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The Polycystic Ovary Syndrome Status – A Risk Factor for Future Cardiovascular Disease

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1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age with a prevalence estimated at 4–8% (Azziz et al., 2004; Moran & Teede, 2009). It is associated with a range of reproductive, obstetric, metabolic and psychological features. Reproductive and obstetric manifestations include hyperandrogenism, menstrual dysfunction, infertility and pregnancy complications, such as early pregnancy loss, gestational diabetes, pregnancy-induced hypertensive disorders and neonatal complications (Boomsma et al., 2006). Additionally, women with PCOS cluster risk factors for cardiovascular disease (CVD) and type 2 diabetes mellitus (DM) as well as the metabolic syndrome (MS). As a consequence, metabolic complications and potential major long-term sequelaes include: an elevated risk of impaired glucose tolerance (IGT), type 2 DM (Legro, 1999, as cited in Moran & Teede, 2009), as well as an increased rate of hypertension and CVD (Shaw et al., 2008). Although there are still aspects to be cleared regarding the long-term consequences of these well-known cardiovascular (CV) risk factors, the research that has been conducted so far seems to indicate that patients with PCOS are at increased risk for adverse CV morbidity and mortality. Of utmost importance is the fact that these women are likely to develop CV disease early, and that even very young and nonobese patients may be affected (Moran & Teede, 2009; Lorenz & Wild, 2007).

However, there is a wide variety of diagnostic criteria generating several reproductive diagnostic phenotypes and the best diagnostic criteria for PCOS as well as the metabolic implications of newer non-National Institute of Health (NIH) PCOS phenotypes are still under intense debate. Although the prevalence of the MS is likely to vary according to PCOS and MS definition and ethnicity, its occurrence is estimated to be substantially higher in women with PCOS compared with the general population. Endothelial dysfunction and increased arterial stiffness are early markers of atherosclerosis, which has been recognized as a chronic inflammatory state. Increased carotid intima-media thickness (IMT) has been reported to occur relatively early in the atherosclerotic process and to represent a powerful predictor of coronary and cerebrovascular events. These alterations were first found in middle-aged women with PCOS and then demonstrated also in young ones. Moreover, coronary artery calcium, a radiographic marker for atherosclerosis, correlates with the extent of coronary atherosclerotic plaque. Up to the present, the results of the literature have

been controversial as far as the presence of arterial structural and functional alterations or of chronic inflammation in PCOS are concerned. However, most lines of investigation point to increased CV risk and sustain the presence of sub-clinic CVD among women with PCOS. Therefore, medical intervention should target the reduction of the risk factors that cluster in women with this disorder. It has even been suggested that if pathologic values of IMT are found, these patients should be aggressively treated, starting with lifestyle programs and eventually with insulin-sensitizing or also statins (Carmina, 2009). Furthermore, the oral contraceptive pills (OCPs), the most common drugs used in PCOS, may exacerbate the metabolic profile of women with this disorder, as they were associated in some studies with a deterioration of carbohydrate metabolism and lipid profile. Women using OCPs have higher highly sensitive C-reactive protein (hsCRP) concentrations than non-users and the ethinyl-estradiol –cyproterne acetate pill has been shown to significantly increase serum CRP levels in PCOS subjects. Though controversies still exist, there are important reports providing suggestions that increased CV risk among women with PCOS indeed increases likelihood of them developing CVD and CV events (Shaw et al., 2008). PCOS thus constitutes a significant health and economic burden estimated at over $4 billion in the USA with ~40.5% of costs related to treatment of type 2 DM. (Azziz et al., 2005). If PCOS status were recognized as an independent risk factor for subsequent CV events, this would, then, justify earlier risk-factor intervention.

2. Phenotypes of the polycystic ovary syndrome and the risk for cardiovascular disease

Although it is widely recognised that PCOS is a diagnosis of exclusion, the optimal diagnostic criteria for PCOS remain controversial. Several definitions are in use today: one arising from an expert conference sponsored by the National Institute of Health (NIH1990 criteria) and the other from another expert conference sponsored by the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) in 2003 in Rotterdam (Rotterdam 2003 criteria). To date, there is limited understanding of the relative prevalence of risk factors for metabolic diseases (type 2 DM and CVD) across the reproductive diagnostic phenotypes of PCOS and clearly the prevalence and the long-term morbidity of PCOS will depend, to some degree, on the criteria used to define this disorder. Clarification of this will aid in determining whether to include non-NIH phenotypes as part of the complex condition of PCOS and in identifying if specific reproductive PCOS phenotypes have elevated metabolic risks (Moran & Teede, 2009).

NIH diagnostic criteria have been used for the past 15 years based on biochemical or clinical hyperandrogenism and anovulation (excluding other secondary causes including thyroid dysfunction, non-congenital adrenal hyperplasia or hyperprolactinaemia) (Zawdaki & Dunaif, 1992). According to these criteria, 4–8% of women in a general population have PCOS (Asuncion et al., 2000; Azziz et al., 2004; Diamanti-Kandarakis et al., 1999; Knochenhauer et al., 1998, as cited in Moran & Teede, 2009). The Rotterdam criteria (ESHRE/ASRM, 2004), which are more extensive, were formulated as two of the three criteria of hyperandrogenism, polycystic ovaries (PCO) on ultrasound and irregular anovulatory periods (Guastella et al., 2010). This introduced two new PCOS phenotypes (non-NIH PCOS) of hyperandrogenic ovulatory women with PCO or non-hyperandrogenic anovulatory women with PCO (2004) (Moran & Teede, 2009). The development of both the ESHRE/ASRM and the Androgen Excess Society (AES) criteria has introduced greater

heterogeneity into PCOS from a reproductive and possibly from a metabolic perspective. In fact, according to these guidelines, the diagnosis of PCOS may present in patients with four different phenotypes: [1] hyperandrogenism, chronic anovulation, and PCO; [2] hyperandrogenism and chronic anovulation but normal ovaries; [3] hyperandrogenism and PCO but ovulatory cycles; and [4] chronic anovulation and PCO but no clinical or biochemical hyperandrogenism (Guastella et al., 2010). In 2006, the AES published a position statement which suggested that androgen excess is the key component of PCOS related to clinical presentation and long-term morbidity. The proposal of the AES was, therefore, to include in the diagnosis of PCOS only the first three phenotypes, excluding the phenotype of PCO and irregular cycles without hyperandrogenism (Azziz et al., 2006, 2009; Guastella et al., 2010; Moran & Teede, 2009).

For simplification, PCOS can be subdivided into four reproductive phenotypes: NIH-diagnosed PCOS either with (phenotype A) or without PCO (phenotype B); biochemical/clinical hyperandrogenism with PCO but no oligo/anovulation (phenotype C), or no biochemical/clinical hyperandrogenism with PCO and oligo/anovulation (phenotype D) (Moran & Teede, 2009).

There is an increasing body of literature devoted to examining the metabolic implications of the reproductive diagnostic phenotypes of PCOS. The majority of literature to date has focused on the NIH diagnosis of PCOS.

