Cardiometabolic aspects of the polycystic ovary syndrome

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List of Abbreviations

AC	Aortic calcification
AE	Androgen excess
AES	Androgen Excess Society
AMPK	Adenosine-monophosphate kinase
ASRM	American Society for Reproductive Medicine
ATM	Activated tissue macrophages
BMI	Body mass index
CAC	Coronary artery calcification
CHD	Congestive heart disease
CIMT	Carotid intima-media wall thickness
CLS	Crown-like structures
CMKLR1	Chemokine-like receptor 1
CRP	C-reactive protein
CT	Computer tomography
CVD	Cardiovascular disease
DEXA	Dual-energy X-ray absorptiometry
DM2	Type 2 diabetes mellitus
ERK 1/2	Mitogen-activated protein kinase pathway
ESHRE	European Society of Human Reproduction and Embryology
FAI	Free androgen index
FMD	Flow-mediated dilation
FSH	Follicle stimulating hormone

GLUT4	Glucose transporter type 4
GnRH	Gonadotropin releasing hormone
HDL-C	High density lipoprotein cholesterol
HMW	High molecular weight
IGT	Impaired glucose tolerance
IL-6	Interleukin 6
IL-18	Interleukin 18
IMT	Intima-media wall thickness
IR	Insulin resistance
IRS-1	Insulin receptor substrate -1
IVF	In-Vitro Fertilization
LDL-C	Low density lipoprotein cholesterol
LH	Lutenizing hormone
MNC	Mononuclear cells
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
NCBI	National Center for Biotechnology Information
NGT	Normal glucose tolerance
NIH	National Institute of Health
NO	Nitric oxide
OCP	Oral contraceptive pill
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NLM	National Library of Medicine
NPY	Neuropeptide Y

OGTT	Oral glucose tolerance test
PCO	Polycystic ovaries
PCOS	Polycystic Ovary Syndrome
RCT	Randomized controlled trial
SHBG	Sex hormone binding globulin
sOB-R	Soluble leptin receptor
TC	Total cholesterol
TG	Triglycerides
TNF-α	Tumor necrosis factor alpha
VLDL-C	Very low density lipoprotein cholesterol
WBC	White blood cells
WHR	Waist-to-hip ratio

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Summary

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder amongst women of reproductive age. Although PCOS is diagnosed exclusively based on reproductive criteria (clinical/biochemical hyperandrogenism, an/oligo-ovulation, and/or polycystic ovaries (PCO) on ultrasound), it is also a metabolic disorder. Insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, obesity and dyslipidemia are more common in women with PCOS than in age-comparable women without PCOS. Many of the metabolic abnormalities that manifest in PCOS, such as insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, and dyslipidemia are worsened by the concurrent incidence of obesity. However, some of these metabolic perturbations occur even in lean women with PCOS and therefore are rightfully recognized as intrinsic to PCOS. The intrinsic factors that produce these metabolic disturbances are reviewed in this thesis paper. Regarding obesity as both an amplifying factor for other disturbances and as a PCOS-induced disturbance is legitimate, as obesity exacerbates the other PCOS-induced disturbances yet, it is itself an intrinsic PCOS-induced disturbance since it arises more frequently in PCOS women than in control cohorts. The consequences of obesity and the other metabolic aberrations are also discussed. Most importantly, these metabolic perturbations lead to chronic low grade inflammation and to cardiovascular impairments that heighten the risk of having cardiovascular disease. Following a review of cardiovascular impairments and cardiovascular events that have been reported in studies on women with PCOS, this paper then proceeds to discuss therapies currently implemented to minimize these cardiovascular risks. Therapies such as oral contraceptives and anti-androgenic medications used to manage the reproductive manifestations of PCOS may themselves be a cause of metabolic perturbations. Therefore, factors influencing the cardiometabolic side effects arising during the treatment of the non-metabolic manifestations of PCOS (hirsutism/anovulation) will also be discussed in an effort to minimize the overall cardiometabolic risk.

Key words: PCOS Insulin resistance Metabolic Syndrome Cardiovascular Disease Cardiovascular Risk Factors

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder amongst women of reproductive age. It is a heterogeneous disorder of uncertain etiology, but there is strong evidence that complex interactions between genetic, environmental and behavioral factors contribute to causing this syndrome (Bargiota and Diamanti 2012). PCOS affects as many as 10% of reproductive-age women when using the NIH criteria for diagnosis, and up to 18% of reproductive-age women are diagnosed with PCOS per the Rotterdam criteria (March et al. 2010). Nevertheless, at least 70% of PCOS cases remain undiagnosed in primary care (Tomlinson et al. 2013).

Although the diagnosis of PCOS is based exclusively on reproductive criteria (hyperandrogenism, oligo/an-ovulation, and/or PCO on ultrasound) (Hoffman and Ehrmann 2008), and management tends to focus primarily on treatment of infertility and hirsutism (Teede et al. 2010), PCOS is also a metabolic disorder. Women with PCOS have an increased risk of presenting with insulin resistance (IR) (Diamanti-Kandarakis and Dunaif 2012), impaired glucose tolerance (IGT) (Moran et al. 2010), type 2 diabetes mellitus (DM2) (Moran et al. 2010), obesity (Diamanti-Kandarakis and Dunaif 2012), and dyslipidemia (Randeva et al. 2012). In addition to presenting with these traditional risk factors for CVD, women with PCOS also show evidence of an increase of nontraditional, novel CVD risk factors, such as subclinical atherosclerosis (Orio et al. 2004a) and an elevation in inflammatory markers (Escobar-Morreale et al. 2011). As PCOS seems to be dominated by metabolic consequences, both as a consequence of the condition and as a vector for further complications, including DM2, CVD, and the exacerbation of the reproductive features of the syndrome (hirsutism and an/oligoovulation) (Teede et al. 2010), it is evident that research on the metabolic and cardiometabolic features of PCOS is needed. The present review is a contribution to this overall effort. The sections that follow discuss the cardiometabolic aspects of PCOS, their potential causes, their associated risks, and possible screening measures and therapies.

The presentation of many of the topics addressed in the present work follows the same logical progression as that of the paper by Randeva et al. (Randeva et al. 2012). Hence many of the top level section headings will resemble those of that paper. However, the technical content

and inner organization within each section are original and substantially different from those of the paper by Randeva et al (Randeva et al. 2012). In particular, many new subjects are addressed and many others are examined in significantly more depth than those of that paper as well as those of reviews by other authors.

Methods

An extensive literature search was conducted to review publications from the late 1980's to the present. Online sources of medical databases included the U.S. National Library of Medicine (NLM), the National center for Biotechnology Information (NCBI) at the NLM, the Helios Group Central Medical Library, PubMed and MedScape. The search was conducted with a combination of terms that included "PCOS," "cardiometabolic," "cardiovascular disease," "metabolic syndrome," "insulin resistance," "obesity," and many others that were assumed to be relevant. Articles were also selected among references in the published papers found in the automated searches. Studies and review articles covering the focused areas were then selected.

Diagnosis of PCOS

Commonly used Criteria

Three different sets of criteria have been used for the diagnosis of PCOS for the past two decades: the National Institutes of Child Health and Human Development (NICHD) or what is known as the NIH criteria (developed in 1990), the Rotterdam criteria (adopted at a PCOS consensus meeting held in 2003), and the Androgen Excess (AE) and PCOS Society (AE-PCOS) criteria (proposed in 2006) (Teede et al. 2010). These criteria are summarized in Table 1.

Table 1: PCOS Diagnostic Criteria, adapted from Teede et al. 2010.

NIH 1990	Rotterdam 2003	AE-PCOS Society 2006
Both of the following: ・ Chronic anovulation, documented by oligo-or amenorrhea and ・ Clinical and/or biochemical signs of hyperandrogenism (with exclusion of other etiologies, e.g., congenital adrenal hyperplasia) with or without PCO on ultrasound	At least two of the following: ・Chronic anovulation, documented by oligo-or amenorrhea ・ Clinical and/or biochemical signs of hyperandrogenism ・ Polycystic ovaries (by ultrasound)	・ Clinical and/or biochemical signs of hyperandrogenism, and at least one of the following: ・ Ovarian dysfunction (Oligo-anovulation and/or polycystic ovarian morphology)

Metabolic profiles of the diagnostic phenotypes

The different diagnostic criteria create several phenotypes of PCOS. Even before the Rotterdam criteria were adopted, it was evident that different subgroups of PCOS existed and it was even suggested that these subgroups differed metabolically (Diamanti and Dunaif 2012). One extensive review by Moran and Teede sought to compare the metabolic profiles among these different reproductive phenotypes (Moran and Teede 2009). For simplification, the phenotypes were divided into four diagnostic groups: Phenotype A (NIH –PCOS of biochemical/clinical hyperandrogenism and oligo/anovulation with PCO); Phenotype B (NIH – PCOS of biochemical/clinical hyperandrogenism and oligo/anovulation without PCO); Phenotype C (non-NIH PCOS with biochemical/clinical hyperandrogenism and PCO but with normal ovulation; Phenotype D (non-NIH PCOS with oligo/an-ovulation and PCO but without any biochemical/clinical hyperandrogenism) (Moran and Teede 2009). The diagnostic criteria of each of these four phenotypes are summarized in table 2.

Table 2: Diagnostic phenotypes of PCOS, adapted from Moran and Teede (2009).

Phenotype A	NIH-PCOS: hyperandrogenism and oligo/anovulation with PCO
Phenotype B	NIH PCOS: hyperandrogenism and oligo/anovulation without PCO:
Phenotype C	Non-NIH PCOS: hyperandrogenism with PCO but with normal ovulation
Phenotype D	Non-NIH PCOS: No hyperandrogenism but with oligo/anovulation and with PCO

Most studies comparing the two subtypes of NIH PCOS, phenotypes A and B, report that women diagnosed under phenotype A present with few, if any, differences in metabolic profiles compared with women having phenotype B PCOS (Moran and Teede 2009). Similarly, most studies limiting comparison to only non-NIH subtypes, phenotypes C and D, also agree that women with phenotype C PCOS do not present with different metabolic risks compared to women with phenotype D PCOS (Moran and Teede 2009). However, most studies conclude that women with NIH PCOS (phenotypes A and B) present with more adverse metabolic profiles (including higher IR, increased prevalence of metabolic syndrome and more adverse lipid profiles) than those with non-NIH PCOS (phenotypes C and D) (Moran and Teede 2009). Studies comparing women categorized under the NIH and non-NIH PCOS groups, but only after matching the subjects for BMI and WHR, found that the metabolic profiles (degree of IR, metabolic syndrome prevalence and lipid profiles) are similar in the NIH and non-NIH PCOS women (Moran and Teede 2009). These results suggest that although NIH phenotypes present with more adverse metabolic profiles, the worse metabolic profile is not an inherent feature of NIH PCOS but is related to excess adiposity, particularly abdominal adiposity, which is more common in NIH PCOS groups (Moran and Teede 2009).

