400 kcal/day diet (25% proteins, 25% fat, and 50% carbohydrates plus 25-30g of fiber per week). Group 2 was prescribed 850 mg of metformin twice in a continuous manner	• Menstrual cyclicity • BMI • LH • Androgen concentrations • Pregnancy rate	• Both groups had a significant improvement in the menstrual cyclicity, reduction in BMI, LH, and androgen (testosterone, androstenedione, dehydroepiandrosterone sulfate) concentrations • The clinical, biochemical, and reproductiveoutcome including menstrual cycle pattern, ovulation, and pregnancy rates were similar in both groups	Amelioration of hyperinsulinemia and hyperandrogenemia with dietary intervention or metformin treatment improves significantly the clinical features and reproductive function in overweight PCOS women
Hoeger *et al*., 2008	79 obese adolescent women with PCOS were randomized to single treatment trial and combination treatment trial: The single treatment trial contains metformin, placebo, LSM,oral contraceptives (OC) groups. The combination treatment trial contains LSM plus OC plus	• Serum concentrations of androgens and lipids	• LSM alone resulted in a 59% reduction in free androgen index with a 122% increase in SHBG • OC resulted in a significant decrease in total testosterone (44%) and free androgen index (86%) but also resulted in an increase in C-reactive protein (39.7%) and cholesterol (14%) • The combination of LSM, OC, and metformin resulted in a 55% decrease in total testosterone, as compared	Both LSM and OC significantly reduce androgens and increase SHBG in obese adolescents with PCOS Metformin, in combination with LSM and OC, reduces central adiposity, reduces total testosterone, but does not enhance overall weight reduction

	metformin, or LSM plus OC plus placebo		to 33% with combined treatment and placebo	
Brown *et al*., 2009	37 sedentary PCOS women were randomized to either an 8-12 week ramp-up followed by a 12-week moderate-intensity exercise program or control (no change in lifestyle)	• Fasting lipoprotein profiles • Lipid parameters	• In exercisers group, significant changes were seen in concentrations of lipoprotein parameters that are associated with decreased insulin resistance	Moderate-intensity exercise without significant weight loss improved several components of the lipoprotein profiles of women with PCOS
Karimzadeh *et al*., 2010	Overweight infertile women with PCOS were assigned to four groups: clomiphene, metformin, clomiphene plus metformin, and LSM. The patients in each group received standardized dietary and exercise advice from a dietitian	• Change in menstrual cycle • Waist circumference measurements • Endocrine parameters • Lipid profile • Clinical pregnancy rate	• Improvement rates of menstrual cycle in the clomiphene (66.6%), clomiphene plus metformin (62.5%), metformin (55.5%), and LSM (66.6%) • Clinical pregnancy rate was 12.2% in clomiphene group, 14.4% in metformin group, 14.8% in clomiphene plus metformin group, and 20% in LSM group • LSM group achieved a significant reduction in waist circumference, total androgen, and lipid profile compared to the other groups	Regarding the side effects of medical treatment for PCOS, such as hyperinsulinemia, hyperanderogenemia, and cardiovascular disorders, LSM proves to be the first and foremost way of dealing with the problem

			• Improvement of SHBG in the LSM and\ metformin groups showed significant differences compared with the other two groups	
Palomba *et al*., 2010	96 overweight and obese clomiphene citrate (CC) resistant PCOS patients were randomized in a three-arm trial: structured exercise training (SET) plus hypocaloric diet for 6 weeks (Group A), 2 weeks of observation followed by one cycle of CC therapy (Group B), or SET plus hypocaloric diet for 6 weeks, with one cycle of CC after the first 2 weeks (Group C)	• Ovulation rate • Anthropometric, hormonal and metabolic data	• After 6 weeks of SET plus hypocaloric diet, the ovulation rate was significantly higher in Group C than in Groups A and B • Compared with baseline, in Groups A and C, a significant improvement in clinical and biochemical androgen and insulin sensitivity indexes was observed. In the same two groups, the insulin sensitivity index was significantly better than that in Group B	In overweight and obese CC resistant PCOS patients, a 6-week intervention of SET and a hypocaloric diet was effective in increasing the probability of ovulation under CC treatment
Nybacka *et al*., 2011	4-month trial with three interventions and a long-term follow-up (dietary management,	• Ovarian function • Endocrinologic, and metabolic status • Body composition	• Body mass index was reduced 6% by the dietary management, 3% by the exercise, and 5% by the combined interventions	LSM is suggested as the first line of treatment to improve reproductive and metabolic health in

	physical exercise, or both)		• Lower body fat and lean body mass were significantly decreased in the dietary groups • Upper body fat was lowered and lean body mass maintained by exercise alone • Menstrual pattern was significantly improved • Follow-up of one-half of the patients for a median of 2.8 years revealed sustained weight reduction and improvement in menstrual pattern	overweight or obese patients with PCOS
Barr *et al*., 2013	12-week low-glycemic index (GI) dietary intervention, preceded by a 12-week habitual diet control phase and proceeded by a 12-week follow-up phase	• Change in insulin sensitivity • Changes of lipids, body composition, and estimated macronutrient intake	• No significant changes occurred during the 12-week habitual diet control phase • During the dietary intervention phase, dietary GI significantly decreased with a concurrent small reduction in saturated fat intake • Measures of insulin sensitivity and non-esterified fatty acid improved after intervention	This is the first study to implement an isocaloric low-GI diet in women with PCOS. Findings warrant further investigation via larger, long-term randomized clinical trials in either hypocaloric or isocaloric conditions to fully elucidate the effects

				of a low-GI diet in PCOS
Jakubowicz *et al*., 2013	60 lean PCOS women randomized into two isocaloric maintenance diets with different meal timing distribution: a BF (breakfast diet) (980 kcal breakfast, 640 kcal lunch and 190 kcal dinner) or a D (dinner diet) group (190 kcal breakfast, 640 kcal lunch and 980 kcal dinner) for 90 days	• Insulinre sistance • Hyperandrogenism	• In the BF group, a significant decrease was observed in both glucose and insulin AUC • In the BF group, free testosterone decreased by 50% and SHBG increased by 105% • No change in these parameters was observed in the D group	In lean PCOS women, a high caloric intake at breakfast with reduced intake at dinner results in improved insulin sensitivity indices and reduced cytochrome P450c17α activity, which ameliorates hyperandrogenism and improves ovulation rate

Appendix 8: Nutritional guidelines for lean PCOS patients

The nutritional guidelines you need to follow

1- You will follow a weight maintenance regimen by consuming adjusted amount of food sizes appropriate to your body fat and muscle analysis composition and energy expenditure.

2- You will need to follow a healthy diet and deviate to healthy food choices(Less fat and sugar, and more fruits, vegetables and whole grains products).

Think about serving sizes and begin with small portion of food.

3- When dining out choose a starter with leafy vegetables.

If you don't feel full and satisfied after your meal add leafy vegetables and finish with a fruit.

4- Take your time to eat and chew your food, this will help you control your portion size of meal. Stop your meal before feeling full.

5- Try to eat a rainbow of fruits and vegetables everyday with every meal.

You need to eat 5 fistful sized fruits and vegetables per day, fruits and vegetables are rich in fibers and enhance your satiety.

These can be taken through preparing salads from different kinds of vegetables.

One counted fistful vegetables is 1/2 cup (1/2 handful) cooked, or 1 cup (1 handful) raw. One fistful fruit is around 1 cup

A fist is about 1 cup.