Hence, a very recent study performed in Brazil in order to assess the NIH PCOS phenotypes (A and B) for metabolic features indicated that PCOS diagnosis based on the presence of hyperandrogenism and ovulatory dysfunction, with or without PCO, is associated with a worse metabolic profile and more insulin resistance than that observed in ovulatory women with the hyperandrogenism +PCO phenotype or with isolated hirsutism, and in ovulatory control women without hirsutism, even after adjustment for BMI (because the prevalence of obesity was higher in the classic PCOS group) (Wiltgen & Spritzer, 2010). However, when weight-matched, the most of data suggest that the metabolic profile of the newer phenotypes is similar to the profile seen in NIH phenotypes (Moran & Teede, 2009). Interestingly, the adiponectin levels, which have been proposed as possible links between reproductive and metabolic anomalies in PCOS (Yilmaz et al., 2009), were significantly lower in groups 1 and 2 (classic PCOS) in comparison with the concentrations in women with the newer phenotype groups 3 and 4, added in accordance with the 2003 criteria. (phenotypes C and D), which were much higher, reaching those of control levels. It is worth mentioning that there was no significant difference in the waist circumference (WC), the waist-to-hip ratio (WHR) and, consequently, the amount of visceral fat between the five groups of women in the study. Thus, adiponectin levels could reflect the distinct PCOS phenotype (Karkanaki et al., 2009). Furthermore, the few studies specifically comparing NIH PCOS with or without PCO (phenotype A versus B) have generally shown similar risk of metabolic disease for the two phenotypes of NIH PCOS. (Dewailly et al., 2006; Diamanti-Kandarakis and Panidis, 2007; Hsu et al., 2007; Shroff et al., 2007, as cited in Moran & Teede, 2009; Moran & Teede, 2009).

Research on the metabolic implications of the newer phenotypes of PCOS introduced by the ESHRE/ASRM is only emerging.

Depending on the population recruited from, up to 18% of women with PCOS by ESHRE/ASRM criteria can have non-hyperandrogenic PCOS (D) and up to 25% of women

can have ovulatory PCOS (C). This indicates an increasing number of women with PCOS who may experience different reproductive and metabolic risks, when compared with those who have NIH PCOS with potential implications for research, screening and clinical practice. There is emerging evidence that these two phenotypes have a less adverse metabolic profile than NIH PCOS (Moran & Teede, 2009).

Regarding the phenotype C and D, particularly, compared with NIH PCOS, the literature, although scarce, suggests less adverse metabolic profiles for both hyperandrogenic ovulatory PCOS and non-hyperandrogenic anovulatory PCOS. Reduced adiposity and abdominal adiposity contribute to a more favourable metabolic profile in these two non-NIH phenotypes. These patients presented with most of the PCOS characteristics but in a milder form. In fact, patients with ovulatory PCOS had intermediate values (between classic PCOS and controls) of BMI, WC, testosterone, insulin, and quantitative insulin sensitivity index (QUICKI) (Guastella et al., 2010; Moran & Teede, 2009). As far as the specific PCOS phenotype- hirsute ovulatory patients with PCO are concerned, there are data indicating a lower prevalence of CV risk factors in hirsute ovulatory patients with PCO and normal androgen levels than with the classic PCOS phenotype, being similar in that regard to women with isolated hirsutism. However, obesity might be implicated in increased susceptibility to insulin resistance in hirsute women, and the monitoring of this group for metabolic comorbidities and CV risk factors is warranted even in the presence of androgen levels within the normal range (Wiltgen & Spritzer, 2010). All in all, both non-NIH (hyperandrogenic ovulatory) PCOS (phenotype C) and non-hyperandrogenic PCOS (phenotype D) generally have lower body weight and body mass index (BMI) and better metabolic profiles compared with NIH PCOS (phenotypes A/B). However, non-NIH (phenotype C and D) PCOS and weight-matched NIH PCOS appear to present with similar metabolic risk profiles, particularly where abdominal fat and total fat are similar between subjects (Moran & Teede, 2009).

In comparison to controls, although not universally observed, women with non-NIH (hyperandrogenic ovulatory) PCOS (phenotype C) seem to be more adversely metabolically affected and this appears to be strongly related to the presence of adiposity and specifically abdominal adiposity. Additionally, some evidence exists to suggest that non-hyperandrogenic anovulatory PCOS matched for abdominal obesity have an adverse metabolic profile compared with controls.

There are even fewer studies comparing the non-NIH phenotypes of hyperandrogenic ovulatory PCOS (phenotype C) and non-hyperandrogenic anovulatory PCOS (phenotype D) and from this limited and conflicting literature, non-hyperandrogenic anovulatory PCOS seems not to display improved metabolic risk factors compared with hyperandrogenic ovulatory PCOS. There is, thus, currently limited evidence to support the exclusion of non-hyperandrogenic PCOS as a phenotype of PCOS based on metabolic presentation (Moran & Teede, 2009).

The current evidence shows that patients with NIH PCOS, who are hyperandrogenic and generally insulin resistant, have the most severe metabolic features. The adverse metabolic profile is strongly connected to obesity and abdominal obesity, the latter being more severe in NIH PCOS than in other non-NIH phenotypes. Both hyperandrogenism and insulin resistance play a role in the adverse metabolic profiles and both may establish the metabolic phenotypes of women with PCOS, either directly or through a high inclination towards

abdominal obesity. The metabolic profile of newer reproductive phenotypes appears to be milder than that of NIH PCOS, but more adverse than that of controls and, again, strongly related to abdominal adiposity. While the ovulatory subgroup is more hyperandrogenic, evidence suggests non-hyperandrogenic women as having a similar metabolic profile to ovulatory PCOS with limited evidence even suggesting they present with more severe IR and dyslipidaemia (Norman et al., 1995a, as cited in Moran & Teede, 2009). In the setting of either hyperandrogenism or insulin resistance, metabolic abnormalities are observed. Consequently, the literature has been, so far, sustaining the inclusion of both newer phenotypes of PCOS based on the ESHRE/ASRM Rotterdam diagnostic criteria, suggesting that these phenotypes are milder forms of PCOS.

There are, however, serious limitations of the literature on the metabolic features of PCOS, such as: ethnic diversity, recruitment sources of participants, consistency in the use of end-points and inconsistently defined controls or the use of CV risk factors instead of clinical disease outcomes (e.g. type 2 DM, coronary artery disease, subclinical or clinical atherosclerosis), to name but a few. Taking all these into account, it has become obvious that we need rigorous, well designed studies with well-defined controls and longitudinal follow-up to efficiently grasp clinical outcomes in order to clarify many of the ambiguous aspects of the metabolic phenotype of PCOS (Moran & Teede, 2009).

3. Cardiovascular risk factors in PCOS

3.1 Traditional cardiovascular risk factors

Several studies have examined the presence of CV risk factors in premenopausal women with PCOS. It has long been established that women with PCOS have an unfavorable cardiometabolic risk profile. Based on National Cholesterol Education Program guidelines, traditional CV risk factors include age > 55 years, current cigarette smoking, diabetes, history of premature coronary artery disease in first-degree relatives (men < 55 years, women < 65 years), hypertension, and dyslipidemia. (Birdsall et al., 1997; Dahlgren et al., 1992; Solomon et al., 2002, as cited in Dokras, 2008).

3.1.1 High blood pressure

The patient's risk is determined by the levels of both systolic (SBP) and diastolic blood pressure (DBP). In fact, of the two readings, the SBP may best predict all the complications related to hypertension.