Cardiometabolic Aspects of PCOS

Insulin Resistance and Hyperinsulinemia

Insulin resistance occurs in 30% of lean women with PCOS (Randeva et al. 2012) and 95% of obese women with PCOS (Wild et al. 2010). Overall, 60-80% of women with PCOS are insulin resistant (Wild et al. 2010). As these statistics illustrate, insulin resistance in PCOS occurs independently of obesity, although excess adiposity, possibly by enhancing the production and secretion of certain adipokines and inflammatory factors (discussed in further sections), exacerbates insulin resistance.

The Impact of Insulin Resistance in PCOS

Insulin resistance (IR) and compensatory hyperinsulinemia produce many complications in PCOS.

Insulin binds to its cognate receptor on ovarian theca cells and enhances LH-stimulated androgen production (Cara and Rosenfield 1988). IR in PCOS interferes with glucose metabolism, without affecting insulin's mitogenic actions, nor insulin-mediated androgen synthesis (Diamanti-Kandarakis and Dunaif 2012). The compensatory hyperinsulinemia that ensues increases ovarian androgen production, contributing in part to the hyperandrogenemia observed in PCOS (Teede et al. 2010). Insulin also inhibits the hepatic production of sex hormone binding globulin (SHBG) (Nestler et al. 1991). Thus, hyperinsulinemia decreases SHBG production, which results in an increase of free, biologically active testosterone levels (Nestler et al. 1991). Interestingly, in a study by Kauffman et al., levels of free testosterone directly correlated with the measures of IR in each woman with NIH-diagnosed PCOS (Kauffman et al. 2008). Although IR can exacerbate hyperandrogenism in PCOS, and there has been debate as to whether hyperandrogenism or IR occurs first, evidence points out that hyperandrogenemism probably occurs before IR. Studies have demonstrated that women with high insulin levels from insulinomas or from exogenous sources do not develop hyperandrogenism, whereas in other studies, testosterone administration to healthy transsexual women or to female rats was followed by IR (Tiras et al. 1999).

Insulin also enhances cholesterol transport into arteriolar smooth muscle cells, and increases cholesterol synthesis in these cells, promoting atherosclerosis (Talbott et al. 2000). Hyperinsulinemia in PCOS, in turn, has been associated with increased intima-media wall thickness (IMT), a marker of subclinical atherosclerosis (Carmina et al 2006b, Talbott et al. 2000). In several studies of women with and without PCOS, IMT showed positive correlations with insulin levels (Carmina et al. 2006b, Folsom et al. 1994, Lasko et al. 1991, Talbott et al. 2000).

Insulin is also a major regulator of many enzymes involved in lipoprotein metabolism (Miccoli et al. 2007, Orio et al. 2004b). Resistance to insulin may contribute, in part, to the dyslipidemia observed in PCOS (Kauffman et al. 2008, Orio et al. 2004b). A detailed description of the steps involved in lipoprotein metabolism is beyond the scope of this review. However, it has been proven that IR increases the hepatic secretion of VLDL and decreases the elimination of VLDL and of chylomicrons (Miccoli et al. 2008). The peristence of VLDL and of chylomicrons in the circulaton provides a major source for triglyceride (TG) production (Miccoli et al. 2008). IR also leads to the more rapid clearance of apolipoprotein-a, a constituent of HDL-C, thus reducing the production and levels of HDL-C (Miccoli et al. 2008). In several population-based studies each involving over 800 enrolled subjects, measures of IR correlated positively with levels of TG and VLDL-C, and negatively with levels of HDL-C (Miccoli et al. 2008). In other studies, Slowinska-Srzednicka and colleagues sought to elucidate the role of insulin resistance in the development of lipid abnormalities in women susceptible to PCOS (Slowinska-Srzednicka et al. 1991). In a group of women with polycystic ovaries, after adjustment for age, BMI and sex hormones, regression analysis showed a strong positive association between fasting insulin levels and TG and VLDL-C levels, and a negative association between fasting insulin levels and levels of the HDL constituent apoliprotien-a (Slowinska-Srzednicka et al. 1991).

The high prevalence of insulin resistance in PCOS renders PCOS women 10 times more likely than controls to develop gestational diabetes, and up to 5 times more likely to develop insulin-related complications such as spontaneous abortion (Randeval et al. 2012).

The Pathogenesis of Insulin Resistance in PCOS

The IR of PCOS is in part independent of obesity; it is primarily a result of intrinsic factors. A post-binding decrease in the phosphorylation of the tyrosine residues and an increase in the phosphorylation of the serine residues of the intracellular domain of the insulin receptor cause resistance to insulin's metabolic actions (Diamanti-Kandarakis and Dunaif 2012). An elevation in serine phosphorylation not only decreases the responsiveness of the insulin receptor to its substrate, but enhances the activity of P450C17, the key enzyme of adrenal and ovarian steroid synthesis (Zhang et al. 1995). The same defect in serine phosphorylation is therefore thought to cause both IR and hyperandrogenism in a subgroup of PCOS patients (Diamanti-Kandarakis and Dunaif 2012). Other possible causes of insulin resistance in PCOS include increased serine phosphorylation of the adaptor protein IRS-1 (Diamanti-Kandarakis and Dunaif 2012). Serine phosphorylation of the latter disrupts intracellular signaling necessary for the translocation of GLUT4 into the plasma membrane (Diamanti-Kandarakis and Dunaif 2012). Reduced expression of GLUT4 has been demonstrated in the plasma membranes of adipocytes of both lean and obese PCOS patients (Rosenbaum et al. 1993). Increased activation of ERK1/2 pathways in muscle cells of PCOS women may also be responsible for resistance to insulin's metabolic actions (Corbould et al. 2006, Diamanti-Kandarakis and Dunaif 2012). Although ERK1/2 pathways are usually involved in insulin's mitogenic actions (Corbould et al. 2006, Diamanti-Kandarakis and Dunaif 2012), enhanced basal activation of ERK1/2 can also inhibit the IRS-1 pathways necessary for GLUT4 translocation to the plasma membrane (Corbould et al. 2006). Increased lipolysis in visceral fat cells may contribute to the hepatic insulin resistance observed in obese PCOS women (Ek et al. 2002). Visceral fat cells of PCOS women demonstrate an enhanced lipolytic response to catecholamines (Ek et al. 2002). Enhanced lipolysis of visceral fat raises fatty acid and glycerol delivery to the portal vein and liver, perturbing liver function, eventually leading to hepatic IR, as well as to hepatic inflammation and to interference with the production of SHBG (Ek et al. 2002).

Impaired Glucose Tolerance and Type 2 Diabetes

The American Diabetes Association has designated PCOS as a non-modifiable risk factor for type 2 diabetes (American Diabetes Association 2004). The prevalence of IGT and DM2 in women with PCOS, assessed in three large ethnically diverse U.S. cross-sectional studies, was 23-35% for IGT and 4-10% for DM2, that is twice the prevalence in age- and weight- matched healthy women without PCOS (Diamanti-Kandarakis and Dunaif 2012). The prevalence of IGT and DM2 among PCOS women from other countries (Italy, Netherlands) was also found to be significantly higher than the prevalence in control women from the same region, although the overall proportion of European PCOS women having IGT or DM2 is nevertheless lower than that of U.S. PCOS women affected by these conditions (Diamanti-Kandarakis and Dunaif 2012). Authors suggest that different diagnostic criteria, diet, race and ethnicity may account for the higher prevalence of IGT and DM2 in U.S. PCOS women (Diamanti-Kandarakis and Dunaif 2012, Kaufmann et al. 2008). When the PCOS women from European and U.S. studies were stratified according to BMI and comparisons were limited to women in comparable BMI categories, the differences in the prevalence of IGT and DM2 between U.S. and European PCOS women still persisted but decreased, highlighting the contribution of lifestyle to the disparities in IGT and DM2 observed between U.S. and European women (Diamanti-Kandarakis and Dunaif 2012). Additionally, a study of two PCOS populations in the U.S., one urban ethnically diverse and one rural ethnically homogeneous, showed similar proportions of women with IGT and DM2 in each of the two populations, therefore demonstrating that PCOS may be a more important risk factor for IGT and DM2 than factors such as race and ethnicity (Legro et al. 1999). These general tendencies towards a deterioration of glucose metabolism have been confirmed by a meta-analysis indicating a higher prevalence of IGT (odds ratio 2.54) and DM2 (odds ration 4) in PCOS women than in BMI matched controls (Moran et al. 2010).

Studies have also reported higher conversion rates from normal glucose tolerance (NGT) to IGT and from IGT to DM2 in PCOS women (Celik et al. 2014, Legro et al. 2005). IGT is an independent predictor of developing DM2 (Barr et al. 2006), CVD (Barr et al. 2006, Tominaga et al. 1999) and of suffering mortality from CVD (Barr et al. 2006, Tominaga et al. 1999). Early identification and treatment of IGT with lifestyle intervention and/or metformin have been

shown to improve outcomes (Knowler et al. 2002). These observations have led experts at the most recent ESHRE/ASRM-sponsored PCOS consensus workshop to suggest the implementation of an annual screening of all PCOS women for IGT with the OGTT (Fauser et al. 2012, Tomlinson et al. 2013), the most sensitive test for assessing IGT in PCOS (Diamanti-Kandarakis and Dunaif 2012).

Chronic hyperinsulinemia *per se* exacerbates IR, leading to a higher demand for insulin production and eventually to β-cell burnout, thereby accelerating the progression to IGT and DM2 (Teede et al. 2010). Although women with PCOS have higher basal insulin secretion conditioned by chronic IR, they demonstrate β-cell secretory defects, manifested by reduced insulin secretory response to meals (O'Meara 1993) and eventually an overall secretion of insulin that is inadequate for the degree of IR (Dunaif and Finegood 1996). IR, β-cell secretory defects and eventual β-cell burnout contribute to the development of IGT and DM2 in PCOS (O'Meara et al. 1993).

Obesity in PCOS

The prevalence of obesity among women with PCOS in the U.S. is 70 to 80%, almost twice as much as in the general U.S. female population (Carmina et al. 2003, Diamanti-Kandarakis and Dunaif 2012). Most studies report the prevalence of obesity in affected women outside the U.S. to be between 38 to 50% (Carmina et al. 2003, Diamanti-Kandarakis 2012). Differences in diagnostic criteria, environmental factors, ethnicity, and lifestyle contribute to these variations (Carmina et al. 2006a, Diamanti-Kandarakis and Dunaif 2012, Kauffman et al. 2008). Although less obesity is reported outside the U.S., the prevalence of obesity among PCOS women outside the U.S. is still higher than that of women in the general population outside the U.S. (Carmina et al. 2003). As an example, 38% of Italian PCOS women are reported to be obese (Carmina et al. 2003), but the reported prevalence of obesity in the general Italian female population is only 8% (Gallus et al. 2013), highlighting a possible contribution of PCOS *per se*, in addition to lifestyle and other factors cited above, to the pathogenesis of obesity.