6- Your diet should contain 50 % of healthy carbohydrates such as brown bread, multi-cereals bread, brown pasta, brown rice, whole grain products and bread made of oats.

Reduce white unhealthy carbohydrates such as white bread, white toast, and

cake, candies of different forms, white rice, white pasta, donuts and sugar products.

The plate figure will help you understand your daily nutrition needs.

7- Your proteins daily consumption should be around 20 % of your daily caloric intake chosen from lean meat products such as turkey meat (breast , no skin) , chicken (breast , no skin) ,fish (filet , no skin) and red meat (filet , no fat).

Dairy products protein sources should from white cheese (ex hallumi cheese and traditional white cheese...), skimmed milk and milk products.

Vegetarian or vegan individuals are advised to eat grains such as beans, peas, edamame, quinoa, chia seeds and soy.

8- Avoid consuming organ meat such as intestine due to their high content of unhealthy fat (Saturated fat responsible for cardiac disease)

Avoid consuming high fat milk, full fat soft cheese, meat or chicken with skin and fat on it, sausages, burgers, salamis, palm oil, coconut oil and packaged cakes and biscuits.

9- Your daily fat intake should be around 30 % from your daily caloric of which:

10 %of which should be from polyunsaturated fat such as seeds, almonds, nuts, walnuts, salmon fish.

Mono-unsaturated fat should encompass around 13 % taken from olive oil, olives and avocado.

Saturated fat intake should not exceed 7 % of the total fat allowance (such as butter, lard, margarine and grease).

Reduce from your diet saturated fats such as animal sources and whole milk products.

Reduce trans-fat such as candies, fried food, baked goods, margarines and crackers.

Add to your diet mono-unsaturated fats such as plants oil, avocados, nuts and seeds with limited quantities of maximum 3 tablespoons per day.

10- Consume twice a week fish meat with one of these portionsis from oily fish such as tuna, salmon and mackerel.

The portion should weigh between 120 grams and 140 grams.

11-Replace frying food by grilling or boiling to decrease the unhealthy fat intake caused by frying.

12-Avoid consuming high caloric meals (containing more than 500 calories with more than 40 % sugar and unhealthy fat) such as sweets, cakes, chocolates bars, donuts and pastries, fried food and junk meals.

13-Eat daily 3main meals (divided as breakfast, lunch and dinner) and 2 snacks on regular time of the day to regulate your metabolism.

Make sure to leave 3-4 hours between each meal.

A healthy breakfast can boost your metabolism.

The below table is an example of your meals composition.

Eat smaller meals during the day rather than larger meals to keep having energy in your body.

14-Breakfast is important as it will regulate your appetite during the day and prevent you from consuming large amounts of food on lunch and dinner time.

15-Avoid any meal after 7 pm because the body metabolism decreases by 70 % after 7 o'clock.

16-Regular amount of sleep helps you regulate your appetite. So try to sleep an average of 8 hours per day.

17-Exercise is able not only able to decrease your body weight but it has also shown its effectiveness in PCOS treatment. Exercise has numerous health benefits

Physical activity for at least 30 minutes at least 3 days per week is recommended.

You can be engaged in aerobic, endurance, suppleness and flexibility and resistance training activity types.

Your daily menu plan:

	Breakfast	Snack	Lunch	Snack	Dinner
Carbohydrates	2 servings	1 serving	3 servings		2 servings
Fruits	1 serving	1 serving	1 fruit		

Vegetables	1 serving		2 servings		1 serving
Meat or meat substitutes			4 servings		2 servings
Milk	1 serving			1 serving	
Fat	2 servings		2 servings		1 serving

1 serving of vegetables = 1 cup of raw vegetables = 1 fistful vegetable

Thee palm not including fingers and thumb is about 3 ounces of meat.

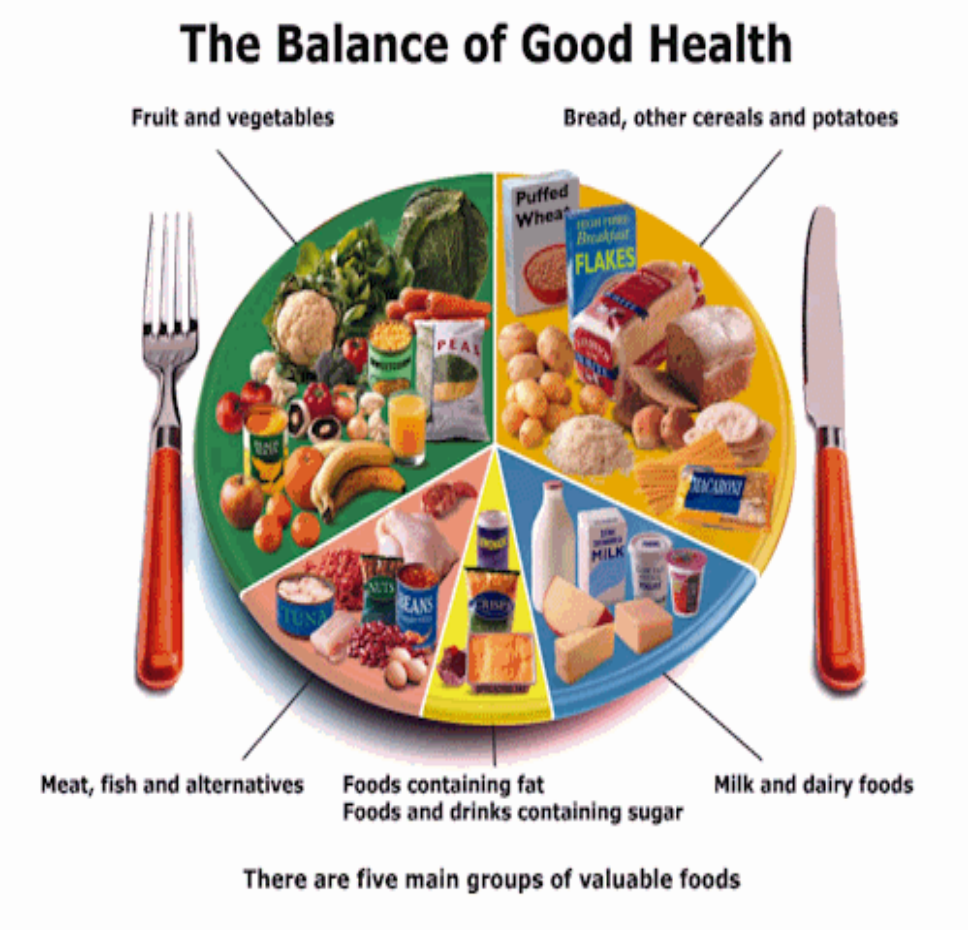
Bread Serving Size Bagel, large (about 4 oz) 1/4 (1 oz)	**Fruit** 1 serving = Apple, unpeeled,	**Lean Meats and Meat Substitutes** 1 serving =	**Fat-Free (skim) or Low-Fat (1%) Milk**	**SERVING CHOICES Monounsaturated Fats 1 serving=**