In spite of the fact that blood pressures (BP) are generally within the normal range in young women with PCOS and not very different from that of age and weight matched controls (Dokras, 2008) abnormalities in the regulation of BP are common in these patients. Prehypertension, defined as SBP 120 to 139 mm Hg or DBP 80 to 89 mm Hg, is associated with a twofold increased risk of CV mortality (Chobanian et al., 2003, as cited in Dokras, 2008). 24-hour ambulatory BP readings showed that women with PCOS have an increased risk of prehypertension (Holte et al., 1996, as cited in Dokras, 2008; Lo et al.,2006). Moreover, although both SBP and DBP are normal in PCOS women in many studies, there are reports in which mean arterial pressures and ambulatory SBP (e.g. Holte et al., 1996, as cited in Cho et al., 2007) or the prevalence of hypertension (Cho et al., 2007) are increased in women with PCOS compared with controls (Hoffman & Ehrmann, 2008). Confirming these results, both

mean arterial BP and the risk of preeclampsia have been found to be higher in women with PCOS (Akram et al., 2010; Boomsma et al., 2006).

According to a study carried out in 2005, there is no difference in ambulatory or office-based BP measurements between women with PCOS and control individuals; however, women with PCOS had a less significant overnight drop in mean arterial BP, a phenomenon also noted in obese adolescents with PCOS (Wild et al., 2005, as cited in Hoffman & Ehrmann, 2008). A Taiwanese study showed that the characteristic hyperandrogenemia in PCOS is associated with an elevated SBP and DBP independent of age, insulin resistance, obesity, or dyslipidemia (Chen et al., 2003). Overall, it appears that elevated SBP is detected after the third decade of life and may be independent of obesity (Dokras, 2008). Moreover, in a very recent study that targeted 113 PCOS women, the frequency of women with BP values above the normal limit was significantly higher in the PCOS group than in the control group. In the PCOS group, the values of SBP and DBP were positively correlated with age, BMI, WC, and triglycerides (TG) ($p<0.05$) to a significant degree. These results underline the importance of preventive strategies in PCOS women in anticipating pathological events related to the cardiovascular system (Azevedo et al., 2011).

Women with PCOS are under an increased risk of developing hypertension later in life, as proved by retrospective data. Follow-up data from the Pittsburgh case-control study proved physician-diagnosed hypertension in 23% of women with PCOS whereas the percentage of cycling control women in this situation was of only 6.9% (Talbott et al., 2001). In an earlier retrospective cohort study of 33 older women with histopathology consistent with PCOS on wedge resection 22 to 31 years earlier, Dahlgren et al. (1992) found the diagnosis of hypertension to be three times more common in women with PCOS than in normal age-matched controls (Dahlgren et al., 1992, as cited in Sukalich & Guzick, 2003). Preliminary data from the Nurses' Health Study demonstrate that the risk of developing hypertension was twice higher in women with increased or highly irregular menstrual cycle length (a possible surrogate for a clinical diagnosis of PCOS) than in women with regular cycles (Rich-Edwards et al., 1998, as cited in Sukalich & Guzick, 2003). A significant difference in the prevalence of hypertension was still noted after correction for BMI.

In spite of noted characteristics that typically accompany PCOS (e.g. insulin resistance, obesity etc.) the exact mechanisms responsible for hypertension in women with PCOS are yet to be clarified. Insulin resistance causes secondary hyperinsulinemia. Hyperinsulinemia may produce enhanced sodium retention (Zavaroni et al., 1995, as cited in Cho et al., 2007), increasing intracellular sodium and calcium and augmenting sympathetic activity (Reaven et al., 1996, as cited in Sukalich & Guzick, 2003) which may have a role in the development of hypertension (Sukalich & Guzick, 2003). Insulin also stimulates the release of insulin-like growth factor (IGF-1) that may contribute to the development of hypertension by determining vascular smooth muscle hypertrophy (Cho et al., 2007). Furthermore, the obesity that is common in PCOS adds to the risk of hypertension. The higher level of androgens seems to be strongly related to BP in women with PCOS who are not obese. Although the mechanisms by which hyperandrogenemia mediates the higher BP in women with PCOS remain to be determined, it is possible that androgens may directly stimulate endothelin-1 (ET-1) or may stimulate the rennin-angiotensin system (RAS) to increase ET-1, thus leading to the expression of two powerful vasoconstrictors that could impact BP in these women (Reckelhoff, 2007).

In conclusion, PCOS women seem to be at increased risk for hypertension development, if not during their reproductive years, then at least later in life (Hoffman & Ehrmann, 2008). Accordingly, very recently (2010), the AE-PCOS Board committee *has been recommending* a BP routine check at each visit. Ideal BP is 120 mm Hg systolic and 80 mm Hg diastolic or lower, and prehypertension should be detected and treated (Cushman, 2007). BP control has the largest benefit for reducing CVD (Wild et al., 2010).

3.1.2 High blood cholesterol and related lipid problems

Dyslipidemia is a major determinant of progression of atherosclerosis. Atherosclerosis starts at a very young age, and PCOS may represent an important model of lipid alterations starting during adolescence or fertile age (Wild et al., 1985; Wild et al., 2011). Actually, lipid abnormalities have been reported in up to 70% of PCOS patients and displayed different patterns, depending on several factors such as: the PCOS phenotype, the presence of obesity and the associated effects of IR and hyperandrogenism that combine with environmental (diet, physical exercise) and genetic factors (Essah et al., 2008; Valkenburg et al., 2008; Wild et al., 2011). The dyslipidaemia occurs independent of BMI (Wild et al., 1985; Wild & Bartholomew, 1988, as cited in Teede et al., 2010a; Talbott et al., 1995, as cited in Sukalich & Guzick, 2003; Teede et al., 2010a) and several studies have confirmed adverse lipid alterations in both obese and nonobese women with PCOS compared with weight-matched control women (Sukalich & Guzick, 2003). Consequently, PCOS might be considered the most common cause of dyslipidemia in women under 40.

Wild and colleagues (Wild et al., 1985) were the first to present data suggesting that women with PCOS had a more adverse lipid profile than control subjects and there are many studies that have analyzed this aspect ever since. Lipid abnormalities include low high-density lipoproteins cholesterol (HDL-C), high low-density lipoprotein cholesterol (LDL-C), high TG levels and small dense LDL particles (Lambrinoudaki, 2011; Wild et al., 2011), a combination closely linked to insulin resistance and an independent predictor of myocardial infarction (MI) and CVD. Increased TG/HDL-C ratio is also a marker for atherogenic, small, dense LDL-C particles and it may be also used as a simple metabolic marker to identify overweight individuals who are insulin-resistant (McLaughlin et al., 2003, Brehm et al., 2004, as cited in Dokras, 2008). This relationship has been demonstrated in women with PCOS by identifying a negative correlation between TG/HDL-C and QUICKI (Dokras et al., 2005). Recently, it has been proved that TG/HDL C > 3.2 and respectively > 3.5 identify both the MS as well as insulin-resistant and dyslipidemic patients whose chances to be at an increased risk for CVD are extremely high (Dokras, 2008). As mentioned earlier, prevalent MS in women with PCOS (Apridonidze et al., 2005; Dokras et al., 2005; Moran et al., 2010) has concentrated attention of most authors on changes in TG and HDL-C (that are components of the metabolic syndrome) with relatively little attention to other lipid changes, although LDL-C and nonHDL-C are considered to be the primary and secondary targets to reduce CVD and atherosclerosis as described by the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines (NCEP-ATP III, 2002, as cited in Wild et al., 2011). However, qualitative disorders of LDL-C have also been identified in women who have PCOS and there have been a large number of studies during the past decade who found increased LDL-C levels in women with this disorder (Carmina et al., 2005; Legro et al., 2001; Rizzo et al., 2009; Wild et al., 2011) and LDL particles smaller than in matched controls (Dejager et al., 2001). Small, dense LDL particles are associated