Consequences of Obesity in PCOS

Obesity plays a role in the expression of metabolic features and other clinical manifestations of PCOS (Diamanti-Kandarakis and Dunaif 2012, Randeva et al. 2012). IR appears in normal weight PCOS women, but the frequency and magnitude increases with obesity (Dunaif et al. 1989, Moran et al. 2012).The magnitude of IR, quantified in one study by the insulin to glucose ratio, demonstrated a strong, positive and linear correlation with body mass index (BMI) of the PCOS subjects (Moran et al. 2012). Hepatic insulin resistance, characterized by reduced sensitivity to insulin's suppression of endogenous glucose production, only occurs in obese PCOS women (Sam et al. 2007). Obese PCOS women also have a 10-fold increase in their risk of suffering from DM2 and a 7-fold increase of IGT compared with normal weight (BMI <25 kg/m^2) PCOS women. (Norman et al. 2001). An accelerated rate of conversion from IGT to DM2 is strongly dependent upon BMI (Ehrmann et al. 1999).

Excess BMI also exacerbates the reproductive abnormalities of PCOS. One study found an inverse correlation between FSH levels and BMI in obese PCOS patients, as well as an increased prevalence of menstrual disorders (anovulation, oligo-ovulation) among obese PCOS women compared to non-obese ones (Kiddy et al. 2008). Obese PCOS women have responded less frequently to ovulation induction treatment than their non-obese counterparts (White et al. 1996). Other studies comparing obese and non-obese PCOS women reported lower rates of implantation after IVF in the obese PCOS sub-group (Fedorcsák et al. 2001 and McCormick et al. 2008). Weight reduction of 5-10% by lifestyle modification has been shown to restore ovulation in anovulatory PCOS women (Huber-Buchholz et al. 1999).

Obesity in PCOS also increases the patient's risk of developing cardiovascular disease. Among PCOS women, the prevalence of the metabolic syndrome, as in the general population, increases with increasing BMI and is highest in obese women with PCOS (Carmina et al. 2006a). Studies report the prevalence of the metabolic syndrome in PCOS women in the U.S. to be 43-47%, twice more than in the age- and BMI- matched control population, suggesting that PCOS *per se*, possibly by promoting abdominal fat accumulation, increases the risk of acquiring the metabolic syndrome (Carmina et al. 2006a). The effect of obesity, of causing chronic low-grade inflammation (Attie and Scherer 2009) with an elevation of inflammatory markers (such as CRP,

TNF-α and IL-6) that increase the risk of cardiovascular disease (Lind 2003), is even more pronounced in PCOS. In PCOS and non-PCOS women, levels of TNF-α (Gonzalez et al. 1999, Samy et al. 2009), IL-6 (Samy et al. 2009) and CRP (Samy et al. 2009) correlate directly with BMI, but overweight and obese PCOS women in some studies have presented with significantly higher levels of these inflammatory markers than their BMI-matched non-PCOS counterparts (Escobar-Morreale et al. 2011, Samy et al. 2009).

The pathogenesis of obesity in PCOS

The pathogenesis of obesity in PCOS is likely multifactorial (Faulds et al. 2003).

One study reported that in a group of lean PCOS women, serum glycerol levels, which reflect lipolytic activity, were lower than glycerol levels in BMI-matched control women (Fauld et al. 2003). The same authors also showed that the subcutaneous adipocytes of the lean PCOS women were larger in size and exhibited a lower response to catecholamine-stimulated lipolysis than the adipocytes of the BMI-comparable control women, suggesting that decreased lipolysis of subcutaneous adipocytes is an early alteration in PCOS, leading to enlarged subcutaneous fat cells, and later to the development of obesity (Faulds et al. 2003).

Another contributor to the high prevalence of obesity in PCOS might be mutations in the peroxisome proliferator-activated receptor-ɤ gene (Orio et al. 2003). A higher frequency of C→T substitution in exon 6 of the peroxisome proliferator-activated receptor-ɤ gene has been reported in PCOS women than in BMI-matched controls (Orio et al. 2003). This substitution enhances the differentiation of fibroblasts and undifferentiated cells into mature adipocytes, possibly leading to obesity (Orio et al. 2003).

Several studies also report that women with PCOS (McCluskey et al. 1991, Hirschberg et al. 2004) or with PCO morphology on ultrasound (Jahanfar et al. 1995) have a higher prevalence of bulimic behavior, in part because of increased androgens (Naessém et al. 2006), which increase appetite and inhibit impulse control (Naessém et al. 2006). After treatment with flutamide (Sunbald et al. 2005) or with anti-androgenic oral contraceptives (Naessén et al. 2007), a reduction of binge eating and meal-related hunger, respectively, has been reported in bulimic women, supporting the idea that androgens may play a role in appetite dysregulation and in the

development of obesity in PCOS (Naessém et al. 2006). Women with PCOS, in comparison to BMI-matched controls, also have reduced secretion of the gastro-intestinal satiety peptide cholecystokinin (Hirschberg et al. 2004) and have dysregulated secretion of the appetite-regulating gut hormone ghrelin (Barber et al. 2008a, Moran et al. 2004) that is independent of diet. These alterations may cause the reduction in satiety that has been reported by PCOS patients in comparison to BMI-matched control women (Hirschberg et al. 2004, Moran et al. 2004).

Additionally, it is widely believed that ghrelin's actions are mediated centrally by neuropeptide Y (NPY) and by the system of NPY fibers (Barber et al. 2008a). NPY acts centrally to increase appetite (Kokot and Ficek 1999). In a study on PCOS women, NPY levels were reported to be higher in obese and lean women with PCOS than in BMI-comparable control women (Baranowska et al. 1999). NPY has also been shown to augment the population of LH and of GnRH secreting fibers (Subdehar et al. 2005), while diminished NPY secretions have been linked with a lower surge-amplitude of LH and of GnRH (Sahu and Kalra 1998). It is possible that elevated levels of this neuropeptide in PCOS may also lead to high LH levels. NPY may therefore represent a link between reproductive dysfunction and obesity, and a fundamental contributing cause of PCOS. However, this hypothesis is purely speculative, as relatively few studies were found that have evaluated NPY levels in PCOS (and searching for additional studies is beyond the scope of this thesis). Future investigations focusing on the metabolic aspects of PCOS should consider evaluating NPY levels, as well as whether these levels correlate with BMI and with gonadotropic hormone levels (especially if such studies cannot be found in an extensive literature search on this particual subject).

The Distribution of Adipose Tissue in PCOS

Subcutaneous abdominal fat and visceral fat both contribute to the development of IR (Smith et al. 2001). Visceral fat furthermore creates a chronic low-grade inflammation (Carmina et al. 2007) and is a surrogate marker for ectopic fat accumulations (Huang et al. 2013), which are responsible for many of the harmful effects of obesity (Fabbrini et al. 2009, Klöting et al. 2010). Many studies, based on anthropometric measures such as the waist-to-hip (WHR) ratio, suggest that there is a tendency in PCOS towards the accumulation of fat in these harmful areas

such as the abdominal visceral region. It is known that fat distribution in the abdominal area is associated with more adverse metabolic profiles in PCOS (Manneras-Holm et al. 2011, Moran and Teed 2009). However, there is debate whether abdominal fat storage occurs more in PCOS than in weight-matched controls. Several studies quantifying abdominal subcutaneous and visceral adipose tissue by MRI (Barber et al. 2008, Manneras-Holm et al. 2011), CT (Yalamanchi et al. 2012) and DEXA (which only quantifies total central abdominal fat) (Good et al. 1999) found no difference in the volume of total abdominal fat or visceral fat between PCOS women and BMI-matched controls. However, in other studies, MRI (Huang et al. 2013) showed an increase in visceral and subcutaneous abdominal fat and DEXA (Kirchengast and Huber 2001) showed an increase in the proportion of upper body fat in PCOS women compared to BMI-matched controls. The different results may depend on the small number of patients and controls and may also be related to the degree of obesity (Huang et al. 2013). Studies in which the majority (Carmina et al. 2007, Huang et al. 2013, Puder et al. 2005) or all (Kirchengast and Huber 2001) of the women were non-obese have reported a higher quantity of central abdominal fat in PCOS women than in BMI-matched controls. Results from a more highly powered study with over 200 patients and controls evaluated by DEXA support the suggestion that the disparities reported by different authors are related to the degree of obesity (Carmina et al. 2007). When the 220 subjects from the study were stratified according to BMI into obese and non-obese sub-groups, there was no difference in the quantity of central abdominal fat between obese PCOS women and obese control women, but when limiting the comparison to the non-obese women, the quantity of central abdominal fat was higher in non-obese PCOS women compared to the quantity in non-obese control women (Carmina et al. 2007). These observations demonstrate that when obesity is present, most subjects display abdominal obesity, independent of being afflicted with PCOS or not (Carmina et al. 2007). However, when obesity is not present, PCOS patients stock a higher portion of their total adiposity in the abdominal region than do BMI-comparable controls (Carmina et al. 2007). Abdominal adiposity may therefore be a risk factor in non-obese PCOS women that confers on them adverse metabolic profiles compared to their BMI-matched non-PCOS counterparts (Carmina et al. 2007). Studies report that the quantity of central abdominal fat positively correlates with the degree of IR in non-obese PCOS women (Carmina et al. 2007) and to the level of inflammatory markers (Puder et al. 2005). DEXA accurately quantifies fat in different regions, is not operator dependent and, unlike MRI,

can be used on large populations (Carmina et al, 2007). DEXA may therefore be a useful screening tool for non-obese PCOS women susceptible to central abdominal fat accumulation, and, hence, to the adverse metabolic complications associated with centripetal fat distribution (Carmina et al. 2011, Moran and Teed 2009).

Adipokines

The increased incidence and severity of cardiovascular risk factors and of metabolic disturbances in PCOS may be in part related to the abnormal production and release of adipokines and inflammatory factors by adipose tissue (Randeva et al. 2012). Although traditionally regarded as a storage organ, emerging evidence also strongly suggests that adipose tissue is an endocrine organ (Randeva et al. 2012), whose altered function may produce widespread cardiometabolic disturbances in PCOS. As alluded to earlier, it is believed that dysregulated adipocyte function and obesity play a pathophysiological role in PCOS (Faulds et al. 2005, Teede et al. 2010).

Leptin

Leptin, a protein secreted by adipocytes, suppresses an individual's appetite and promotes energy expenditure (Kumar and Clark 2009). Serum leptin levels are elevated in obese patients, who are considered leptin resistant (Kumar and Clark 2009).

Hyperleptinemia: Data from a study by Wallace et al. with over 1000 study participants suggested that hyperleptinemia is an independent, positive risk factor for cardiovascular disease (Wallace et al. 2001). Knudson et al. showed that leptin receptors are present on coronary endothelium (Knudson et al. 2005). Excessive leptin may cause coronary endothelial dysfunction (Knudson et al. 2005) followed by the subsequent development of atherosclerosis (Reilly et al. 2004).

Leptin and reproductive disorders:: Some authors have suggested that leptin plays a role in reproduction. Leptin deficiency in mice, induced by the ob/ob genetic mutation (Barash et al. 1996) or by starvation (Ahima et al. 1996), produces amenorrhea and hypogonadism, whereas leptin administration to such mice restores menstruation and treats hypogonadism (Barash et al. 1996, Ahima et al. 1996). Although leptin deficient mice have low levels of LH and FSH, leptin

levels in most studies of PCOS women did not correlate with LH or FSH levels (Mantzoros et al. 1997, Hahn et al. 2006, Rouru et al. 1997, Telli et al. 2002).