Or Biscuit, 2.5" wide 1 Or Bread (white/wheat/rye) 1 slice (1 oz) Or Cornbread, 1.75" cube (1 1/2 oz) Or English muffin 1/2 Or Hot dog or hamburger bun 1/2 (1 oz) Or Pancake, 4" wide, ¼" thick 1 Or Pita pocket bread (6" across) 1/2 Or Roll, plain, small 1 (1 oz) Or Tortilla, corn or flour (6" across) 1 Or Waffle, 4" square or round 1 **Cereals and Grains Serving Size** Or Cereals, cooked (oats, oatmeal) 1/2 cup	small 1 (4 oz) Or Applesauce, unsweetened 1/2 cup Or Banana, extra small 1 (4 oz) Or Berries Blackberries 3/4 cup Or Blueberries 3/4 cup or Raspberries 1 cup or Strawberries 1.25 cup whole berries Or Cantaloupe, small 1/3 melon or 1 cup cubed (11 oz) Cherries, sweet fresh 12 (3 oz)	Beef, Select or Choice grades—Ground round, roast (chuck, rib, rump), round, sirloin, steak (cubed, flank, porterhouse, T-bone), tenderloin 1 oz Or Cheeses with < 3 grams of fat per oz 1 oz Or Cottage cheese 1/4 cup Or Egg whites 2 Or Fish, fresh or frozen, plain: catfish, cod, flounder, haddock, halibut,	**and Yogurt** 1 serving = Milk, buttermilk, acidophilus milk, Lactaid 1 cup Or Evaporated milk 1/2 cup Or Yogurt, low-fat, plain or flavored with a low calorie sweetener 2/3 cup or Yogurt, fat-free, plain or flavored with a low calorie	Avocado, medium 2 Tbsp (1 oz) Or Nut butters (trans-fat free) 1.5 tsp Nuts Or Almonds, cashews 6 nuts Or Macadamia 3 nuts Or Peanuts 10 nuts Or Pecans 4 halves Or Oil—Canola, olive, peanut 1 tsp Or Olives, black (ripe) 8 large Or Olives, green (stuffed) 10 large **Polyunsaturated Fats** **1 serving =** Margarine Lower-fat spread 1 Tbsp Or Stick, tub or squeeze 1 tsp Or Mayonnaise Reduced-fat 1 Tbsp Or Regular 1 tsp

Or Cereals, unsweetened, ready-to-eat 3/4 cup Or Granola, low-fat 1/4 cup Or Pasta, cooked 1/3 cup Or Rice, white or brown, cooked 1/3 cup **Starchy Vegetables Serving Size** Or Corn, cooked 1/2 cup Or Corn on cob, large 1/2 cob (5 oz) Or Hominy, canned 3/4 cup Or Peas, green, cooked 1/2 cup	Or Dried fruits (blueberries, cherries, cranberries, mixed fruit, raisins) 2 Tbsp or Grapefruit, large 1/2 (11 oz) or Grapes, small 17 (3 oz) or Guava 1/2 cup or Kiwi 1 (3 1/2 oz) or Mandarin oranges, canned 3/4 cup or Mango, small 1/2 fruit (5.5 oz) or 1/2 cup or Orange, small 1 (6 1/2 oz) or Papaya 1/2 fruit or 1	orange roughy, salmon, tilapia, trout, tuna 1 oz or Hot dog with < 3 grams of fat per oz 1 or Pork, lean—Canadian bacon, rib or loin chip/roast, ham, tenderloin 1 oz or Poultry, without skin 1 oz or Processed sandwich meats with < 3 grams of fat per oz 1 oz Or Tuna, canned in	sweetener 1 cup or Hot cocoa mix, sugar-free 1 envelope Hot cocoa mix, sugar-free 1 envelope	Or Oil—Corn, cottonseed, flaxseed, grape seed, safflower, soybean, sunflower 1 tsp or Salad dressing Reduced-fat 2 Tbsp Or Regular 1 Tbsp

	cup cubed (8 oz) or Passion fruit 1/4 cup or Peaches (fresh, medium) 1 (6 oz) orPears (fresh, large) 1/2 (4 oz) or Pineapple (fresh) 3/4 cup or Plums Small fresh 2 (5 oz) Or Dried (prunes) 3 Or Watermelon 1 slice or 1.25 cups cubes (13.5 oz) **Fruit Juice Serving Size** Or Apple, grapefruit, orange,	water or oil, drained 1 oz **Plant-Based Proteins Serving Size** Baked beans 1/3 cup Or Beans/lentils, cooked (black, garbanzo, kidney, lima, navy, pinto) 1/2 cup or Peas, cooked 1/2 or cup Hummus 1/4 cup or Nut spreads—Almond butter, cashew butter, peanut butter, soy nut butter 1 Tbsp		

	pineapple 1/2 cup Or Grape, prune, and 100% fruit juice blends	or Tempeh 1/4 cup or Tofu 4 oz (1/2 cup)		



If you have further questions please do not hesitate to contact the researcher (CarolineHamadi) to provide you with the information needed.

Appendix 9: Nutritional guidelines for overweigh and obese PCOS patients

The nutritional guidelines you need to follow

1- You will follow a low calorie diet by consuming adjusted amount of food sizes appropriate to your body fat and muscle analysis composition and energy expenditure.
2- You will need to follow a healthy diet and deviate to healthy food choices (Less fat and sugar, and more fruits, vegetables and whole grains products). Decrease your meal portion size by half and use smaller plates rather than

large plates in order to decrease the amount of your meal.

Think about serving sizes and begin with small portion of food.

3- When dining out choose a starter with leafy vegetables.

If you don't feel full and satisfied after your main meal add leafy vegetables and finish with a fruit.

4- Take your time to eat and chew your food, this will help you control your portion size of meal.

Stop your meal before feeling full.

5- Try to eat a rainbow of fruits and vegetables everyday with every meal.

You need to eat 5 fistful sized fruits and vegetables per day, fruits and vegetables are rich in fibers and enhance your satiety.

These can be taken through preparing salads from different kinds of vegetables.

One counted fistful vegetables is 1/2 cup (1/2 handful) cooked, or 1 cup (1 handful) raw. One fistful fruit is around 1 cup.

A fist is about 1 cup.

6- Your diet should contain 50 % of healthy carbohydrates such as brown bread, multi-cereals bread, brown pasta, brown rice, whole grain products and bread made of oats.

Reduce white unhealthy carbohydrates such as white bread, white toast, and cake, candies of different forms, white rice, white pasta, donuts and sugar products.

The plate figure will help you understand your daily nutrition needs.

7- Your proteins daily consumption should be around 20 % of your daily caloric intake chosen from lean meat products such as turkey meat (breast , no skin) , chicken (breast , no skin) ,fish (filet , no skin) and red meat (filet , no fat).

Dairy products protein sources should from white cheese (ex hallumi cheese and traditional white cheese..) , skimmed milk and milk products.

Vegetarian or vegan individuals are advised to eat grains such as beans, peas, edamame, quinoa, chia seeds and soy.

8- Avoid consuming organ meat such as intestine due to their high content of unhealthy fat (Saturated fat responsible for cardiac disease)

Avoid consuming high fat milk, full fat soft cheese, meat or chicken with skin and fat on it, sausages, burgers, salamis, palm oil, coconut oil and packaged cakes and biscuits.

9- Your daily fat intake should be around 30 % from your daily caloric of which:

10 %of which should be from polyunsaturated fat such as seeds, almonds, nuts, walnuts, salmon fish.

Mono-unsaturated fat should encompass around 13 % taken from olive oil, olives and avocado.

Saturated fat intake should not exceed 7 % of the total fat allowance (such as butter, lard, margarine and grease).

Reduce from your diet saturated fats such as animal sources and whole milk products.

Reduce trans-fat such as candies, fried food, baked goods, margarines and crackers.

Add to your diet mono-unsaturated fats such as plants oil, avocados, nuts and seeds with limited quantities of maximum 3 tablespoons per day.

10- Consume twice a week fish meat with one of these portionsis from oily fish such as tuna, salmon and mackerel.