with increase in cardiovascular risk independent of total LDL (Austin et al., 1988, as cited in Sukalich & Guzick, 2003). However, the wide variability of LDL-C values between individual studies indicate that LDL-C values are influenced by many variables, including ethnic groups, severity of the syndrome (anovulatory versus ovulatory forms), quality of food, and body weight (Essah et al., 2008; Wild et al., 2011). When subset analyses including only BMI-matched patients were performed, LDL-C was still higher in women with PCOS. It clearly indicates that PCOS per se is responsible for increased lipid values, although the absolute value and the related cardiovascular risk may be different among individual patients (Wild et al., 2011).

Only one study reported mean values of LDL-C <100 mg/dL. Four studies reported mean concentrations in PCOS women ≥130 mg/dL, whereas no study showed mean LDL-C values >130 mg/dL in control subjects. Considering the reported variances, it is not uncommon for individual PCOS patients to have LDL concentrations >160 mg/dL. Elevated nonHDL-C was reported as a common abnormality in PCOS women included in the studies. 21 of the 31 comparisons, registered nonHDL-C >130 mg/dL and the mean difference was still considerably higher with BMI matching (16 mg/dL vs. control subjects). Similarly to what was found for LDL-C, higher nonHDL-C levels were generally reported in PCOS subjects whose mean BMI was higher. There were clear differences with PCOS even when the BMI was in the nonobese or nonoverweight categories. This suggests that obesity or overweight (and higher insulin resistance) is not the only factor that accounts for elevated LDL-C and nonHDL-C in PCOS (Carmina, 2009; Wild et al., 1985).

However, though decreased HDL-C (particularly HDL2, the most antiatherogenic HDL subtype) (Legro et al., 2001; Diamanti-Kandarakis et al., 2007, as cited in Hoffman & Ehrmann, 2008) may represent the most common lipid alteration in PCOS (Essah et al., 2008), and many studies have reported elevated levels of TG as compared to controls, a very recent meta-analysis performed by Wild et al. (2011) shows that altered levels of TG are not commonly identified in many populations (Carmina et al., 2003, as cited in Wild et al., 2011; Essah et al., 2008). Only three studies reported TG concentrations that exceeded 150 mg/dL, and these were found in women who were overweight or obese (Wild et al., 2011).

However, two main patterns of lipid alteration were described separately or combined (Wild et al., 2011). The most common pattern is probably classic atherogenic dyslipidemia (increased TG, low HDL-C, and increased small dense LDL subclasses + increased nonHDL-C). This lipid pattern is similar to that found in type 2 DM, and it is mainly the consequence of insulin resistance that impairs the ability of insulin to suppress lipolysis, thereby increasing mobilization of free fatty acids (FFA) from adipose stores. Consequently, increased hepatic delivery of FFAs impairs insulin inhibition of hepatic very low-density lipoprotein 1 synthesis, causing altered catabolism of very low-density lipoprotein (Brunzell et al., 2003 as cited in Wild et al., 2010). Additionally, the ability of insulin resistance to alter the expression of lipoprotein lipase and hepatic lipase may also contribute to this lipid pattern (Wild et al., 1985). Sustaining this hypothesis, insulin resistance was linked with hyperlipidemia in most of the studies including women with PCOS (Slowinska-Srzednicka et al., 1991, as cited in Sukalich & Guzick, 2003). Due to the fact that excessive adipose tissue increases insulin resistance, this pattern is likely to be found in obese patients with PCOS. These lipid abnormalities are further augmented among those women who develop glucose intolerance in association with PCOS. The magnitude of androgen elevation, race and

ethnicity are considered additional modifiers of dyslipidemia in women with PCOS (Ehrmann et al., 2006, as cited in Hoffman & Ehrmann, 2008). The lipid profile of both lean and obese women with PCOS is improved by treatment with the androgen-receptor blocker flutamide. Moreover, both in vitro and in vivo studies have included hyperandrogenemia in the pathogenesis of low HDL-C levels, possibly by upregulating the genes involved in the catabolism of HDL-C (Hoffman & Ehrmann, 2008). However, Wild et al. found that hyperinsulinemia has a more significant effect on lipids than hyperandrogenemia in hirsute women (Wild et al., 1992, as cited in Sukalich & Guzick, 2003). This lipid pattern was reported in about 70% of American women with classic PCOS but is less commonly met in other countries where mean body weight is lower (Essah et al., 2008). However, also in Mediterranean countries, about one half of women with PCOS have low HDL-C and a small dense LDL phenotype. However, because of the common increase of LDL-C, a second mechanism related to altered LDL quality has been demonstrated (Wild et al., 2010). Many studies have also demonstrated an increase in LDL-C in women with PCOS (Essah et al., 2008; Legro et al., 2001; Valkenburg et al., 2008). However, its prevalence in PCOS is generally lower than that found for the atherogenic dyslipidemia and ranges from 24 to 40% (Essah et al., 2008; Valkenburg et al., 2008). It depends on body weight to a lesser extent and may be at least partially related to the hyperandrogenism (Wild et al., 2010). In fact, several studies have shown that in female postmenopausal populations, increased circulating values of LDL are related to higher testosterone and free androgen index (Mudali et al., 2005, Liu et al., 2001, as cited in Wild et al., 2011).

In conclusion, dyslipidemia is likely to be found in women with PCOS. Consequently both the American College of Obstetricians and Gynecologists (ACOG) (ACOG, 2009 as cited in Wild et al., 2011) and the Androgen Excess and PCOS Society (Wild et al., 2010) guidelines have recently pointed to the fact that an individual CV risk assessment in women with PCOS is highly necessary and that the CV risk assessment of patients with this disorder should include a complete fasting lipid and lipoprotein evaluation including LDL-C and nonHDL-C, as well as for TG and HDL-C. In addition, the Androgen Excess and PCOS Society guidelines have indicated different LDL-C cutoff values depending on the degree of CV risk of PCOS patients (Wild et al., 2010). In the prevention of CV risk, the first goal is to bring LDL-C to normal levels by using lifestyle intervention and medication if necessary. The second goal is keeping nonHDL-C reduced (Wild et al., 2011).

3.1.3 Obesity

Obesity and excess weight are among the most widely met chronic diseases in the Western world countries. The prevalence of increased BMI ranges between 30% and 80% among women with PCOS (Vribkova & Hainer, 2009). Moreover, it has been widely proved that 50–60% of women with PCOS have a body fat distribution of the android type irrespective of their BMI (Barber et al., 2006) and that patients with PCOS had a central fat excess independent of total fat mass (Lambrinoudaki, 2011; Puder et al., 2005; Ilie et al., 2008).