Leptin levels in PCOS: These findings, including the possible effects of leptin resistance on the pituitary-gonadal axis (Telli et al. 2002), have led authors to investigate the possible role of leptin in PCOS. Although some studies have found leptin levels to be elevated in PCOS women compared to controls (Brzechffa et al. 1996, Yildizhan et al. 2011), the general consensus reported by the majority of published studies is that there is no difference in circulating leptin levels in PCOS subjects in comparison to BMI-matched controls (Gennarelli et al. 1998, Hahn et al. 2006, Iuorno et al. 2007, Mantzoros et al. 1997, Pirwany et al. 2001, Rouru et al. 1997, Sepillian et al. 2006, Svendsen et al. 2012, Telli et al. 2002). The different results might be explained by differences in ethnicity, heterogeneity in criteria used to classify PCOS, and low number of PCOS subjects and controls (Chen et al. 2013, Telli et al. 2002). Furthermore, the studies by Brzechffa et al. and Yildizhan et al. did not match properly for BMI (Telli et al. 2002). Further investigation with more participants may be needed to more precisely elucidate the role of leptin in PCOS.

In support that leptin levels do not differ between PCOS women and controls are two important studies. One of these is the study by Telli et al. which used uniform criteria (hyperandrogenism, oligomenorrhea and PCO on ultrasound) to recruit PCOS subjects and matched adequately for BMI. The second of these is the study by Hahn et al., which included over 200 participants, more than the other studies. Both of these studies found no differences in leptin levels in PCOS women in comparison to BMI-matched controls (Hahn et al. 2006, Telli et al. 2002). Furthermore, other studies report the levels of circulating leptin to be no different in ovulatory PCOS patients and in non-ovulatory ones (Carmina et al. 2009, Pirwany et al. 2001); the same finding applies between oligomenorrheic and amenorrheic PCOS women (Hahn et al. 2006).

Interestingly, Hahn et al. reported that despite similar levels of total leptin, PCOS women had lower levels of the soluble leptin receptor sOB-R and therefore higher levels of free biologically active leptin (Hahn et al. 2006). However, in a similar study, Sepilian et al. reported no differences in the level of sOB-R or free leptin index between PCOS women and controls (Sepilian et al. 2006). Results from a study of leptin levels and sOB-R levels in twins suggests

that BMI influences total leptin levels while genetic components regulate sOB-R levels (Hahn et al. 2006, Li et al. 2005), suggesting that genetic background may be responsible for lower sOB-R levels and higher free leptin levels in a subgroup of PCOS women (Hahn et al. 2006). However, given the differing and varied results among studies, further investigations are needed to elucidate the role of sOB-R in PCOS.

Leptin and adiposity in PCOS: Most studies report that adiposity, quantified by BMI, is the main correlative component and determinant of leptin levels in PCOS women (Gennarelli et al. 1998, Hahn et al. 2006, Iuorno et al. 2007, Mantzoros et al. 1997, Pirwany et al. 2001, Rouru et al. 1997, Sepillian et al. 2006, Svendsen et al. 2012, Telli et al. 2002). Leptin mRNA expression in adipocytes did not differ between PCOS women and BMI-matched controls (Svendsen et al. 2012), providing further evidence that obesity, rather than PCOS *per se*, affects leptin production and circulating levels.

Leptin, androgens and PCOS: After adjustment for BMI, some authors report that leptin levels do correlate minimally with the free androgen index (Geneneralli et al. 1998, Hahn et al. 2006, Laughlin et al. 1997, Pirwany et al. 2001), but nevertheless do not differ between visibly hirsute and non-hirsute women with PCOS (Gennarelli et al. 1998).

Leptin and insulin levels in PCOS: Most studies also addressing leptin and insulin report that after adjustment for BMI, leptin levels in PCOS women do not correlate with the chronic insulin levels (Genneralli et al. 1998, Laughlin et al. 1997, Mantzoros et al. 1997, Sepilian et al. 2006, Svendsen et al. 2012), while others report that leptin levels in PCOS women did correlate with measures of insulin resistance (Hahn et al. 2006, Yildizhan et al. 2011). However, in further support of findings that negate a significant correlation between insulin resistance and leptin levels, treatment of chronically hyperinulinemic insulin resistant PCOS women with the thiazolidinediones troglitazone (Manntzoros et al. 1997) or rosiglatazone (Sepilian et al. 2006) was shown to lower insulin levels but did not alter leptin levels in these patients.

Adiponectin

Adiponectin, which is secreted exclusively by adipose tissue, exerts insulin sensitizing actions both indirectly (Kadowaki et al. 2006) and directly by activating tyrosine phosphorylation of the skeletal muscle insulin receoptor (Stefan et al. 2002). Adiponectin levels

are reduced in insulin resistance states such as DM2 across all ethnic groups (Weyer et al. 2001). Low levels are also associated with a faster progression towards DM2 in at-risk individuals (Weyer et al. 2001) and with higher risk of CHD in women (Zyriax et al.2008). Low levels are also possibly associated with high LH/FSH ratios and impaired ovulation because adiponectin in normal levels reduces secretion of LH through AMPK phosphorylation without affecting FSH secretion (Lu et al. 2008).

A meta-analysis has demonstrated that adiponectin levels are lower in PCOS women than in control women of comparable BMI (Toulis et al. 2009). A more recent meta-analysis has indicated that the T45G polymorphism in the adiponectin gene is associated with PCOS (Gao et al. 2012). Although few studies exist focusing on high molecular weight adiponectin and the earlier meta-analysis did not specifically evaluate levels of high molecular weight (HMW) adiponectin (Toulis et al.2008), which is considered to be a more potent mediator of insulin sensitivity (Pajvani et al. 2004), it has been reported that levels of HMW adiponectin and the ratio of HMW adiponectin to total adiponectin are both lower in PCOS women than in age and BMI comparable controls (Wickham et al. 2011).

Visfatin

Visfatin is a cytokine secreted, among other cell types, by adipocytes (Fukuhara et al. 2005). It stimulates glucose uptake by cells, thus inducing insulin-mimetic effects (Fukuhara et al. 2005). A meta-analysis established that plasma visfatin levels are significantly increased in subjects with obesity, DM2, metabolic syndrome and CVD (Chang et al 2011). Furthermore, in diabetics, serum visfatin levels increase with progressive β-cell deterioration (Lòpez-Bermejo et al. 2006). Haider et al demonstrated that insulin inhibited visfatin release from adipocytes in healthy subjects, suggesting that elevated visfatin levels may reflect insulin resistance (Haider et al. 2006).

Given visfatin's insulin-mimetic actions, some authors have suggested visfatin may be elevated to compensate for insulin resistance (Tan et al. 2006) and to prevent further resistance to insulin (Kowalska et al. 2007, Zahorska-Markiewicz et al. 2007). However, elevated visfatin levels may produce harmful effects. Rising visfatin levels correlate with the degree of endothelial dysfunction, quantified by the decline in flow mediated vasodilation and impaired renal clearance (Takebayashi et al. 2007). Visfatin activates nuclear transcription factor NF-kB in

vascular endothelial cells (Adya et al. 2008) and in lipid-laden macrophages of atherosclerotic lesions (Fan et al. 2011), culminating in the activation of metalloproteinase 2 (Adya et al. 2008) and metalloproteinase -9 (Fan et al. 2011, Dahl et al. 2007), leading to vascular inflammation and plaque destabilization, respectively.

In patients undergoing carotid endarterectomy or percutaneous coronary interventions, visfatin expression is higher in the atherosclerotic lesions of symptomatic patients than in the lesions of unsymptomatic patients, further emphasizing the role of this adipokine in plaque destabilization and acute cardiovascular events (Dahl et al. 2007). Likewise, elevated visfatin levels in PCOS may also signal heightened cardiovascular risk in certain women with this syndrome, particularly in those with insulin resistance.

Given its associations to insulin resistance and vascular inflammation, several studies have undertaken to elucidate the role of visfatin in PCOS. Higher levels of serum visfatin and visfatin mRNA in adipocytes have been reported in PCOS women compared to BMI-matched controls (Chan et al. 2007, Kowalska et al. 2007, Ozkaya et al. 2010, Panidis et al. 2008, Tan et al. 2006). Serum visfatin levels were found to correlated with BMI (Tan et al. 2006, Chan et al. 2007), insulin resistance (Kowalska et al. 2007, Seow et al. 2011, Tan et al. 2006), free androgen index (Kowalska et al. 2007) and LH levels (Panidis et al. 2008).

It has been observed that metformin treatment for 3 months lowered visfatin levels (Ozkaya et al. 2010). However, the investigations demonstrated many inter-study variations in parameters such as BMI, IR, FAI and LH that significantly correlated with visfatin levels in some studies but not in others. These variations may be attributed to the small number of participants, less than 30 PCOS women in all but one (Kowalska et al. 2010) of these investigations, and to interracial variations in the phenotypic expression of PCOS (Tan et al. 2006).

More recent studies have reported no differences in visfatin levels between PCOS women and controls (Lajunen et al. 2012, Olszanecka-Glinianowicz et al. 2012), therefore necessitating further inquiries with more participants to clarify the role of this adipokine in PCOS.

Chemerin

Chemerin is a chemotactic protein secreted by adipocytes (Bozaoglu et al. 2007, Sell et al. 2009) that is necessary for adipocyte differenciation (Roh et al. 2007). It is able to attract macrophages, which express the chemerin receptor CMKLR1 (chemokine-like receptor 1) (Zabel et al. 2005). In view of its chemo-attractant properties, this adipokine may be one factor underlying the link between obesity and chronic inflammation (Zabel et al. 2005). Chemerin also induces insulin resistance in peripheral tissues such as skeletal muscle by activation of ERK-1/2 and NF-kB pathways, culminating in inhibited cellular glucose uptake (Sell et al. 2009). Insulin stimulates chemerin secretion, promoting a vicious circle increasing insulin resistance (Tan et al. 2009). This protein is thought to possibly present a link between obesity and diabetes (Sell et al. 2009). Serum chemerin levels have been found to correlate with BMI (Bozaoglu et al. 2007, Sell et al. 2009), WHR (Boazoglu et al. 2007, Sell et al. 2009), triglycerides (Boazoglu et al. 2007), elevated blood pressure (Boazoglu et al. 2007), and adipocyte volume (Sell et al. 2009). Adipocyte volume has been found to be higher in PCOS patients, even in lean PCOS patients when compared to BMI-matched controls (Faulds et al. 2003). Chemerin levels in PCOS are of interest, as this may be one of the factors underlying insulin resistance so common in PCOS. Chemerin is also implicated in inflammation, which may be responsible for vascular damage leading to CVD. Chemerin levels have been reported to be higher in obese PCOS women than in BMI and WHR matched controls (Guzel et al. 2014, Tan et al. 2009) as well as in lean PCOS women compared to BMI matched controls (Guzel et al. 2014). Treatment of the PCOS patients with metformin for 6 months has lowered chemerin levels and improved insulin resistance, without changing BMI (Tan et al. 2009).

Proinflammatory and Macrophage-Derived Factors

This section presents a survey of macrophages and proinflammatory factors such as tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), Interleukin-6 (IL-6), Interleukin – 18 (IL-18) and white blood cells (WBC).