The portion should weigh between 90 grams and 100 grams.

11- Replace frying food by grilling or boiling to decrease the unhealthy fat intake caused by frying.

12- Avoid consuming high caloric meals (containing more than 500 calories with more than 40 % sugar and unhealthy fat) such as sweets, cakes, chocolates bars, donuts and pastries, fried food and junk meals.

13- Eat daily 3main meals (divided as breakfast, lunch and dinner) and 2 snacks on regular time of the day to regulate your metabolism.

Make sure to leave 3-4 hours between each meal.

A healthy breakfast can boost your metabolism.

The below table is an example of your meals composition.

Eat smaller meals during the day rather than larger meals to keep having energy in your body.

14- Breakfast is important as it will regulate your appetite during the day and prevent you from consuming large amounts of food on lunch and dinner time.

15- Avoid any meal after 7 pm because the body metabolism decreases by 70 % after 7 o'clock.

16- Regular amount of sleep helps you regulate your appetite. So try to sleep an average of 8 hours per day.

17- Exercise is able not only able to decrease your body weight but it has also shown its effectiveness in PCOS treatment. Exercise has numerous health benefits

Physical activity for at least 30 minutes per day at least 5 days per week is recommended.

You can be engaged in aerobic, endurance, suppleness and flexibility and resistance training activity types.

Your daily menu plan:

	Breakfast	Snack	Lunch	Snack	Dinner
Carbohydrates	1 serving	1 serving	2 servings		1 serving
Fruits	1 serving	1 serving			
Vegetables	1 serving		2 servings		1 serving
Meat or meat substitutes			2 servings		2 servings
Milk	1 serving			1 serving	
Fat	1 serving		1 serving		1 serving

1 serving of vegetables = 1 cup of raw vegetables = 1 fistful vegetable

Thee palm not including fingers and thumb is about 3 ounces of meat.

Carbohydrates 1 serving = **Bread Serving Size** Bagel, large (about 4 oz) 1/4 (1 oz) Or Biscuit, 2.5” wide 1 Or Bread (white/wheat/rye) 1 slice (1 oz)	**Fruit** 1 serving = Apple, unpeeled, small 1 (4 oz) Or Applesauce, unsweetened 1/2 cup Or Banana, extra small 1 (4 oz) Or Berries	**Lean Meats and Meat Substitutes** 1 serving = Beef, Select or Choice grades—Ground round, roast (chuck, rib, rump), round, sirloin, steak	**Fat-Free (skim) or Low-Fat (1%) Milk and Yogurt** 1 serving = Milk, buttermilk, acidophilus milk, Lactaid 1 cup	**SERVING CHOICES Monounsaturated Fats 1 serving=** Avocado, medium 2 Tbsp (1 oz) Or Nut butters (trans-fat free) 1.5 tsp Nuts Or Almonds, cashews 6 nuts Or Macadamia 3 nuts

Or Cornbread, 1.75” cube (1 1/2 oz) Or English muffin 1/2 Or Hot dog or hamburger bun 1/2 (1 oz) Or Pancake, 4” wide, ¼” thick 1 Or Pita pocket bread (6” across) 1/2 Or Roll, plain, small 1 (1 oz) Or Tortilla, corn or flour (6” across) 1 Or Waffle, 4” square or round 1 **Cereals and Grains Serving Size** Or Cereals, cooked (oats, oatmeal) 1/2 cup Or Cereals, unsweetened, ready-to-eat 3/4 cup	Blackberries 3/4 cup Or Blueberries 3/4 cup or Raspberries 1 cup or Strawberries 1.25 cup whole berries Or Cantaloupe, small 1/3 melon or 1 cup cubed (11 oz) Cherries, sweet fresh 12 (3 oz) Or Dried fruits (blueberries, cherries, cranberries, mixed fruit, raisins) 2 Tbsp or Grapefruit,	(cubed, flank, porterhouse, T-bone), tenderloin 1 oz Or Cheeses with < 3 grams of fat per oz 1 oz Or Cottage cheese 1/4 cup Or Egg whites 2 Or Fish, fresh or frozen, plain: catfish, cod, flounder, haddock, halibut, orange roughy, salmon, tilapia, trout, tuna 1 oz or Hot dog with < 3	Or Evaporated milk 1/2 cup Or Yogurt, low-fat, plain or flavored with a low calorie sweetener 2/3 cup or Yogurt, fat-free, plain or flavored with a low calorie sweetener 1 cup or Hot cocoa mix, sugar-free 1 envelope	Or Peanuts 10 nuts Or Pecans 4 halves Or Oil—Canola, olive, peanut 1 tsp Or Olives, black (ripe) 8 large Or Olives, green (stuffed) 10 large **Polyunsaturated Fats** **1 serving =** Margarine Lower-fat spread 1 Tbsp Or Stick, tub or squeeze 1 tsp Or Mayonnaise Reduced-fat 1 Tbsp Or Regular 1 tsp Or Oil—Corn, cottonseed, flaxseed, grape seed, safflower, soybean, sunflower 1 tsp or Salad dressing Reduced-fat 2 Tbsp

Or Granola, low-fat 1/4 cup Or Pasta, cooked 1/3 cup Or Rice, white or brown, cooked 1/3 cup **Starchy Vegetables Serving Size** Or Corn, cooked 1/2 cup Or Corn on cob, large 1/2 cob (5 oz) Or Hominy, canned 3/4 cup Or Peas, green, cooked 1/2 cup	large 1/2 (11 oz) or Grapes, small 17 (3 oz) or Guava 1/2 cup or Kiwi 1 (3 1/2 oz) or Mandarin oranges, canned 3/4 cup or Mango, small 1/2 fruit (5.5 oz) or 1/2 cup or Orange, small 1 (6 1/2 oz) or Papaya 1/2 fruit or 1 cup cubed (8 oz) or Passion fruit 1/4 cup or Peaches (fresh, medium) 1 (6 oz)	grams of fat per oz 1 or Pork, lean—Canadian bacon, rib or loin chip/roast, ham, tenderloin 1 oz or Poultry, without skin 1 oz or Processed sandwich meats with < 3 grams of fat per oz 1 oz Or Tuna, canned in water or oil, drained 1 oz **Plant-Based Proteins Serving Size** Baked beans 1/3 cup		Or Regular 1 Tbsp

	orPears (fresh, large) 1/2 (4 oz) or Pineapple (fresh) 3/4 cup or Plums Small fresh 2 (5 oz) Or Dried (prunes) 3 Or Watermelon 1 slice or 1.25 cups cubes (13.5 oz) **Fruit Juice Serving Size** Or Apple, grapefruit, orange, pineapple 1/2 cup Or Grape, prune, and 100% fruit juice blends	Or Beans/lentils, cooked (black, garbanzo, kidney, lima, navy, pinto) 1/2 cup or Peas, cooked 1/2 or cup Hummus 1/4 cup or Nut spreads—Almond butter, cashew butter, peanut butter, soy nut butter 1 Tbsp or Tempeh 1/4 cup or Tofu 4 oz (1/2 cup)		

If you have further questions please do not hesitate to contact the researcher (Caroline Hamadi) to provide you with the information need.

Appendix 10:Food frequency questionnaire

FOOD FREQUENCY QUESTIONNAIRE

Think about your eating patterns during the past year while answering this questionnaire. Please indicate your usual intake of each of the following food items per Day, Week, or Month.

For example: Apple. If you consume 3 apples daily, write 3 in the "Day" column, if you think you average 3 apples a week over the year, write 3 in the "Week" column. However, if you rarely consume a food, let's say once or twice a year, then tick below "Rarely/Never".