Obesity also increases hyperandrogenism, hirsutism, insulin resistance and metabolic disorders, infertility and pregnancy complications both independently and by exacerbating PCOS phenotypic expression (Balen et al., 1995, Kiddy et al., 1990, as cited in Teede et al., 2010a). Therefore, in spite of the lack of epidemiological data, the increasing epidemic of obesity worldwide is thought to facilitate the high prevalence of PCOS in the general

population. It is of utmost importance to underline the fact that the defining characteristic of PCOS is visceral fat, rather than subcutaneous one, as visceral fat plays an important role in the proinflammatory response. Accumulation of visceral fat leads to insulin resistance, endothelial dysfunction and a proinflammatory status through fat-derived metabolic products, hormones (adiponectin, resistin, FFA) and cytokines (interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), tumor necrosis factor-α (TNF-α). Although many researches (Puder et al., 2005; Cascella et al., 2008; Svendsen et al., 2008, as cited in Penaforte et al., 2011) have advanced the idea that women with PCOS accumulate fat mainly in the upper body compared to controls matched for weight and age (Gambineri et al., 2002, as cited in Penaforte et al., 2011), other studies, including ours, did not detect any differences in total body fat, abdominal fat, visceral fat or trunk fat (Faloia et al., 2004; Glintborg et al., 2006; Barber et al., 2008, as cited in Penaforte et al., 2011; Carmina et al., 2007; Ilie et al., 2011), or trunk to peripheral fat (arm fat + leg fat + head fat) ratio in obese women with and without the syndrome (Svendsen et al., 2008, as cited in Penaforte et al., 2011), either.

We have already underlined the fact that the presence of obesity has a big impact on the development and expression of PCOS (for instance, in a woman who has PCO but does not meet diagnostic criteria for PCOS, obesity can cause disorders such as: menstrual irregularity and hirsutism, thus completing the PCOS diagnosis) (Kiddy et al., 1990 as cited in Farrel &.Antoni, 2010). There is evidence that molecules secreted by adipose tissue such as leptin, TNF-α, IL-6 might also influence adrenal and ovarian function (Escobar-Morreale et al., 2007). In fact, PCO is present in approximately 20% of all women and many of these women do not display other syndrome features (i.e., hirsutism, irregular menstrual cycles, and elevated testosterone), which would likely appear if a PCO woman were to become overweight or obese (Polson et al., 1998 as cited in Farrel &.Antoni, 2010). The prevention of obesity, then, can be a crucial factor against the increased incidence of PCOS and its associated physiological abnormalities, especially during childhood and preadolescent years (Farrel & Antoni, 2010). Whether obesity represents a factor amplifying intrinsic hormonal and metabolic components of PCOS or, alternatively, whether it has a direct pathophysiological role is still a matter of debate. However, overall, all the reports suggest that obesity (especially abdominal obesity) and PCOS interact to promote premature atherosclerosis and increase CV mortality (Guzick et al., 1996; Birdsall et al., 1997; Christian et al., 2003, as cited in Amato et al., 2011; Pierpoint et al., 1998; Wild et al., 2000; Shaw et al., 2008).

3.1.4 Insulin resistance, glucose intolerance, diabetes mellitus

The American Heart Association and American Diabetes Association (ADA) consider the diagnosis of DM a CV disease equivalent to a prior MI. Insulin resistance is considered one of the main pathogenic factors behind the development of PCOS (Svendsen et al., 2010). Hyperandrogenism and insulin resistance were first linked in 1921 when Achard and Thiers (1921) published their classic description of a bearded woman with diabetes. This link has been confirmed ever since by many investigators (Legro, 2006; Svenden et al., 2010) and insulin resistance is now considered the main pathogenic factor in the development of PCOS. Many of the late complications of PCOS, primarily diabetes, dyslipidemia, and CVD also seem to be connected to insulin resistance (Dunaif, 1997, as cited in Svendson et al., 2010). Moreover, IGT and MS, as predictors of type 2 DM and premature CVD mortality, are more widely met in women with PCOS (odds ratio, approximately 4:1) (Ehrmann et al., 1999, Legro et al., 1999, as cited in Wild et al., 2010).

3.1.4.1 Insulin resistance and abnormal glucose metabolism

Insulin resistance occurs in around 50% to 80% of women with PCOS (Wild et al., 2000, as cited in Wild et al., 2010), and in 95% of obese women with PCOS (Carmina & Lobo, 2004, DeUgarte et al., 2005, as cited in Wild et al., 2010), primarily in the more severe NIH diagnosed PCOS. Lean women and milder Rotterdam diagnosed PCOS seem to be less affected by severe insulin resistance. Ethnicity may also be an independent factor that contributes to the risk for insulin resistance and glucose intolerance. For example, though there are few data related to the relatively higher prevalence of PCOS among women of South Asian origin, there is some evidence that the latter group of women are more likely to be affected by insulin resistance than Caucasian women (Rodin et al., 1998, Wijeyaratne et al., 2002, Balen et al., 2005, Bhathena, 2007, as cited in Bathena, 2011). Women with PCOS manifest IR independently and additively with obesity, with PCOS and obesity acting synergistically to impair insulin sensitivity (Dunaif et al., 1989, as cited in Wild et al., 2010).

A family history of type 2 diabetes is also more widely met among PCOS women with IGT or type 2 DM compared with those with normal glucose tolerance (Ehrmann et al., 2005, as cited in Dokras, 2008). Furthermore, in spite of the variability in reports concerning the prevalence of prediabetes and DM among women with PCOS, most studies confirm that women with PCOS, especially obese PCOS women, have a higher prevalence of impaired fasting glucose (IFG), IGT and DM (Chang & Wild, 2009), and a risk to develop the disease at an earlier age than the general population (Sukalich & Guzick, 2003). PCOS is now recognized by the ADA as a leading risk factor for DM screening in adolescent girls (Palmert et al., 2002, as cited in Dokras, 2008) and premenopausal women irrespective of race and ethnicity (Dokras, 2009).

Mechanisms involved in insulin resistance are likely to be complex with genetic and environmental contributors. Specific abnormalities of insulin metabolism identified in PCOS include reductions in secretion, reduced hepatic extraction, impaired suppression of hepatic gluconeogenesis and abnormalities in insulin receptor signaling. Interestingly, there is a paradoxical expression of insulin resistance in PCOS whereby insulin-stimulated androgen production persists while its role in glucose metabolism is impaired (Dunaif, 1997). Therefore, insulin resistance in PCOS results in hyperinsulinaemia with its associated diverse and complex effects on regulating lipid metabolism, protein synthesis and modulation of androgen production.

Lean women with PCOS often have abnormalities of insulin secretion and action compared to weight-matched control subjects. An overweight woman with PCOS may also demonstrate extrinsic insulin resistance associated with adiposity, which can be mechanistically distinct from the insulin resistance present in lean women with PCOS. Only a subgroup of women with insulin resistance and PCOS develops coexistent pancreatic insufficiency with β cell failure followed by type 2 (non-insulin-dependent) DM. In this setting, insulin output cannot overcome resistance and hyperglycaemia develops (Dunaif et al., 1989, Dunaif and Finegood, 1996, Ehrmann et al., 1999, Legro et al., 1999, Kelly et al., 2000, Goodarzi and Korenman, 2003, Balen et al., 2005, Ehrmann, 2005, as cited in Bahthena, 2011).