Macrophages

Adipose tissue inflammation mediated by activated tissue macrophages (ATM) is a major pathway culminating in the development of obesity-related insulin resistance (Lumeng et al . 2011, Wentworth et al. 2010). CD11c is a marker specific to these ATMs that infiltrate adipose tissue in obese individuals and secrete cytokines such as TNF- and IL-6, both associated with insulin resistance. In contrast, the markers CD206, CD14 and CD163 are expressed by less inflammatory macrophages (Wentworth et al. 2010). CD11c-expressing macrophages cluster around dead adipocytes, forming histologically defined crown-like structures (CLS) (Wentworth et al. 2010). The density of CLS has been found to correlate with the degree of insulin resistance and obesity (Wentworth et al. 2010). CD11c and CLS density is significantly higher in lean and obese PCOS women than in BMI-comparable non-PCOS controls (Huang et al. 2013). The observation that CD11c macrophages and CLS occur more frequently in obese men than in obese women has lead to the suggestion that the increase observed in lean and obese PCOS women is likely a result of hyperandrogenism (Huang et al. 2013). CD11c adipose tissue infiltration and CLS may be an early change in lean hyperandrogenic PCOS women leading to the development of insulin resistance in this group (Huang et al. 2013) that is comparable to the insulin resistance in obese controls (Dunaif et al. 1989).

TNF-α

TNF- α plays a role in the pathogenesis of insulin resistance (Hotamisligil et al. 1995). It inhibits tyrosine phophorylation of the insulin receptor and of IRS-1 in muscle and fat cells (Hotamisligil et al. 1995), and has also been shown to down-regulate the expression of the GLUT4 transporter necessary for cellular entry of glucose (Stephens and Pekala 1991). Serum levels of TNF- α are elevated in both obesity and DM2 (Hotamisligil et al. 1995).

Studies report that TNF- α levels in PCOS correlate with BMI (Escobar-Morreale et al. 2003a, Gonzalez et al. 1999, Puder et al. 2005, Samy et al. 2009) and that circulating TNF- α levels are elevated in both non-obese (Gonzalez et al. 1999, Sayin et al. 2003) and obese (Samy et al. 2009) PCOS women when compared with BMI-matched controls. However, in other studies, these differences in the levels of TNF- α between PCOS women and controls diminished after adjusting for BMI and abdominal adiposity (Escobar-Morreale et al. 2003a, Puder et al. 2005), thus questioning whether TNF- α elevations are related to PCOS or are a function of excess adiposity. A meta-analysis found no significant difference in TNF- α levels between

PCOS subjects and BMI-matched controls (Escobar-Morreale et al. 2011). However, the authors caution against over-interpretation of these results, as their study also revealed evidence of a publication bias favoring publication of studies that underestimate the differences in TNF- α levels between PCOS women and controls (Escobar-Morreale et al. 2011). An important difference between PCOS and control subjects may be the lie in the TNF-α receptor (Peral et al. 2002). Most of the metabolic effects of TNF-α are mediated through the TNF-α receptor 2 (Peral et al. 2002). Although TNF-α receptor 2 levels were increased in obesity, no differences in levels were observed between PCOS women and controls (Peral et al. 2002). However, a the methionine 196 arginine polymorphism in exon 6 of the gene encoding the TNF-α receptor 2 was reported to be significantly more frequent in women with PCOS than in controls, suggesting that TNF-α plays a role in the development of metabolic pathologies in PCOS, and that this might be related to a structural change in the TNF-α receptor that confers a more responsive phenotype rather than to circulating TNF-α levels (Peral et al. 2002). Authors agree that larger and more highly powered studies are needed to clarify the role of TNF-α in PCOS (Escobar-Morreale et al. 2011, Peral et al. 2002).

CRP

CRP has been proven to be a strong independent predictor of cardiovascular events in healthy asymptomatic as well as symptomatic women in the general population (Danesh et al. 2004, Ridker et al. 2000). Obesity is associated with elevations in CRP (Yudkin et al. 1999).A meta-analysis of 26 studies matching carefully for BMI revealed that CRP is elevated in PCOS independently of obesity (Escobar-Morreale et al. 2011). This elevation of CRP in PCOS is more pronounced when obesity is present, further heightening the risk of cardiovascular events in this group of women (Escobar-Morreale et al. 2011). However, the authors caution against over attributing increased cardiovascular risk to PCOS *per se* because after adjusting for BMI, the elevation in CRP attributable to PCOS is relatively small (Escobar-Morreale et al. 2011).

Interleukin-6

Interleukin-6 (IL-6) is released by mononuclear leucocytes and adipose tissue (Escobar-Morreale et al. 2011), with levels being elevated in obesity (Escobar-Morreale et al. 2011). It directly stimulates hepatic CRP synthesis (Escobar-Morreale et al. 2011). Although IL-6 elevations have been reported in lean and obese PCOS women in relation to BMI comparable controls (Escobar-Morreale et al. 2011), a recent meta-analysis proved no significant difference

in circulating IL-6 levels between PCOS women and BMI-matched controls (Escobar-Morreale et al. 2011), suggesting that elevated IL-6 in PCOS is primarily related to obesity.

However, a promoter region polymorphism (G/C) at position -174 of the gene encoding IL-6 has been found to be strongly associated with DM2 in the Caucasian population (Vozarova et al. 2003). This same polymorphism has been reported to occur more frequently in PCOS patients (Erdogan et al. 2008, Villuendas et al. 2002). Furthermore, a certain microsatellite CA-repeat polymorphism in the locus encoding the α-subunit of the IL-6 receptor is associated with obesity, while the Arg148 allele in the region encoding the gp130 subunit of the IL-6 receptor gene is more common in normoandrogenic subjects than hyperandrogenic ones (Escobar-Morreale et al. 2003b). These observations suggest that genetically determined hypersignaling defects in the IL-6 receptor, rather than only circulating IL-6 levels, may be implicated in the pathogenesis of metabolic hyperandrogenic disorders such as PCOS (Escobar-Morreale et al. 2003b). However, relatively little is known about this field and authors agree that larger studies are needed (Escobar-Morreale et al. 2003b, Escobar-Morreale et al. 2011).

Other inflammatory mediators - IL-18 and WBC

Studies on IL-18 and MNC are insufficient in numbers to perform a meta-analysis, but the limited existing evidence suggests that elevations of IL-18 and WBC in PCOS are independent of obesity (Escobar-Morreale et al. 2011).

Dyslipidemia

Dyslipidemia is the most common metabolic abnormality in PCOS (Hoffman and Ehrmann 2008, Randeva et al. 2012), and polycystic ovary syndrome is the leading cause of dyslipidemia in reproductive-age women (Sam and Dunaif 2003). Observations of PCOS affected women and their relatives have shown that the probability of developing dyslipidemia is 1.8 fold larger in the PCOS individuals (Randeva et al. 2012). Overall, studies of PCOS patients report slightly decreased levels of cardioprotective HDL-C, with slightly elevated levels of TG, VLDL-C and LDL-C (Hoffman and Ehrmann 2008, Randeva et al. 2012). PCOS women display the lipid profile observed in insulin resistant states such as DM2, and characterized specifically by elevated TG and lowered HDL-C (Legro et al. 2001, Randeva et al. 2012). The main

determinant of heart disease risk is the total cholesterol (TC) to HDL-C ratio (Wild et al. 2010). This ratio is also slightly elevated in PCOS patients (Randeva et al. 2012).

Elevated LDL-C (Graf et al. 1990, Legro et al. 2001, Sam et al. 2005, Talbot et al. 1998) and VLDL-C (Graf et al 1990) in PCOS are further elevated when excess adiposity is present, but, as confirmed by a recent meta-analysis, the higher levels occur in PCOS independently of obesity (Wild et al. 2011). Elevated LDL-C levels are linked with hyperandrogenemia (Graf et al. 1990, Sam et al. 2005, Wild et al. 1985). It is unclear whether hyperandrogenemia and elevated LDL-C have a causal relationship or whether these are closely related genetic traits inherited together (Sam et al. 2005).

Although LDL-C and VLDL-C are elevated in PCOS independently of obesity, obesity is thought to be the major determining factor for elevations in TG levels and for the reduction of HDL-C levels that are observed in PCOS (Joharatnam 2011, Legro et al. 2001, Sam et al. 2005, Wild et al. 2011). In a study comparing lipid profiles between PCOS probands, their sisters with and without PCO morphology on ultrasound, and controls, the elevated TG levels and reduced HDL-C levels in probands relative to the other groups disappeared after controlling for BMI (Joharatnam et al. 2011), suggesting that BMI is the predominant determinant of TG and HDL-C levels in PCOS (Joharatnam et al. 2011), both of which are strong independent risk factors for death from cardiovascular disease (Bass et al. 1993). However, after adjustment for BMI, age, and centripetal obesity in another large study of PCOS women, HDL-C levels still remained significantly lower in PCOS women when compared to controls, though only slightly so. This indicates that factors other than BMI and centripetal obesity are contributors to the lowering of HDL-C levels in PCOS women, although, of course, BMI is observably a significant determinant of lipid profiles (Glueck et al. 2009).

In a study which did not control for diet, HDL-C levels were unexpectedly higher in obese PCOS women than in obese controls (Legro et al. 2001). This inconsistency signals that in addition to BMI (Joharatnam et al. 2011), other factors such as age, ethnicity, genetic influences and environment also modulate lipid profiles of women with PCOS (Carmina et al. 2006a, Legro et al. 2001, Essah et al. 2008, Talbot et al. 1998). The importance of environmental (diet, activity level) and genetic contributions to dyslipidemia is evidenced by the fact that TG elevations in

American PCOS women compared to Italian PCOS women persist even after controlling for BMI (Carmina et al. 2006, Essah et al. 2008).

Even PCOS women with normal lipid profiles may be at increased risk for cardiovascular events. This is because significantly higher levels of lipoprotein-a and a higher proportion of small, dense LDL have been found in PCOS patients compared to controls (Berneis et al. 2009), although TC and total LDL-C levels did not differ between PCOS and control women (Berneis et al. 2009). This similarity in TC and total LDL-C levels makes the PCOS women appear to have normal lipid profiles. Such PCOS women are at higher risk for cardiovascular events, because certain lipoproteins, such as lipoprotein-a and small, dense LDL-C, are more atherogenic (Berneis et al. 2009).

Another atherogenic shift in PCOS is the lipid-to-protein ratio of HDL-C particles. The lipid-to-protein ratio in an HDL-C particle reflects the capacity of the particle to remove cholesterol from tissues (Rajkhiova et al. 1997). A reduction of this ratio signals a decreased or impaired capacity to remove cholesterol and prevent atherosclerosis (Rajkhiova et al. 1997).

In one study, the lipid-to-protein ratio in the HDL-C was found to be lower in obese PCOS women than in obese women without PCOS (Rajkhiova et al. 1997). The latter finding signals a drop in the anti-atherogenic properties of HDL-C of PCOS women (Rajkhiova et al. 1997).

Despite slight changes in lipid profiles in PCOS, most women with PCOS are young and have normal blood pressure, and hence do not qualify for primary prevention of cardiovascular disease (Randeva et al. 2012). Nevertheless, it is suggested to perform at least one measurement of lipid profiles in PCOS in conjunction with an assessment for other cardiovascular risk factors such as smoking and family history of CVD (Randeva et al. 2012).