Please be precise as much as you can.

Remember! The accuracy of the study results depends on the accuracy of your answers.

Food item	Serving size	Day	Week	Month	Rarely / Never
Example: Apple	1 item		3		
Bread and Cereals					
1. White bread (1 slice)	1 slice (30g)				
2. Brown or whole wheat bread	1 slice				
3. Bread, markouk	¼ loaf (30g)				
4. Breakfast cereals, regular/ bran	1 cup				
5. Sugar coated cereals	1 cup				
6. Rice, white, cooked	1 cup				
7. Pasta, plain, cooked	1 cup				
8. Wheat, whole, cooked / Bulgur	1 cup				
9. Popcorn	1 cup				
Dairy products					
10. Low-fat milk (2% fat)	1 cup				

11. Whole fat milk	1 cup				
12. Milk, condensed and sweetened	1 cup				
13. Fat free / low fat yogurt	1 cup				
14. Whole fat yogurt	1 cup				
15. Cheese regular yellow (Kashkawan, cheddar,etc..)	1 slice (30g)				
16. Cheese low fat yellow (Kashkawan, cheddar,etc..)	1 slice (30g)				
17. Cheese white (Akkawi, feta,etc..)	1 slice (30g)				
18. Cheese white low fat (Akkawi, feta,etc..)	1 slice (30g)				
19. Cheese spread (picon, Kraft,etc.)	1 slice (30g)				
20. Cheese spread low fat	1 slice (30g)				
21. Labneh	2 Tbsp				
Fruits & Juices					
22. Apple, fresh, small	1 item				
23. Banana, medium	1 item				
24. Orange (1 item) / Grapefruit (1/2 item)	1 serving				
25. Kiwi	1 small				
26. Mango	1 item				
27. Grapes, fresh	1 cup				
28. Dried fruits: raisins (2 Tbsp), dates (2), apricots (4)	1 serving				
29. Canned fruits	1 cup				
30. Fresh fruit juice	1 cup				
31. Fresh vegetable juice: tomato / other vegetables	1 cup				
32. Fruit drinks: canned/bottled	1 cup				
Food item	**Serving size**	**Day**	**Week**	**Month**	**Rarely / Never**

Vegetables					
33. Salad – green: lettuce, celery, green peppers, onions	1 cup				
34. Dark green vegetables (e.g.: spinach, silq, hindbeh,…)	1 cup				
35. Carrots, raw or cooked	1 cup				
36. Tomatoes, fresh, medium	1 item				
37. Cucumber	1 cup				
38. Corn / green peas, cooked	1 cup				
39. White potato, baked / boiled / mashed	1 item				
40. Squash, summer (kussa), Eggplant /cooked	1 cup				
41. Cauliflower/ Cabbage/ broccoli	1 cup				
Meat & Alternates					
42. Legumes: lentils, broad beans, chickpeas, etc., cooked	1 cup				
43. Nuts and seeds: peanuts, almonds, sunflower seeds, etc.	1 cup				
44. Beef	1 item (90gr.)				
45. Lamb	1 item (90gr.)				
46. Chicken	1 item (90gr.)				
47. Fish, canned with oil: tuna, sardines	1 serving (90gr.)				
48. Tuna canned with water	1 serving (90gr.)				
49. Shellfish: shrimp, lobster, clams	1 cup				
50. Fish, fresh	1 serving (90gr.)				
51. Eggs, whole, large	1 item				
52. Organ Meats(Liver, kidneys, brain)	1 cup				

53. Luncheon meats: Bologna, salami, etc.	1 slice (20g)				
54. Sausages, makanek, hot dogs	1 item (30g)				
Fats and oils					
55. Vegetable oil: corn / sunflower / soy	1 Tbsp				
56. Olive oil	1 Tbsp				
57. Olives	1 item				
58. Vegetable ghee	1 Tbsp				
59. Butter	1 Tbsp				
60. Mayonnaise	1 Tbsp				
61. Lard / animal ghee	1 cup				
Sweets &Desserts					
62. Cookies: chocolate chips, oatmeal, peanut butter, etc.	1 small item				
63. Doughnut / muffin	1 item				
64. Cake	1 item				
65. Pudding or custard, regular	1 cup				
66. Ice cream	1 cup				
67. Chocolate bar	1 item				
68. Sugar,	1 Tbsp				
69. Halawa	1 potion (40g)				
70. Molasses , honey, jam	1 Tbsp				
71. Arabic sweets, baklawa, maamoul, Knefeh	1 item (40g)				

Food item	**Serving size**	**Day**	**Week**	**Month**	**Rarely / Never**
Beverages					
72. Soft drinks, regular (1 can = 1½ cup)	1½ cup (11 fl. oz)				

73. Soft drinks, diet (1 can = 1½ cup)	1½ cup (11 fl. oz)				
74. Turkish coffee (1 small cup = ¼ cup)	¼ cup (2 floz)				
75. Coffee/Nescafe or tea	1 cup				
76. Coffee or tea, decaffeinated	1 cup				
77. Hot chocolate or cocoa	1 cup				
78. Beer, regular (1 can = 1½ cup)	1½ cup				
79. Wine: red, white, or blush	½ cup (4 fl. oz)				
80. Liquor: whiskey, vodka, gin, rum	1/6 cup (1.5 fl oz.)				
Miscellaneous					
81. Manaeesh, zaatar, cheese	1 large				
82. French fries	1 cup				
83. Chips: potato, corn, tortilla	1 cup				
84. Falafel sandwich, medium	1 item				
85. Chawarma sandwich, medium	1 item				
86. Burgers(Beef, chicken, fish)	1 item				
87. Burgers with cheese	1 item				
88. pizza	2 slices				
89. Croissant (plain, cheese, zaatar, chocolat)	1 item				

Are there any other foods not mentioned above that you usually eat at least once per week?

Example: pâté, cream sauce, fava beans, etc (do not include dry spices). Do not list foods that have been listed in the previous section.

Other foods that you usually eat at least once /week	Usual serving size	Servings/week

Appendix 11: 24 hour recall

Questions	Menu	Ingredients	Amount in household measure	Consistency (Dry/Liquid/ Thick/Soft/NA)	Final Weights *(for investigators use only)*
Did you eat or drink anything after you got up in the morning yesterday? What did you have?	**1.**				
	2.				
Did you eat /drink anything else in the morning? *(if working ,then specify; "before going for work")* What did you have?	**1.**				
	2.				
	3.				
Did you eat/drink anything in the afternoon? What did you have?	**1.**				
	2.				
	3.				
	1.				