3.1.4.2 Impaired glucose tolerance (IGT) and type 2 diabetes mellitus

Overall, the risk of IGT in premenopausal PCOS women in the United States may be 25 to 40% and that of type 2 DM 4 to 10%, irrespective of race (Dokras, 2008). Solomon et al.

examined the risk for type 2 DM development in the 106,052 women enrolled in the Nurses' Health Study. Women with menstrual cycles greater than 40 days or irregular cycles had an age-adjusted relative risk of type 2 DM of 2.42 (95% confidence interval, 1.81-3.24) compared with normally cycling controls (Solomon et al., 1998, as cited in Sukalich & Guzick, 2003). But do women with PCOS switch to DM at higher rates?

Women with PCOS in the United States who have IGT have been reported to convert to DM anywhere from 6% over 3 years to 13.4% over 8 years in older women (Ehrmann et al., 1999, Legro et al., 2005, as cited in Chang & Wild, 2009). Smaller samples have reported rates of 29% (4 of 14) over 2 years and 54% (7 of 13) after 6 years (Norman et al., 2001, as cited in Chang & Wild, 2009).

Additionally, the rate of conversion from IGT to type 2 DM in a general Australian population was estimated in the large cohort Australian Diabetes, Obesity and Lifestyle (AusDiab) study at 2.9% per year for young females (Barr et al., 2005, as cited in Teede et al., 2010a). Another Australian study has reported a substantially higher conversion rate (8.7% per year over 6.2 years) in women with PCOS (Norman et al., 2001, as cited in Teede et al., 2010a). However, this has not been uniformly reported (Legro et al., 2005, as cited in Teede et al., 2010a). In the United States, women with normal glucose tolerance at baseline had a 16% conversion to IGT per year, and those with baseline IGT had a 6% conversion rate over ~3 years, or 2% per year (Legro et al., 2005, as cited in Dokras, 2009). In another study, classic PCOS patients had a 5-fold risk of developing type 2 DM over 8 yr *vs.* age- and weight-matched controls, although only 12% of PCOS patients without obesity developed glucose abnormalities (Wild et al., 2010).

Comparatively, other studies reported conversion rates to DM of 25% and 66% over 5 and 10 years in the high-risk Pima Indian population (Saad et al., 1988, as cited in Chang & Wild, 2009) and 50% over 5 years in Latina women with a previous history of gestational diabetes (Kim et al., 2002, as cited in Chang & Wild, 2009). Conversion rates for normoglycemic women to IGT vary from 9 to 16%, to as high as 40% (Chang & Wild, 2009). Collectively, these data may support high rates of conversion from IGT to DM and normal glucose tolerance to IGT, but the rates are not higher than those of other at-risk populations. These data are also incomplete due to the fact that the presence or absence of PCOS status according to race has not been defined in the populations referred to. Finally, with the RR of developing DM 6.90 (95% CI, 4.35 to 10.94) over 8 years in women with the MS (established in much larger cohorts though also among older women), the assessment of the MS and the prevention of conversion to DM are of high clinical relevance for women with or without PCOS (Chang & Wild, 2009).

Alarmingly, IGT and type 2 DM are highly prevalent among PCOS adolescents (Palmert et al., 2002, as cited in Wild et al., 2010). Although incident data are not rigorous, up to 40% of women with classic PCOS develop IGT or type 2 DM by the fourth decade of life and their glycemic control is seriously affected by age and weight gain. (Boudreaux et al., 2006, Legro et al., 2005, Norman et al., 2001, as cited in Wild et al., 2010).

Women with PCOS also have higher gestational diabetes (GDM) risk, with a recent meta-analysis reporting an odds ratio (OR) of 2.94 (Boomsma et al., 2006). The risk of GDM occurs both independent of and is exacerbated by obesity (Boudreaux et al., 2006, Legro et al., 1999, as cited in Teede et al., 2010a). Though there are few studies to adequately assess the natural

history of IGT, DM2 and CVD in PCOS and further research is needed, the International Diabetes Federation (IDF) has identified PCOS as a major non-modifiable risk factor associated with type 2 DM (Alberti et al., 2007, as cited in Teede et al., 2010a).

It has become more and more obvious that IGT has significant clinical relevance and its early identification and intervention improve long-term outcomes and can prevent IGT progression to DM, including in high-risk PCOS women.

There are currently no generic guidelines for IGT screening, only for type 2 DM based on fasting glucose or more recently on HbA1c as a first line. However, impaired fasting glucose cannot accurately predict IGT in women, in general, and in PCOS (Teede et al., 2010a). As a consequence, and taking into account the high prevalence of insulin resistance, IGT and type 2 DM in PCOS, as well as their implications in the pathogenesis of this disease and especially in the onset of type 2 DM and of CVD, recently, the AE-PCOS Board recommend that a 2-h post 75-g oral glucose challenge be performed in PCOS women with a BMI greater than 30 kg/m^2, or alternatively in lean PCOS women with advanced age (>40 yr), personal history of GDM, or family history of type 2 DM (Salley et al., 2007). Those with IGT should be screened annually for developing type 2 DM, acknowledging efficacy of treating IGT, but not necessarily impaired fasting glucose, to prevent type 2 DM (Salley et al., 2007). Hemoglobin A1c above 6.5% has been proposed as the defining criterion for diabetes (Lorenzo & Haffner, 2010). This criterion was proposed for risk assessment, but further studies will be needed to determine whether this criterion is useful in implementing lifestyle interventions and medical management for CVD prevention (Wild et al., 2010).

3.2 Other risk factors-nontraditional cardiovascular risk factors

Besides the traditional CVD risk factors, a large number of markers that have been proposed as "nontraditional" CVD risk factors (adiponectin, leptin, CRP, IL-6, plasminogen activator inhibitor 1 (PAI-1) or serum amyloid A) and were shown to contribute to accelerated atherosclerosis in diabetes might also be involved in the pathogenesis of vascular disease in PCOS. Platelet function abnormalities, alterations in the coagulation cascade or a prothrombotic state (reduced fibrinolysis and raised level of PAI-1 or elevated clotting factors such as fibrinogen) can be seen with diabetes and hyperinsulinemia. Elevated levels of CRP, or of inflammatory cytokines such as TNF-α and IL-6, IL-18, of matrix metalloproteinases, fibrinogen, ET-1, of white blood cell and platelet counts have been reported in women with PCOS (Wild et al., 2010; Jovanovic et al., as cited in Lambrinoudaki, 2011; Lorenz & Wild et al., 2007; Essah et al., 2007). Talbott et al. also identified significantly increased mean PAI-1 levels in women with PCOS (28 ng/mL) compared with controls (19 ng/mL), a relationship that remained after adjustments for BMI and insulin levels (Talbott et al., 2000b). Moreover, the increased level of PAI activity in PCOS was directly correlated with insulin resistance and it decreased with improvement in insulin sensitivity, either through weight loss or through the use of sensitizing agents, thus implicating it as a contributing cardiovascular risk factor (Cho et al., 2007). No differences, however, in PAI-1 between women with PCOS and controls have been identified by other studies (Atiomo et al., 2000, Yarali et al., 2001, as cited in Sukalich & Guzick, 2003). Further research regarding PAI-1 in women with PCOS is needed to resolve this discrepancy.