Traditional and novel cardiovascular risk factors in PCOS

Traditional Cardiovascular Disease Risks Factors in PCOS

Traditional risk factors for cardiovascular disease, such as IGT, DM2, dyslipidemia, obesity, and elevated blood pressure, are more prevalent in women with PCOS than in control women of similar age (Wild et al. 2010).

Markers of Atherosclerosis

Calcification of the coronary arteries assessed by electron beam computed tomography correlates with the degree of atherosclerosis found on histopathological exam and was found to predict the incidence of cardiovascular events in asymptomatic women (Arad et al. 2000). The prevalence and extent of coronary artery calcification (CAC) were found by several studies to be higher in both younger (aged 30 to 45 years) and older (aged over 40 years) women with PCOS than in controls, independently of age and BMI (Christian et al. 2003, Shroff et al. 2007, Talbott et al. 2004). It has been suggested that the reported increase in CAC among PCOS women is related to the parameters that were different in PCOS women in relation to the control women in the studies - increased LDL-C (Christian, et al. 2003, Talbot et al. 2004), lower HDL-C (Talbot et al. 2004) and hyperinsulinemia (Talbot et al. 2004). Among the women with PCOS, BMI was a significant predictor of whether the women would have CAC (Christian et al. 2003, Talbott et al. 2004, Shroff et al. 2007), leading to the suggestion that obese women with PCOS should be targeted for aggressive treatment and prevention of cardiovascular disease (Christian et al. 2003, Shroff et al. 2007). Talbott et al. also reported higher prevalence and extent of aortic calcification (AC) in women with PCOS (Talbott et al. 2004). The investigators of the latter study found that total testosterone was an independent risk factor for greater AC (Talbott et al. 2004). In animal models, testosterone exacerbated atherosclerosis in female monkeys but conferred a protective effect in males (Talbott et al. 2004). Similarly, a large study reported that men with the highest total testosterone levels had a reduced risk of AC, but conversely, women with elevated testosterone levels had the highest risk for CAC (Hak et al. 2002, Talbott et al. 2004), leading authors to suggest that the aorta in women may be more sensitive to the effects of endogenous testosterone (Hak et al. 2002, Talbott et al. 2004).

Increased intima-media wall thickness (IMT) is an early marker of atherosclerosis (Lorenz et al. 2007). Increased carotid intima-media wall thickness (CIMT) is a strong independent predictor of the occurrence of major cardiovascular events later in life (Lorenz et al. 2007). Higher CIMT has been reported in both younger (age 20 to 35 years) (Carmina et al. 2006b, Luque-Ramirez et al. 2007) and older (over 45 years) (Talbott et al. 2004) patients with PCOS in comparison to controls of similar age and BMI. A recent meta-analysis indicated that women with PCOS had a 0.072 to 0.084 mm higher CIMT compared to controls (Meyer et al. 2012). Every 0.10 mm increase in CIMT has been estimated to increase the risk of a myocardial infarction (MI) by 15% and the risk of stroke by18% (Meyer et al. 2012). The increase in CIMT in PCOS relative to controls of comparable age and BMI has been associated in different studies to higher levels of insulin (Carmina et al. 2006b), hyperandrogenism (Luquez-Ramirez et al. 2007), Il-18 (Meyer et al. 2012), LDL-C (Meyer et al. 2012), and abdominal obesity (Meyer et al. 2012), although the contribution of each of these factors to increased CIMT in PCOS has not been systematically evaluated (Meyer et al. 2012). However, CIMT increases with age in PCOS, as in the general population (Meyer et al. 2012).

Vascular Endothelial Dysfunction

Several studies have demonstrated decreased brachial artery flow mediated dilation (FMD), a marker of endothelial function, in young normal weight, overweight and obese women with PCOS compared to body mass matched controls (Carmina et al. 2006a, Orio et al. 2004a). The decreased FMD was observed even in normal weight PCOS women who were also normotensive and had normal lipid profiles (Orio et al. 2004a) and who therefore lacked many of the traditional cardiovascular risk factors (Orio et al. 2004a). It has been suggested that elevated androgen levels in the PCOS women relative to controls contributed to the observed decline in endothelial function (Carmina et al. 2006b). When obese PCOS and control women of similar age, BMI, LDL-C and TC levels received intra femoral artery infusions of the endothelial-dependent vasodilator methacholine chloride (MCh), the leg blood flow was 50% less in the PCOS women compared to the controls, suggesting impaired nitric oxide (NO) production in the endothelial cells of PCOS women (Paradisi et al. 2001). The degree of decrease in leg blood flow was strongly associated with free testosterone levels (Pardisi et al. 2001). This, in conjunction with the observation that androgen deprivation in men has enhanced endothelial-dependent vasodilation (Herman et al. 1997), has led to the suggestion that elevated androgen levels in

PCOS women may be a major contributor to endothelial dysfunction and macrovascular disease (Paradisi et al. 2001).

Several molecules implicated in endothelial dysfunction have been linked to PCOS. A recent meta-analysis indicated that homocysteine, a mediator of endothelial injury, is in higher levels in PCOS women than in controls of similar age and BMI (Murri et al. 2013). The same study also demonstrated that levels of asymmetric dimethylarginine (ADMA), a competitive inhibitor of endothelial NO synthase and an independent risk marker for cardiovascular morbidity and mortality (McDowell and Lang 2000), are higher in PCOS women than in age and BMI matched controls (Murri et al. 2013). Several studies have found that in comparison to age- and BMI- comparable control women, PCOS women also exhibit elevated levels endothelin-1 (Caramina et al. 2006b, Diamanti-Kandarakis 2012), a by-product of endothelial damage and a potent vasoconstrictor (Carmina et al. 2006b). Plasminogen activator inhibitor-1, which inhibits fibrinolysis and in higher levels predisposes to accelerated development of atherosclerosis (DeLoughrey 1999), has been shown to be elevated in normal weight young PCOS women relative to controls (Yildiz et al. 2002).

Cardiac Dysfunction

Studies report that compared to age and BMI matched controls, young PCOS women have increased left ventricular mass index (LVMi) (Orio et al. 2004b), a predictor of CVD morbidity and mortality (Randeva et al. 2012), and decreased diastolic filling (Orio et al. 2004b, Tiras et al. 1999). Both of these abnormalities occur independently of excess weight, presenting in lean as well as overweight and obese PCOS patients. Additionally, decreased left ventricular ejection fraction has been reported in young overweight and obese women with PCOS compared to controls (Orio et al. 2004b).

The risk of cardiovascular events in PCOS

There are conflicting results from studies evaluating the risk of cardiovascular events in PCOS.

A 49-year follow-up study of 786 women diagnosed with PCOS based only on ovarian wedge section found that the risk of fatal cardiovascular events was no different between PCOS women and controls (Pierpoint et al. 1998). Similarly, a retrospective study of 319 women diagnosed with PCOS based on more stringent criteria (an/oligo-ovulation and

hyperandrogenism) reported that there was no difference in cardiovascular mortality risks between PCOS women and age matched-controls (Wild et al. 2000), although the PCOS women demonstrated a higher risk for non-fatal cerebrovascular events, even after adjustment for BMI (Wild et al. 2000).

Another investigation followed 82,439 women aged 20-35 for 14 years (Solomon et al. 2002). Compared with women reporting a history of regular menses, women reporting a history of very irregular menses had a significantly higher risk for nonfatal and fatal cardiovascular disease, even after adjustment for BMI, age, menopausal status, and smoking (Solomon et al. 2002). Although these women were not diagnosed with PCOS, it is estimated that 80-90% of women reporting menstrual irregularity have PCOS (Randeva et al. 2012). Furthermore, a recent meta-analysis indicated a 2-fold increased risk of coronary heart disease (CHD) and stroke for patients with PCOS relative to women without PCOS (de Groot et al. 2011). The meta-analysis found that there is a 55% increase in the risk for CHD and stroke in PCOS women using only studies that adjusted for BMI, showing that BMI is not the sole cause of increased risk for cardiovascular events in women with PCOS (de Groot et al. 2011).

The risk of cardiovascular events also appears to be higher in postmenopausal women with a history of PCOS than those without. The Women's Ischemia Syndrome Evaluation study reported that the cumulative 5-year event free survival for women without a history of PCOS is 88.4%, and only 78.9% for those with a premenopausal history of PCOS (Shaw et al. 2008).

A retrospective study found similar results. The study evaluated the incidence of CV events (MI, angina, heart failure, stroke, CV death) in a cohort of 2300 PCOS women between 1988 and 2009 (Mani et al. 2013). Overall, CV events were not any more prevalent in the cohort than in the local female population (Mani et al. 2013). However, when the cohort was stratified by age and comparisons were limited to age-similar groups in the local female population, PCOS showed an association with CV events within each age group (Mani et al. 2013). The age-specific prevalence of CV events was significantly higher in PCOS patients over 45 compared with the local female population, with odds ratio as high as 12.88 in women over 65 with a pre-menopausal history of PCOS (Mani et al. 2013). Factors in the cohort associated with an increased risk of CV events were age, hypertension, obesity, smoking and having DM2 (Mani et al. 2013).

Management

This section reviews the therapies used to manage the cardiometabolic disturbances observed in PCOS. The treatment of other manifestations of PCOS, such as hirsutism and anovulation, can itself be a cause of cardiometabolic risks (as will be shown later in the sub-section on oral contraceptive pills (OCPs) and in the sub-section on anti-androgenic therapies). Therefore, this section also discusses the cardiometabolic risks that have manifested during management with OCPs and anti-androgenic agents. Factors influencing whether or not metabolic side effects will arise during OCP use are also discussed so that such side effects can be minimized.

Lifestyle Interventions

Lifestyle change is the first line of treatment for most overweight and obese women with PCOS (Teede et al. 2010). Additionally, normal weight PCOS women are advised to prevent excessive weight gain (Teede et al. 2010). A weight loss of 5-10% through lifestyle modification restores ovulation in many obese and overweight women with PCOS (Lefebvre et al. 1997, Huber 1999), reduces androgen levels, improves hirsutism (Levebre et al. 1997) and improves IR (Huber et al. 1999). Although current recommendations propose an energy reduced diet consisting of low fat, moderate protein and high carbohydrate content with low glycemic indexes, there is a shift towards increasing the protein proportion and lowering the carbohydrate one, as this approach has been shown to lead to more fat loss (Mikkelsen et al. 2000, Kasim-Karakas et al. 2009) and improve lipid profiles in PCOS women more than carbohydrate-based diets (Kasim-Karakas et al. 2009). Even moderate exercise alone with no change in diet, although not resulting in any weight loss, has been shown to reduce IR and improve lipid profiles of PCOS women (Brown et al. 2013).