Did you eat/ drink in the evening *(if working, then specify; "after coming from work")*					
	2.				
What did you have?	**3.**				
Did you eat/drink anything at night?	**1.**				
	2.				
What did you have?	**3.**				
	4.				
Did you eat or drink anything else just before going to bed?	**1.**				
	2.				
What did you have?	**3.**				
Is there anything else	**1.** **2.**				

that you ate/drank yesterday which you haven't told me already? What did you have?	**3.**				

Appendix 12

Table 21 : Average change in food items intake for the sub-population recruited in the scale-up phase

Food type	PCOS			Non-PCOS		
	Intervention	Control	p-value	Intervention	Control	p-value
White bread	-2.5 (±0.5)	3.0 (±1.0)	<0.001	-4.0 (±4.0)	5.0 (± 3.0)	**<0.001
Brown bread	-3.0 (±1.0)	2.0 (±1.0)	<0.001	0.0 (±4.0)	6.5 (±0.5)	**<0.001
Markouk	0.0 (±0.0)	1.0 (±1.0)	<0.001	-4.0 (±7.0)	2.0 (±2.0)	**<0.001
Cereals	-2.0 (±2.0)	1.0 (±0.0)	<0.001	-3.5 (±3.5)	0.0 (±0.0)	**<0.001
Sugar Cereals	-2.5 (±0.5)	2.0 (±0.0)	<0.001	-2.0 (±1.0)	0.5 (±0.5)	**<0.001
Rice	-2.0 (±0.0)	3.0 (±1.0)	<0.001	-5 (±2.0)	1.5 (±2.5)	**<0.001
Pasta	-4.0 (±2.0)	2.5 (±1.5)	<0.001	-2.5 (±1.5)	1.0 (±2.0)	**<0.001
Wheat	-0.5 (±0.5)	1.0 (±0.0)	<0.001	-4.0 (±2.0)	1.0 (±1.0)	**<0.001
Pop corn	0.0 (±0.0)	2.5 (±0.5)	<0.001	0.0 (±0.0)	2.5 (±2.5)	**<0.001
Low fat milk	5.0 (±2.0)	-1.5 (±0.5)	<0.001	1.5 (±0.5)	0.0 (±0.0)	**<0.001
Whole fat milk	-2.0 (±2.0)	2.5 (±1.5)	<0.001	-1.5 (±0.5)	1.5 (±1.5)	**<0.001
Condensed milk	-1.5 (±1.5)	1.5 (±0.5)	<0.001	-1.5 (±0.5)	2.5 (±2.5)	**<0.001
Fat free yogurt	3.0 (±1.0)	-1.0 (±1.0)	<0.001	1.5 (±1.5)	0.0 (±0.0)	**<0.001
Whole fat yogurt	-1.5 (±1.5)	2.5 (±0.5)	<0.001	-2.0 (±0.0)	0.0 (±2.0)	**<0.001
Regular cheese	-4.0 (±3.0)	5.5 (±2.5)	<0.001	-2.0 (±0.0)	-0.5 (±1.5)	**<0.001
Low fat cheese	-1.0 (±3.0)	0.0 (±0.0)	0.012	2.0 (±3.0)	1.0 (±1.0)	0.018
White cheese	4.0 (±1.0)	3.0 (±1.0)	<0.001	1.0 (±1.0)	-1.5 (±1.5)	**<0.001
Low fat white cheese	5.5 (±2.5)	-2.0 (±5.0)	<0.001	3.0 (±1.0)	2.0 (±2.0)	*0.001
Spread cheese	-2.5 (±2.5)	2.5 (±0.5)	<0.001	-4.0 (±3.0)	0.0 (±1.0)	**<0.001
Low fat spread cheese	2.0 (±0.0)	-1.0 (±1.0)	<0.001	5.0 (±1.0)	2.0 (±1.0)	**<0.001
Labneh	0.0 (±0.0)	0.5 (±0.5)	<0.001	0	0	-
Apple	-2.5 (±3.5)	2.0 (±1.0)	<0.001	-1.5 (±2.5)	0.5 (±0.5)	**<0.001
Banana	-1.5 (±1.5)	0.0 (±1.0)	<0.001	-4.0 (±3.0)	0.5 (±0.5)	**<0.001

Orange	0.0 (±0.0)	1.5 (±3.5)	0.002	0.0 (±1.0)	0.5 (±0.5)	*0.001
Kiwi	-0.5 (±0.5)	-1.5 (±2.5)	0.003	-0.5 (±0.5)	0.0 (±0.0)	**<0.001
Mango	0.0 (±0.0)	1.5 (±0.5)	<0.001	-1.0 (±1.0)	-0.5 (±0.5)	*0.001
Grapes	-1.0 (±1.0)	5.0 (±1.0)	<0.001	-5.0 (±5.0)	2.0 (±2.0)	**<0.001
Dried fruits	-1.0 (±1.0)	4.0 (±0.0)	<0.001	-2.0 (±1.0)	-0.5 (±0.5)	**<0.001
Canned fruits	-2.0 (±2.0)	1.5 (±0.5)	<0.001	-2.0 (±1.0)	-0.5 (±0.5)	**<0.001
Fresh juice	-2.5 (±4.5)	4.0 (±0.0)	<0.001	-3.0 (±3.0)	-0.5 (±0.5)	**<0.001
Fresh vegetables juice	-0.5 (±0.5)	1.5 (±2.5)	<0.001	-2.0 (±1.0)	0.0 (±0.0)	**<0.001
Fruit drink	-4.0 (±4.0)	4.0 (±0.0)	<0.001	-3.0 (±2.0)	0.5 (±0.5)	**<0.001
Green salad	5.0 (±4.0)	-3.5 (±0.5)	<0.001	5.0 (±2.0)	-0.5 (±0.5)	**<0.001
Dark green vegetables	2.0 (±2.0)	-0.5 (±0.5)	<0.001	4.0 (±2.0)	0.0 (±0.0)	**<0.001
Carrots	2.5 (±1.5)	5.0 (±1.0)	<0.001	4.0 (±1.0)	0.0 (±0.0)	**<0.001
Tomato	3.0 (±3.0)	1.0 (±1.0)	<0.001	2.5 (±1.5)	0.0 (±0.0)	**<0.001
Cucumber	3.5 (±3.5)	0.0 (±0.0)	<0.001	3.0 (±3.0)	0.0 (±0.0)	**<0.001
Corn/ peas/	-4.0 (±4.0)	0.5 (±2.5)	<0.001	3.0 (±4.0)	0.0 (±0.0)	**<0.001
Potato	-5.5 (±3.5)	3.0 (±1.0)	<0.001	-0.5 (±4.5)	0.0 (±0.0)	***0.397**
Squash	-2.0 (±1.0)	0.5 (±0.5)	<0.001	0.1 (±1.8)	0.1 (±0.1)	**0.836**
Cauliflower	-3.0 (±3.0)	-0.5 (±1.5)	<0.001	3.0 (±0.0)	0.6 (±1.4)	**<0.001
Legumes	2.5 (±1.5)	-4.0 (±1.0)	<0.001	3.0 (±1.0)	-1.0 (±1.0)	**<0.001
Nuts	-3.0 (±1.0)	1.5 (±1.5)	<0.001	1.5 (±2.5)	0.5 (±2.5)	*0.032
Beef	2.5 (±0.5)	-2.0 (±2.0)	<0.001	-2.0 (±3.0)	-1.0 (±1.0)	*0.018
Lamb	-3.5 (±3.5)	4.0 (±2.0)	<0.001	-5.5 (±2.5)	-1.0 (±2.0)	**<0.001
Chicken	1.0 (±1.0)	2.0 (±2.0)	0.001	-2.0 (±4.0)	0.5 (±0.5)	**<0.001
Fish	-4.0 (±4.0)	1.0 (±1.0)	<0.001	-4.0 (±3.0)	-1.5 (±1.5)	**<0.001
Tuna	3.5 (±2.5)	-1.0 (±1.0)	<0.001	3.5 (±2.5)	1.0(±1.0)	**<0.001
Shellfish	2.0 (±1.0)	0.0 (±0.0)	<0.001	0.5 (±0.5)	1.0 (±1.0)	*0.001
Fresh fish	0.5 (±0.5)	0.0 (±0.0)	0.003	0.5 (±2.5)	0.0 (±0.0)	*0.002
Eggs	-3.0 (±3.0)	2.0 (±0.0)	<0.001	-2.5 (±0.5)	-0.5 (±2.5)	**<0.001
Organ meats	-2.5 (±2.5)	0.0 (±0.0)	<0.001	0.5 (±0.5)	0.5 (±0.5)	**1.000**
Luncheon meat	-1.5 (±1.5)	0.0 (±0.0)	<0.001	0.0 (±0.0)	0.0(±1.0)	**1.000**
Sausage	-1.0 (±1.0)	2.5 (±1.5)	<0.001	-2.0 (±2.0)	0.0 (±1.0)	**<0.001
Vegetable oil	-1.5 (±2.5)	1.0 (±1.0)	<0.001	-0.5 (±3.5)	1.5 (±0.5)	**0.001
Olive oil	-5.0 (±4.0)	0.5 (±0.5)	<0.001	-2.0 (±4.0)	1.5 (±0.5)	**<0.001
Olives	-2.5 (±2.5)	0.0 (±0.0)	<0.001	-2.5 (±5.5)	0.5 (±0.5)	**<0.001