Elevated concentrations of circulating homocysteine have been identified as another cardiovascular risk factor, causing endothelial oxidative stress and platelet aggregation and

consequently leading to atherosclerosis. Significantly elevated concentrations of homocysteine have been documented in both lean and obese women with PCOS versus normal controls (Yarali et al., 2001, as cited in Sukalich & Guzick, 2003; Lorenz & Wild, 2007). Several but not all studies have found insulin resistance to be the most important predictor of increased homocysteine (Lorenz & Wild, 2007).

Adiponectin, a crucial adipocytokine, may also have a protective role in vascular damage in PCOS. Adiponectin has been shown to inhibit endothelial inflammation, to stimulate the production of NO in the endothelium, protecting blood vessels from the damage associated with insulin resistance. The majority of studies performed on women with PCOS demonstrate decreased level of adiponectin, compared to controls (Escobar-Morreale et al., 2006; Ardawi et al., 2005; Carmina et al., 2006b). Moreover, it was shown that hypoadiponectinemia is present in PCOS independent of BMI, with WHR, WC and free testosterone levels as the major determinants of decreased concentrations of adiponectin (Escobar-Morreale et al., 2006; Gulcelik et al., 2008; Ilie et al., 2008). These studies suggest that abdominal adiposity is characteristically associated with hypoadiponectinemia; hyperandrogenemia may also have a role, probably by facilitating an abdominal deposition of fat. A negative correlation was found between levels of adiponectin and IMT in a PCOS group compared with matched controls, apparently independent of the well known association of carotid change with insulin resistance and BMI, suggesting that the decrease in adiponectin may be an independent risk factor for the development of endothelial damage in PCOS (Carmina et al., 2006b).

Obstructive sleep apnea (OSA) is an independent CV risk factor and women with PCOS are 5-30 times more likely to have this disorder than are controls (Hoffman & Ehrmann, 2008). Moreover, it was suggested that the prevalence of OSA among women with PCOS is equal to, or may even exceed that in men. As previously noted, OSA is characterized by the combination of episodic sleep disruption and hypoxemia, each of which can trigger at least three major hormonal responses: activation of the hypothalamic-pituitary-adrenal (HPA) axis with increased cortisol production/secretion, increased catecholamine output from sympathetic nervous system stimulation, and increased release of adipokines from adipose tissue. These responses appear to contribute to the metabolic abnormalities associated with OSA, particularly to the decline in insulin sensitivity and glucose tolerance (Nitsche & Ehrmann, 2010).

The risk of OSA is increased as a function of both total body fat mass as well as body fat distribution. Visceral fat appears to be more metabolically active and the quantity of visceral fat has been shown to highly correlate with OSA risk (Nitsche & Ehrmann, 2010). Furthermore, disordered breathing during sleep has been found to be more common in PCOS than controls, even when controlled for BMI (Gopal et al., 2002; Fogel et al., 2001, as cited in Cho et al., 2007). In two studies (Vgontzas et al., 2001, Gopal et al., 2002, as cited in Nitsche & Ehrmann, 2010), the severity of sleep apnea did not correlate with BMI and in a third one (Fogel et al., 2001, as cited in Nitsche & Ehrmann, 2010), even after controlling for BMI, PCOS women were as much as 30 times more likely to have sleep disordered breathing and 9 times more likely than controls to have daytime sleepiness (Nitsche & Ehrmann, 2010). Overall, all these data indicate that the high prevalence of OSA in women with this disorder cannot be fully attributed to excess adiposity and that additional factors (e.g. hyperandrogenemia that is characteristic of PCOS or insulin resistance) might explain the

high prevalence. These factors are particularly relevant to the pathogenesis of OSA in women with PCOS (Nitsche & Ehrmann, 2010). Both WHR, a measure of central obesity, and circulating total testosterone were correlated with AHI. The androgenization of the PCOS women promotes development of central obesity, which has been established in other studies as a predictor for OSA. However, others have suggested that insulin resistance was the strongest predictor of OSA, stronger than were age, BMI or circulating testosterone (Vgontzas et al., 2001, as cited in Cho et al., 2007).

Nitsche (2010) has even proposed that there may be two "subtypes" of PCOS, i.e. PCOS with or without OSA, and these two subtypes may be associated with distinct metabolic and endocrine abnormalities. PCOS women with OSA may be at much higher risk for diabetes and CVD than PCOS women without OSA and may benefit from therapeutic interventions targeted to decrease the severity of OSA (Nitsche & Ehrmann, 2010).

4. Metabolic syndrome in PCOS

Metabolic syndrome is a conglomeration of multiple interrelated risk factors for CVD and type 2 DM, occurring 'more often than by chance alone'. These include atherogenic dyslipidemia, elevated blood pressure and blood glucose levels, along with central obesity (Bhattacharya, 2011). Although there is a general agreement regarding the main components of the MS, at least six diagnostic definitions have been proposed, including those formulated by the Adult Treatment Panel-III (ATP-III), the IDF and the World Health Organization (WHO). This variation requires different cut-off points and inclusion criteria (Day, 2007, as cited in Kandaraki et al., 2009). The definition proposed by the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATPIII) (Grundi et al., 2004, as cited in Kandaraki et al., 2009) is the most commonly used for clinical and research purposes. The IDF has proposed the most recent criteria, which resemble the NCEP ones, with the exception that central obesity, assessed according to ethnicity-specific cut-offs, is an integral part of the IDF definition (Kandaraki et al., 2009).

Along with the epidemic of obesity, the prevalence of MS is increasing worldwide, both in the developing and developed countries. As noted previously, MS is associated with a risk of CVD and is a common early abnormality in the development of type 2 DM. In addition, MS plays a well-recognised role in the development of OSA, erectile dysfunction, PCOS and malignant tumours (Baranova et al., 2011).

MS and PCOS are undoubtedly common afflictions in women of reproductive age in the general population. The significant interconnection between MS and PCOS when studied in combination is noteworthy. Namely, MS is significantly more prevalent in women with PCOS than in their age-matched counterparts from the general population. This PCOS-MS overlap is not singularly met in Caucasian women with PCOS. A highly prevalent MS has also been identified in Brazilian, Chinese, Korean, Indian and in multiracial PCOS populations, at least in overweight/obese patients, in spite of the fact that there are variations in the MS prevalence rates dependent on ethnic/racial regions, age, the diagnostic criteria and the comparison group studied (Fig.1) (Soares et al., 2008; Cheung et al., 2008; Park et al., 2007; Bhattacharya, 2008; Glueck et al., 2003; Apridonidze et al., 2005; Ehrmann et al., 2006; Dokras et al., 2005).