Pharmacological Management

Insulin-sensitizing drugs

Thiazolidinediones

Studies of the use of thiazolidinedione drugs in PCOS demonstrate conflicting results. A systematic Cochrane Review reported that the use of rosiglitazone in PCOS women improves IR, endothelial function and lowers androgen concentrations (Lord et al. 2003a). However, in a randomized control trial among non-obese PCOS women with study groups consisting of placebo, metformin alone, rosiglitazone alone and combination metformin and rosiglitazone therapy, it was observed that although rosiglitazone alone lowered androgen levels, it lead to weight gain and did not improve insulin resistance (Baillargeon et al. 2004). In the latter study, insulin resistance improved only with metformin therapy or with combination metformin-rosiglitazone therapy (Baillargeon et al. 2004). Furthermore, thiazolidinediones exacerbate the risk of bone fracture, exacerbate pre-existing congestive heart disease (CHD), and the thiazolidinedione troglitazone was pulled off of the market because of hepatocellular toxicity (Wild et al. 2010). Therefore, thiazolidinedione use is limited (Wild et al. 2010).

Metformin

Metformin is incontrovertibly known to lower IR and increase insulin-mediated glucose disposal (Bargiota and Diamant-Kandarakis 2012, Ibáñez et al. 2000, Lord et al. 2003a, Moghetti et al. 2000, Pasquali et al. 2000, Santana et al. 2004). One study of PCOS women taking metformin demonstrated a reduction of the annual NGT to IGT conversion rate in PCOS women by 11-fold (from 16% to 1.4%) and an increase in the annual IGT to NGT reversion rate from 2.25 % (Legro et al. 2005) to 11% (Sharma et al. 2007). A meta-analysis demonstrated that metformin reduces the onset of DM2 in at risk individuals, including in women with PCOS, by 40% (Salpeter et al. 2008). Other beneficial effects in PCOS after the initiation of metformin therapy include reductions of CRP (Morin-Papunen et al. 2003a) and IMT (Orio et al. 2003a), and an enhancement of FMD (Diamanti-Kandarakis et al. 2005).

However, metformin does not always effectively improve IGT (Bargiota and Diamanti-Kandarakis 2012). A study that followed a large number of women with PCOS and under

metformin treatment showed that the effectiveness of metformin in preventing progression to diabetes is a function of baseline glucose levels and insulin sensitivity (Glueck et al. 2008).These observations highlight the importance of early intervention. Metformin is also not as effective in morbidly obese patients (Bargiota and Diamanti-Kandarakis 2012). If a patient is morbidly obese, or if early intervention was not undertaken and the impairment of glucose metabolism is long-existing, metformin therapy will improve IGT, if at all, only at higher doses (Bargiota and Diamanti-Kandarakis 2012). However, the dangers of high doses preclude this practice from being implemented. These observations point out the need for development of ways to manage this group of resistant patients.

Metformin use in obese PCOS women not undergoing lifestyle changes has been shown to induce no (Moghetti et al. 2000) or only minimal (2.7 kg or 2.9%) (Nieuwenhuis-Ruifro et al. 2009) weight loss. RCTs have shown that the combination of metformin and lifestyle changes in obese PCOS women produce slightly more weight loss and a significantly higher reduction in visceral fat than lifestyle changes only (Gambineri et al. 2006, Pasquali et al. 2000), with the effect of metformin on inducing weight loss being dose dependent (Harbourne et al. 2005). Metformin may reduce appetite in obese PCOS women by lowering neuropeptide Y levels (Orbetzova et al. 2011). The Androgen Excess Society (AES) recommends the introduction of metformin therapy in obese women with PCOS in whom lifestyle intervention did not appreciably improve IGT (Wild et al. 2010). The AES also recommends metformin therapy in PCOS women with IGT who are in the normal weight range and in whom weight loss would therefore not be appropriate (Wild et al. 2010).

Conflicting results exist concerning the effect of metformin on lipid profiles. Metformin impacts the lipid profile, either directly, by inhibiting hepatic acetyl-CoA carboxylase and activating mitochondrial fatty acid oxidation, or indirectly, through the inhibition of thecal androgen production (Bargiota and Diamanti-Kandarakis 2012). However, the final impact on lipid profile is multifactorial, and probably dependent on the BMI of the patient (Bargiota and Diamanti-Kandarakis 2012). Some studies have demonstrated a significant rise in HDL-C levels (Ibanez et al. 2000, Fleming et al. 2008, Santana et al.2004) with a drop in levels of TG (Ibanez et al. 2000, Rautio et al. 2005) and TC (Ibanez et al. 2000, Santana et al. 2004) during metformin therapy in PCOS. Others have reported no significant changes in levels of HDL-C (Luque-

Ramirez et al. 2007), TC (Banaszewska et al. 2009, Flemming et al. 2008, Luque-Ramirez et al. 2007, Moghetti et al. 2000, Tang et al. 2006), and TG (Banaszewska et al. 2009, Flemming et al. 2008, Luque-Ramirez et al. 2007, Moghetti et al. 2000, Santana et al. 2004, Tang et al. 2006). Differences in the study subjects and the doses of metformin may account for the contrasting results (Bargiota and Diamanti-Kandarakis 2012). Obese PCOS women have been more resistant to improvements in lipid profiles (Fleming et al. 2008, Luque-Ramirez et al. 2007, Rautio et al. 2005, Tang et al. 2006). A meta-analysis by Lord et al. 2003 detected no significant effect of metformin on TC, HDL-C and TG levels, whereas LDL-C levels were significantly reduced during treatment (Lord et al. 2003b). The RCTs included in the meta-analysis demonstrated many inter-study variations, thus limiting definitive conclusions. Some studies have reported no changes or even minor non-significant elevations in LDL-C levels during metformin therapy of both non-obese (Banaszewska et al. 2009, Luque-Ramirez et al. 2007, Moghetti et al. 2000) and obese (Luque-Ramirez et al. 2007, Rautio et al. 2005) women with PCOS. Due to these conflicting results and the dangers of rising nonHDL-C levels, the expert committee of the AES has recommended that metformin not be used when LDL-C or non-HDL-C levels are elevated (Wild et al. 2010).

The use of metformin appears to have uncontroversial positive effects on many factors of relevance to cardiovascular risk while the effects on other factors remain uncertain. The large uncertainties and discrepancies in the outcomes of the various studies reviewed stem from the lack of consistency between studies and call for more rigorously designed RCTs. In particular, future studies should span longer periods of time and follow cohorts over periods that are sufficient to observe outcomes. It is imperative that the studies track and factor in all possible factors that may affect outcome, such as lifestyle changes, diet (including details on caloric and carbohydrates intake), and energy expenditure. It is also indispensible that future studies record baseline status such as the degree of obesity and IR, as it appears that baseline conditions have an impact on the effectiveness of metformin.

Anti-obesity agents

Orlistat, an inhibitor of pancreatic lipase and dietary fat absorption, has been evaluated for the treatment of PCOS. Randomized open-label trials comparing metformin and Orlistat therapy in PCOS women have indicated that orlistat therapy alone reduces androgen levels

(Jayagopal et al. 2005, Metwally et al. 2009), restores ovulation (Jayagopal et al. 2005, Metwally et al. 2009) , and lowers BMI (Jayagopal et al. 2005, Metwally et al. 2009) as effectively as metformin. Orlistat therapy alone did not improve IR (Jayagopal et al. 2005, Metwally et al. 2009). However, a combination of Orlistat and energy restricted diet were shown to improve IR (Diamanti-Kandarakis et al. 2007), and, importantly, lowered advanced glycation-end products (Diamanti-Kandarakis et al. 2007) which are implicated in causing micro- and macro- vascular disease. However, clinical experience with Orlistat is limited and therefore the AES does not recommend its use in PCOS (Wild et al. 2010).

OCPs

Use of OCPs in PCOS, through is effective for reducing hirsutism, inducing the regression of male-pattern alopecia and restoring cyclical bleeding (Bargioata and Diamanti-Kandarakis 2012). OCPs have, however, been shown to deteriorate the cardiovascular risk and metabolic profile (Bargiota and Diamanti-Kandarakis 2012), and therefore the use of OCPs in PCOS women may pose concern. A meta-analysis surveying the use of OCPs (with < 50 ug ethinyl-estradiol) in the general female population indicated that second and third generation OCPs increased the risk of MI/stroke (odds ratio 2.01) (Baillargeon et al. 2005). The deterioration of carbohydrate metabolism and of lipid profiles observed while taking OCPs may present a possible link between adverse cardiovascular outcome and OCP use (Nader and Diamanti-Kandarakis 2007).

The effects of OCPs on carbohydrate metabolism in PCOS, as reported by different studies, appear to be wholly contradictory, ranging from improvements to outright diabetes establishment with similar contradictory results with respect to IR (Bargiota and Diamanti-Kandarakis 2012).

To conciliate the many variations, several authors have proposed a unifying hypothesis. They propose that the impact of OCs on glucose metabolism depends of several factors: the estrogen dose; the body type and genetic predispositions of the patient; the progestin component; the environment and the natural progression of PCOS of each particular patient (Mathur et al. 2008, Nader and Diamanti-Kandarakis 2007).

Estrogen has been shown to impair insulin action in a dose-dependent manner (Godsland 1996). In a randomized trial, PCOS women who received 35 μg ethinyl estradiol/ 2 mg cyproterone acetate perorally demonstrated impairments in insulin secretion and action, whereas those who were given transdermal ethinyl estradiol with peroral 2 mg cyproterone acetate showed no changes in these parameters (Vrbíková et al. 2004). Furthermore, a general population study of postmenopausal women showed that endogenous estradiol concentrations were closely and positively associated with rising IR (Kalish et al. 2003). In addition to the adverse contribution of higher estrogen levels, the metabolic perturbations that are potentially created by OCPs are also dependent on body type and genetic predispositions to glucose intolerance (Mathur et al. 2008, Nader and Diamanti-Kandarakis 2007). In randomized clinical trials, 35 μg ethinyl estradiol/2 mg cyproterone acetate was shown to worsen glucose tolerance in obese PCOS women (Morin-Papunen et al. 2000), but had no effect on glucose tolerance in lean PCOS women, although it caused a slight weight gain and an elevation in leptin levels in the latter group (Morin-Papunen et al. 2003). These results suggest that progestin-only formulations, such as cyproterone acetate, may be a more appropriate OCP for obese PCOS women in whom lifestyle modification and metformin failed to restore ovulation, or for obese PCOS adolescents and women desiring contraception but who are also more susceptible to metabolic perturbations or to thromboembolism.

The progestin component of an OCP, especially the more androgen-like progestins, could also influence metabolic parameters (Mathur et al. 2008, Nader and Diamanti-Kandarakis 2007) and can induce IR, either directly or by delaying estrogen metabolism (Nader and Diamanti-Kandarakis 2007). In a randomized clinical trial, after a group of young non-obese PCOS women had received 20 μg ethinyl estradiol/ 75 μg gestodene for over 1 year, half of the women were randomly selected to receive 30 μg ethinyl estradiol/3 μg drospirenone instead (Ibáñez and De Zegher 2004b). After 6 months, abdominal fat increased in the women who had remained on 20 μg ethinyl estradiol/ 75 μg gestodene, whereas abdominal and total fat quantity decreased in those who had been switched to 30 μg ethinyl estradiol/3 μg drospirenone (Ibáñez and De Zegher 2004b). The authors attributed these differences to the more androgen-like properties of gestodene vs. the anti-androgenic ones of drospirenone (Ibáñez and De Zegher 2004b).