Vegetable ghee	-3.5 (±2.5)	0.0 (±0.0)	<0.001	-1.5 (±1.5)	0.0 (±0.0)	**<0.001
Butter	-2.5 (±0.5)	1.5 (±0.5)	<0.001	-2.5 (±1.5)	-1.0 (±1.0)	**<0.001
Mayonnaise	-4.0 (±1.0)	3.0 (±2.0)	<0.001	-3.5 (±1.5)	0.5 (±1.5)	**<0.001
Animal Ghee	-2.5 (±1.5)	0.0 (±0.0)	<0.001	-0.5 (±0.5)	0.0 (±0.0)	**<0.001
Cookies	-5.0 (±4.0)	4.5 (±1.5)	<0.001	-3.0 (±1.0)	-0.5 (±0.5)	**<0.001
Doughnuts	-3.5 (±1.5)	4.5 (±1.5)	<0.001	-1.7 (±1.3)	-0.6 (±0.6)	**<0.001
Cake	-4.0 (±3.0)	4.0 (±0.0)	<0.001	-2.1 (±0.1)	-0.1 (±0.1)	**<0.001
Pudding	-1.5 (±0.5)	2.5 (±1.5)	<0.001	-0.8 (±0.2)	0.0 (±0.0)	**<0.001
Ice cream	-3.5 (±1.5)	2.5 (±1.5)	<0.001	-1.5 (±0.5)	-0.5 (±0.5)	**<0.001
Chocolate	-5.0 (±4.0)	4.0 (±0.0)	<0.001	-2.0 (±0.0)	-1.0 (±1.0)	**<0.001
Sugar	-4.0 (±4.0)	4.0 (±2.0)	<0.001	0.0 (±0.0)	2.5 (±3.5)	**<0.001
Halawa	-1.5 (±0.5)	3.0 (±3.0)	<0.001	-0.5 (±0.5)	-4.0 (±0.0)	**<0.001
Molasses	-2.5 (±1.5)	4.0 (±1.0)	<0.001	-0.5 (±0.5)	-0.9 (±0.9)	*0.012
Arabic sweets	-1.5 (±0.5)	2.5 (±1.5)	<0.001	-0.5 (±0.5)	-0.5 (±1.5)	**1.000**
Soft drinks	-5.5 (±4.5)	3.5 (±3.5)	<0.001	-4.0 (±3.0)	0.5 (±0.5)	**<0.001
Diet soft drinks	0.0 (±0.0)	-2.0 (±2.0)	<0.001	1.5 (±1.5)	0.5 (±0.5)	**<0.001
Turkish coffee	0.5 (±0.5)	-5.5 (±5.5)	<0.001	0	0	**<0.001
Caffeinated beverages	0.0 (±0.0)	-2.0 (±2.0)	<0.001	0	0	**<0.001
Decaffeinated beverages	0.0 (±0.0)	-1.5 (±1.5)	<0.001	0	0	**<0.001
Hot chocolate	-1.5 (±0.5)	0.5 (±0.5)	<0.001	-2.0 (±1.0)	-0.6 (±0.0)	**<0.001
Beer	-1.5 (±1.5)	0.5 (±0.5)	<0.001	-2.0 (±2.0)	0.0 (±0.2)	**<0.001
Wine	-3.5 (±2.5)	0.0 (±0.0)	<0.001	0.0 (±0.0)	-0.2 (±0.4)	**<0.001
Liquor	-2.0 (±2.0)	0.0 (±0.0)	<0.001	-0.05 (±0.05)	0.2 (±0.2)	**<0.001
Manaeesh	-2.5 (±1.5)	5.0 (±4.0)	<0.001	-2.2 (±1.7)	0.2 (±0.05)	**<0.001
French fries	-3.0 (±1.0)	2.5 (±1.5)	<0.001	-3.4 (±2.4)	0.5 (±0.3)	**<0.001
Chips	-1.5 (±0.5)	3.0 (±1.0)	<0.001	-2.0 (±1.0)	0.6 (±0.05)	**<0.001
Falafel	-1.3 (±0.3)	2.0 (±0.0)	<0.001	-0.7 (±0.3)	0.3 (±0.3)	**<0.001

Appendix 13

Table 22 : Average change in food items intake for the sub-population recruited after 12 months

Food type	PCOS		
	Intervention	Control	p-value
White bread	-3.1 (±0.5)	3.1 (±1.0)	**<0.001
Brown bread	-3.4 (±1.0)	2.8 (±1.0)	**<0.001
Markouk	0.0 (±0.0)	1.8(±1.0)	**<0.001
Cereals	-2.5 (±2.0)	1.7 (±0.0)	**<0.001
Sugar Cereals	-2.8 (±0.5)	2.1 (±0.0)	**<0.001
Rice	-2.9(±0.0)	3.8 (±1.0)	**<0.001
Pasta	-4.5(±2.0)	2.9 (±1.5)	**<0.001
Wheat	-1.2 (±0.5)	1.8 (±0.0)	**<0.001
Pop corn	0.5 (±0.0)	2.9 (±0.5)	**<0.001
Low fat milk	5.3 (±2.0)	-1.9 (±0.5)	**<0.001
Whole fat milk	-2.5 (±2.0)	2.9 (±1.5)	**<0.001
Condensed milk	-1.6 (±1.5)	1.9 (±0.5)	**<0.001
Fat free yogurt	3.5 (±1.0)	-2.0 (±1.0)	**<0.001
Whole fat yogurt	-1.6 (±1.5)	2.9 (±0.5)	**<0.001
Regular cheese	-4.7 (±3.0)	6.3 (±2.5)	**<0.001
Low fat cheese	-1.8 (±3.0)	1.0 (±0.0)	*0.012
White cheese	4.7 (±1.0)	4.0 (±1.0)	**<0.001
Low fat white cheese	6.0 (±2.5)	-3.0 (±5.0)	**<0.001

Spread cheese	-2.9 (±2.5)	3.5 (±0.5)	**<0.001
Low fat spread cheese	2.1 (±0.0)	-2.0 (±1.0)	**<0.001
Labneh	0.0 (±0.0)	1.5 (±0.5)	**<0.001
Apple	-2.9 (±3.5)	3.0 (±1.0)	**<0.001
Banana	-1.9(±1.5)	1.0 (±1.0)	**<0.001
Orange	0.0 (±0.0)	2.5 (±3.5)	*0.002
Kiwi	-0.9 (±0.5)	-2.5 (±2.5)	*0.003
Mango	1.2 (±0.0)	2.5 (±0.5)	**<0.001
Grapes	-1.6 (±1.0)	6.0 (±1.0)	**<0.001
Dried fruits	-1.7 (±1.0)	5.0 (±0.0)	**<0.001
Canned fruits	-2.1 (±2.0)	2.5 (±0.5)	**<0.001
Fresh juice	-2.7 (±4.5)	5.0 (±0.0)	**<0.001
Fresh vegetables juice	-0.5 (±0.5)	2.5 (±2.5)	**<0.001
Fruit drink	-4.1 (±4.0)	4.5 (±0.0)	**<0.001
Green salad	5.7 (±4.0)	-4.5 (±0.5)	**<0.001
Dark green vegetables	2.7(±2.0)	-1.5 (±0.5)	**<0.001
Carrots	2.6 (±1.5)	6.0 (±1.0)	**<0.001
Tomato	3.8(±3.0)	2.0 (±1.0)	**<0.001
Cucumber	3.9 (±3.5)	1.0 (±0.0)	**<0.001
Corn/ peas/	-4.1(±4.0)	1.5 (±2.5)	**<0.001
Potato	-5.7(±3.5)	4.0 (±1.0)	**<0.001
Squash	-2.1 (±1.0)	1.5 (±0.5)	**<0.001
Cauliflower	-3.8 (±3.0)	-1.5 (±1.5)	**<0.001
Legumes	2.9 (±1.5)	-5.0 (±1.0)	**<0.001

Nuts	-3.7 (±1.0)	2.5 (±1.5)	**<0.001
Beef	3.5 (±0.5)	-2.0 (±2.0)	**<0.001
Lamb	-4.5 (±3.5)	4.1(±2.0)	**<0.001
Chicken	2.0 (±1.0)	2.1 (±2.0)	*0.001
Fish	-3.4 (±4.0)	1.2 (±1.0)	**<0.001
Tuna	4.0 (±2.5)	-1.5 (±1.0)	**<0.001
Shellfish	2.2 (±1.0)	0.1 (±0.0)	**<0.001
Fresh fish	0.7 (±0.5)	0.1 (±0.0)	**<0.001
Eggs	-3.5 (±3.0)	2.1 (±0.0)	**<0.001
Organ meats	-2.8 (±2.5)	0.1 (±0.0)	**<0.001
Luncheon meat	-1.7 (±1.5)	0.2(±0.0)	**<0.001
Sausage	-1.8 (±1.0)	2.7 (±1.5)	**<0.001
Vegetable oil	-1.9 (±2.5)	1.2 (±1.0)	**<0.001
Olive oil	-5.7 (±4.0)	0.7 (±0.5)	**<0.001
Olives	-2.8 (±2.5)	0.2 (±0.0)	**<0.001
Vegetable ghee	-3.9 (±2.5)	0.8 (±0.0)	**<0.001
Butter	-2.8 (±0.5)	1.9 (±0.5)	**<0.001
Mayonnaise	-4.4 (±1.0)	3.1 (±2.0)	**<0.001
Animal Ghee	-2.9 (±1.5)	0.1 (±0.0)	**<0.001
Cookies	-5.5 (±4.0)	4.9 (±1.5)	**<0.001
Doughnuts	-3.7 (±1.5)	4.5 (±1.5)	**<0.001
Cake	-4.7 (±3.0)	4.1 (±0.0)	**<0.001
Pudding	-1.9 (±0.5)	2.7 (±1.5)	**<0.001
Ice cream	-3.8 (±1.5)	2.6 (±1.5)	**<0.001
Chocolate	-5.4 (±4.0)	4.1 (±0.0)	**<0.001
Sugar	-4.8 (±4.0)	4.1(±2.0)	**<0.001
Halawa	-1.9 (±0.5)	3.1 (±3.0)	**<0.001
Molasses	-2.7 (±1.5)	4.1 (±1.0)	**<0.001

Arabic sweets	-1.9 (±0.5)	2.6 (±1.5)	**<0.001
Soft drinks	-5.8 (±4.5)	3.6 (±3.5)	**<0.001
Diet soft drinks	1.1(±0.0)	-2.1 (±2.0)	**<0.001
Turkish coffee	0.9(±0.5)	-5.6 (±5.5)	**<0.001
Caffeinated beverages	0.9(±0.0)	-2.1 (±2.0)	**<0.001
Decaffeinated beverages	0.9(±0.0)	-16 (±1.5)	**<0.001
Hot chocolate	-1.9 (±0.5)	0.5 (±0.5)	**<0.001
Beer	-1.9 (±1.5)	0.9 (±0.5)	**<0.001
Wine	-3.9 (±2.5)	0.9 (±0.0)	**<0.001
Liquor	-2.1 (±2.0)	0.9 (±0.0)	**<0.001
Manaeesh	-2.9 (±1.5)	5.1 (±4.0)	**<0.001
French fries	-3.1 (±1.0)	2.4 (±1.5)	**<0.001
Chips	-1.9 (±0.5)	3.1 (±1.0)	**<0.001
Falafel	-1.8 (±0.3)	2.1 (±0.0)	**<0.001

All food measures showed statistically significant changes

Appendix 14

Variation in statins intake between the 4 study groups

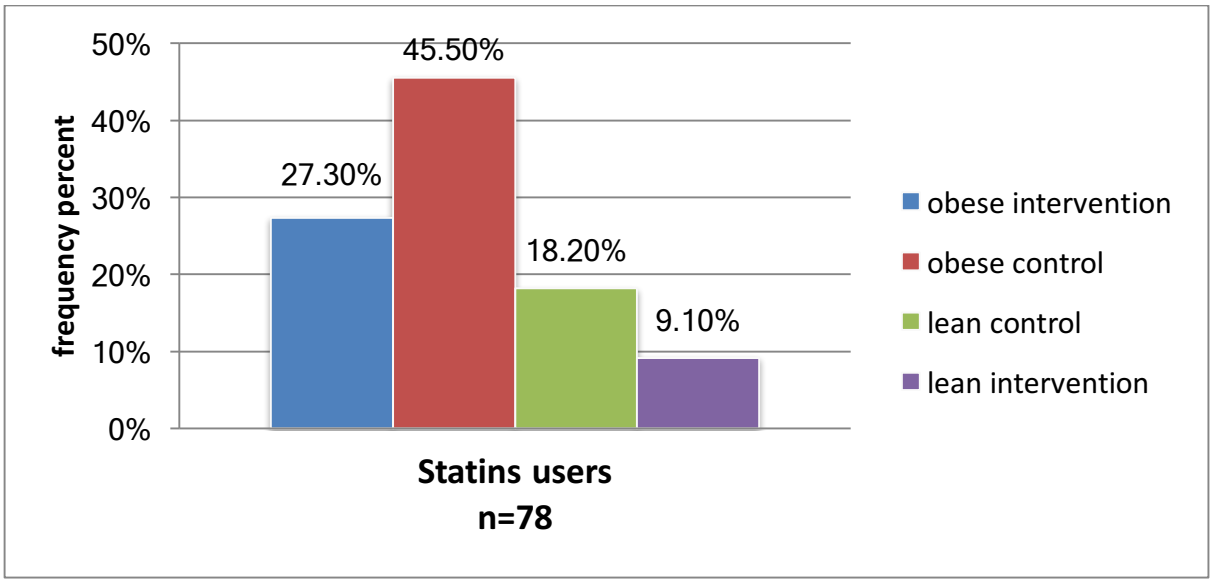


Figure 7 Variation in statins medication intake between the 4 study groups