Perturbation of lipid profiles is the other frequent consequence of OCP treatment that poses concern in PCOS. Increased TG levels are the most common side effect of OCP use in women with PCOS, independently of age and BMI (Bargiota and Diamanti-Kandarakis 2012). A meta-analysis comparing RCTs of OCPs in PCOS indicated a rise in TG associated with OCP use in PCOS (Costello et al. 2007). The estrogen component is thought to cause this by lowering TG clearance by the liver (Bargiota and Diamanti-Kandarakis 2012). Giving PCOS women subdermal estrogen (Vrbíková et al. 2004) resulted in no change in TG levels. However, the estrogen component also has the beneficial effect of raising HDL-C by increasing the hepatic expression of the apoliprotein A-I gene, which in turn enhances HDL-C production. (Bargiota and Diamanti-Kandarakis 2012).

Not only the estrogen component, but also progestins impact the lipid profile (Nader and Diamanti-Kandarakis 2007). In an RCT, 20 μg ethinyl estradiol/75 μg desogestrel was shown to elevate LDL-C (Gaspard et al. 2004), whereas administration of the OCP 30 μg ethinyl estradiol/3 μg drospirenone containing the anti-androgenic drospirenone had no effect on LDL-C and thus lowered the LDL-C to HDL-C ratio (Gaspard et al. 2004). Subsequent to these results, Gaspard et al. and other authors have advocated the use of 30 μg ethinyl estradiol/3 μg drospirenone in PCOS (Gaspard et al. 2004, Ibáñez and De Zegher 2004b), arguing that this combination alleviates many of the metabolic concerns (e.g. dyslipidemia) specific this syndrome (Mathur et al. 2008).

However, 30 μg ethinyl estradiol/3 μg drospirenone may exacerbate low grade inflammation (Ibáñez and De Zegher 2004a) that is associated with cardiovascular risk. In a RCT of young non-obese PCOS women, TG and IL-6 levels increased and adiponectin levels dropped after 6 months of taking 30 μg ethinyl estradiol/3 μg drospirenone (Ibáñez and De Zegher 2004a). Nine months after the beginning of the study, half of the young women were randomly selected to receive flutamide (62.5 mg) and metformin (850 mg) in addition to 30 μg ethinyl estradiol/3 μg drospirenone e (Ibáñez and De Zegher 2004a). TG and adipocytokine levels reverted towards normal in the group supplemented with flutamide (62.5 mg)-metformin (850 mg) but worsened in the non-supplemented group, leading to the suggestion that future research should evaluate the possibility of supplementing OCPs in PCOS with flutamide-metformin (Ibáñez and De Zegher 2004a). However, given the hepatotoxicity of flutamide at higher doses

(750 mg), the authors cautioned against making the suggestion a widespread clinical practice before more investigations are undertaken (Ibáñez and De Zegher 2004a) and advised monitoring hepatic enzymes in those receiving this treatment (Ibáñez et al. 2005).

Results from another 6 month RCT study showed that 35 µg ethinyl estradiol/2 mg cyproterone acetate increased IR by 25% and increased arterial stiffness, whereas 20 ug ethinyl estradiol/100 ug levenorgestrel plus spironolactone 50 mg twice daily demonstrated a neutral effect on IR and on arterial stiffness (Randeva et al. 2012). The results from these previous studies have lead to advocacy that OCPs used by PCOS women should be preparations with a lower estrogen content in combination with anti-androgens having anti-hypertensive effects, such as spirinolactone (Randeva et al 2012) or drospirenone (Gaspard et al. 2004, Mathur et al. 2008). In PCOS women with slight perturbations in the lipid profile, a progestin-only, anti-androgenic OCP may be more appropriate. LDL-C levels > 160 mg/dL or TG levels > 250 mg/dl preclude the use of OCPs.

Anti-androgens

Androgens, as well as causing hirsutism, may contribute to the pathogenesis many of the metabolic abnormalities of PCOS, such as a perturbed lipid profile (Bargiota and Diamanti-Kandarakis 2012, Sam et al. 2005). Through the activation of visceral fat cell lipolysis (Bargiota and Diamanti-Kandarakis 2012) and through interference with insulin signaling pathways (Corbould 2008), androgens may also worsen IR.

A study of the use of the anti-androgenic flutamide in PCOS has shown that in addition to lowering androgen levels and improving hirsutism, this medication improved lipid profiles (Diamanti-Kandarakis et al. 1998). Results from another study comparing diet therapy in PCOS vs. flutamide and diet together showed that flutamide adds a significant effect in decreasing visceral fat, lowering LDL-C and improving IR than diet alone (Gambineri et al. 2004).

Spironolactone exerts both anti-androgenic and anti-mineralocorticoid effects (Bargiota and Diamanti-Kandarakis 2012). It has also been effective in treating hirsutism (Bargiota and Diamanti-Kandarakis 2012). In a clinical trial, lean and overweight PCOS women demonstrated no change of IR after 12 months of taking spironolactone (Zulian et al. 2005). In the group taking spironolactone in association with lifestyle dietary modification, overweight and lean

women demonstrated a reduction of IR, as well as a reduction of TG and an elevation of HDL-C levels, respectively (Zulian et al. 2005). However, in another study of 3 months, women with PCOS women with hirsutism and a slight baseline dyslipidemia were given spironolactone (Nakhjavani et al. 2009). Spironolactone therapy was associated with an elevation of LDL-C and a reduction of HDL-C, suggesting that this drug may deteriorate the lipid profile over the short term and should not be administered to dyslipidemic women (Nakhjavani et al. 2009).

Cholesterol-lowering drugs

Several cholesterol-lowering drugs exist, but only statins have been adequately studied in PCOS women and are recommended by the AES expert committee for lowering cholersterol in PCOS women (Wild et al. 2010). The use of statins is recommended in PCOS women if LDL-C > 160 mg/dl, if non-HDL-C > 190 mg/dl, or if LDL-C remains > 130 mg/dl despite 3 months of lifestyle modification and there is the concomitant presence of 2 other CVD risk factors (Wild et al. 2010). If severe dyslipidemia is not corrected by lifestyle modification and statins, experts recommend dual pharmacotherapy with the addition of fenofibrate (Wild et al. 2010). In the event that TG levels are above 500 mg/dl, the expert committee also advises 4 mg daily of omega-3 fatty acids (Wild et al. 2010).

Bariatric Surgery

Follow-up studies of PCOS women having undergone bariatric surgery have indicated a restoration of ovulation in all PCOS women (Eid et al. 2005, Escobar-Morreale 2005), as well as a significant decrease in androgen levels (Escobar-Morreale 2005), a reduction of hirsutism (Eid et al. 2005, Escobar-Morreale 2005), mean weight loss of 41 kg (Escobar-Morreale 2005) or 56.7% of excess weight in 1-year (Eid et al. 2005), and improvements in IR (Escobar-Morreale 2005). Due to the risk of nutritional deficiencies and infection associated with bariatric surgery, the expert committee of the AES has recommended that such surgery be performed only after standard weight loss strategies have failed in PCOS women with a BMI greater than 40 kg/m^2 or in women with a BMI greater than 35 kg/m^2 and who also have a high-risk obesity-related condition (Wild et al. 2010).

Conclusions

PCOS is an ancient evolutionary trait, most probably present before humans migrated out of Africa, as it is widespread and common to all races. Aggressive behavior induced by elevated testosterone levels, increased bone mineral density, small number of children subsequent to oligo-ovulation, and reduced thermogenesis as a consequence of insulin resistance, may have proffered survival advantages (Azziz et al. 2011). Many PCOS patients today would be sub-fertile or fertile at the low BMI of hunter gatherers (Azziz et al. 2011). However, in the context of modern lifestyles, especially per western standards, PCOS women have an increased risk of presenting with insulin resistance, impaired glucose tolerance, type 2 diabetes mellitus, obesity, and dyslipidemia. These metabolic perturbations lead to chronic low grade inflammation and to vascular impairments that heighten the risk of cardiovascular disease.

Insulin resistance, though obviously not the ultimate cause of PCOS, is prevalent in most obese and many lean PCOS cases. It plays a central role in the evolution and establishment of the syndrome.

Decreased adiponectin levels reported by many of the studies reviewed in this thesis, and pertaining to women from different regions of the world, might represent a more ancient and widespread trait of PCOS that increases susceptibility to IR. Other intrinsic causative factors of IR are more narrowly distributed. They are present in some sub-groups of PCOS women and not in others, and thus may represent more recently acquired traits. These less uniformly observed possible causative factors, among many others that have been reported, include alterations in IRS-1 and IRS-2 signaling, increased activation of ERK ½ pathways, and lipolytic defects of visceral adipocytes.

This review has provided a survey of many intrinsic causative factors that underlie IR, obesity, and the other metabolic perturbations associated with PCOS. Future investigations may elucidate which of these intrinsic causative factors are present in the different phenotypic sub-groups of PCOS women. Further studies may also delineate which of these intrinsic factors are more common in certain geographic regions and associated ethnicities. Finally, and more importantly, through future investigations we may gain an understanding of which of these causative factors are associated with the most severe consequences. This may help foster a better

understanding of the pathophysiology underlying PCOS in different sub-groups and populations. Such knowledge could then be leveraged to devise the most optimal screening and effective management for women from different sub-groups and ethnicities.

Current management of the metabolic manifestations and of the cardiovascular risk in PCOS is tailored to the specific presentation of each patient. Management options, depending on the presentation of the patient, may include lifestyle modification, insulin-sensitizing agents, anti-androgenic agents, anti-hypertensives, statins and bariatric surgery.

The higher cardiometabolic risk of obese PCOS women has lead experts to advocate closer monitoring and more aggressive management of this group of patients. Experts have also advocated the annual screening of all PCOS women for IGT with the OGTT, and more frequent screenings for those with other DM2 risk factors, such as a family history of DM2. Some investigators have also suggested screening lean women with DEXA for excess abdominal fat accumulations, as lean PCOS women with a higher proportion of abdominal fat relative to BMI-comparable control women are more susceptible to developing insulin resistance and may therefore benefit from more aggressive prevention.

Central abdominal fat accumulation has been associated with insulin resistance, chronic inflammation, and harmful ectopic fat accumulations. Studies evaluating abdominal fat accumulation in PCOS women relative to BMI-comparable controls have reported contradictory results. This highlights the need for more studies that would quantify ectopic, visceral and subcutaneous abdominal fat by CT or MRI, in order to provide definitive answers about the relationship between PCOS and visceral fat.

Authors of studies evaluating the effects of OCPs and of metformin on certain aspects of the cardiometabolic profile, such as on the lipid profile, have reported contradictory results. This demonstrates the need for future RCTs evaluating women with PCOS to span longer periods of time, and to track all possible factors that may affect outcome, such as baseline status, lifestyle changes and diet (including details on caloric and carbohydrates intake). In particular, women with long-standing metabolic disturbances and those with the most severe metabolic profiles were reported to be more resistant to current management strategies and medications. This fact points to the need for future studies that focus on devising more effective ways to manage this resistant group of PCOS women.

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Biography

Roya Ougouag was born in Urbana, Illinois. She graduated from high school in Idaho Falls, Idaho, with top honours and was the AP state scholar of her graduating year.