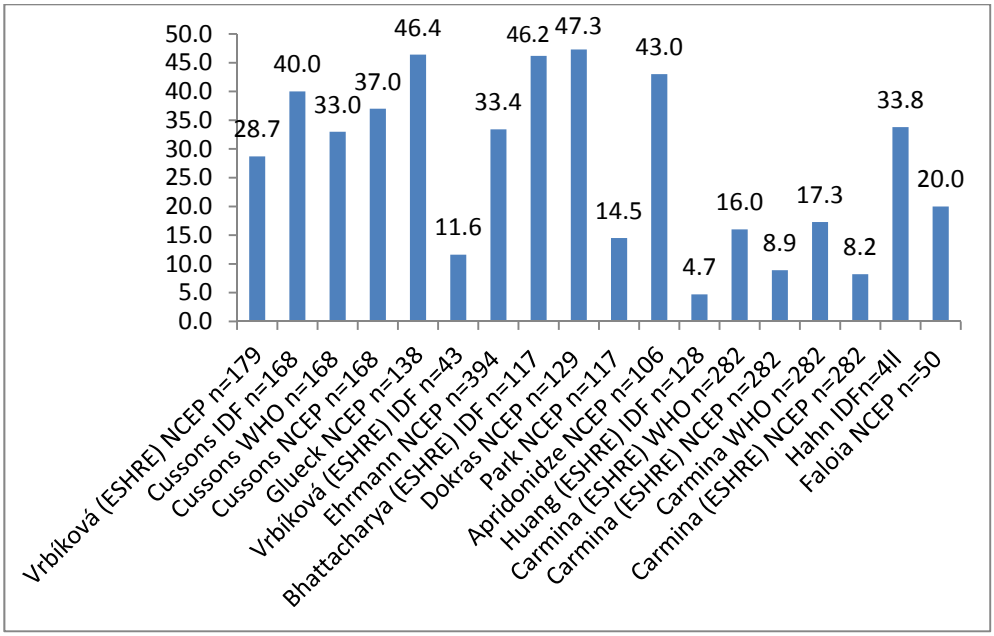

Fig. 1. The prevalence of metabolic syndrome in studies of PCOS

Data are presented as %. Studies are listed on the pattern: first author - the definition of the MS - the number of participants included in the study. Wherever unspecified (in the brackets), the criteria used for the diagnosis of PCOS were the NIH criteria. Except for the studies of Carmina, who examined both NIH and ESHRE/ASRM criteria, the rest of the mentioned researches used either NIH or ESHRE criteria (as mentioned). NIH, National Institute of Health; ESHRE/ASRM, European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine

Four US studies in predominantly obese women with PCOS have reported that 33.4% to 47.3% of these women fulfill the NCEP ATPIII criteria for MS (Glueck et al., 2003; Apridonidze et al., 2005; Ehrmann et al., 2006; Dokras et al., 2005). Apridonidze et al. (2005) performed a study on 106 women with PCOS and recorded a 43% prevalence rate, which is twice higher than the age-adjusted rate of 24% in women of all ages in the general population based on data from the Third National Health and Human Examination Survey III (NHANES III). The prevalence of MS in PCOS women, divided by decade of life registered the following values: women between ages 20 and 29 - 45% and women between ages 30 and 39 - 53%, compared with 6% and 15%, respectively, in women in the general US population of the same age ranges. Notably, the prevalence rate of the MS in women with PCOS between ages 30 and 39 (53%) was even higher than the reported 44% rate observed in women aged 60 to 69 years from the NHANES III study. Similar results were reported by Dokras et al. (2005) who discovered, in a retrospective study carried on 129 women with PCOS and 177 normal controls, that the age-adjusted prevalence rate of the MS was 47.3% compared with a 4.3% rate in controls. They also observed that when compared by age group, MS was significantly more prevalent in PCOS subjects than in controls (Essah et al.,

2007). Despite being obviously higher than the US population-based estimates, these prevalence rates may, to some extent, reflect the impact of the high prevalence of obesity in the above populations – independently of PCOS per se (Glueck et al., 2003; Apridonidze et al., 2005; Ehrmann et al., 2006; Dokras et al., 2005). Furthermore, in three of the above US studies, the included control groups were not specifically selected (Glueck et al., 2003; Apridonidze et al., 2005; Ehrmann et al., 2006). In another US study (Dokras et al., 2005), the apparent preponderance of MS in the PCOS group was eliminated when non-obese PCOS patients were compared with age and BMI- matched controls, while the difference between the obese subgroups of patients and controls was reduced to non-significant levels. Thus, the actual impact of MS in women with PCOS as compared to controls, evenly matched for age and BMI, awaits further investigation.

Moreover, European studies among PCOS populations with lower BMI and also other non-US studies have reported significantly lower prevalence rates of MS than the ones reported by the US studies (Carmina et al., 2006a; Vural et al., 2005; Vrbikova et al., 2005). In spite of these different results, in some studies (Carmina et al., 2006a, c; Vural et al., 2005), but not all (Vrbikova et al., 2005), the MS has been shown to be more prevalent in European PCOS patients than in controls of similar ethnicity and age.

For instance, in Italy, where women with PCOS have a lower mean body weight and less frequently increased serum TG than US PCOS, MS is less common but still 4 times more frequent in PCOS patients than in the general female population of similar age. Patients with mild PCOS phenotype (ovulatory PCOS) have a lower prevalence of MS but, in these patients too, MS is twice more frequent than in the normal population (Carmina, 2006c). Carmina et al. (2006a) sought to determine the prevalence of MS in Italian women using both the ATP-III and the WHO criteria. Using ATP-III criteria, the prevalence of MS was 8.2% and, using WHO criteria, it was 16% in Italian women with PCOS, higher than in controls, where the prevalence was 2.4% using both methods. Regarding the influence of the way in which PCOS is diagnosed, the MS prevalence was higher (8.9% by ATP-III, 17.3% by WHO) in classic PCOS patients than in ovulatory PCOS (5% and 10.6% respectively). Body weight significantly modified prevalence rates (Carmina et al., 2006a). Furthermore, in a very recent study, the same researchers found a relatively low prevalence of MS (7.1%) in Mediterranean PCOS, but higher than in normoweight and BMI-matched controls (2.4% and 3.5%, respectively) (Rizzo et al., 2011). Additionally, in a Czech study, MS (defined by IDF adolescent criteria) was present in five adolescents (11,62%) with PCOS (defined according to the ESHRE criteria) in comparison with one healthy girl. When comparing the prevalence of adolescents with at least one feature of MS, there was no difference between PCOS (17 out of 43) and healthy controls (27 out of 48) (VrbÍková et al., 2010a). Moreover, using ATP III criteria this time, MS was detected in 28.7% of those 179 women with PCOS. The most frequent features were an increased WC, decreased concentration of HDL - C (both in 96%), and increased BP (88%). Increased TG (49%) and impaired fasting blood glucose or type 2 DM (37.3%) were less common (VrbÍková et al., 2010b).

Another study performed on Caucasian women also found an approximate 4-fold increase in the prevalence of MS in women with PCOS compared with the age-matched female population (33% by WHO, 37% by NCEP-ATP-III and 40% by IDF criteria in PCOS subjects, compared with 10% by NCEP-ATP-III and 13% by IDF in controls) (Cussons et al., 2008). A similar prevalence was found also in a German study using IDF criteria (Hahn et al., 2007, as

cited in Cussons et al., 2008). The most common individual component of the MS present in the PCOS group was an elevated WC, followed by reduced HDL, then insulin resistance indicated by the homeostasis model assessment of insulin resistance (HOMA-IR). The least prevalent individual component was an elevated fasting glucose (Cussons et al., 2008). The adverse metabolic milieu of the syndrome can affect not only adults but also adolescents with PCOS (Diamanti-Kandarakis et al., 2008, as cited in Kandaraki et al., 2009). This is extremely likely to generate an increased prevalence of MS in this age group of patients, too. To explore this intuitively plausible concept, a few studies have addressed the prevalence of MS in adolescents with PCOS and the results are contradictory (Coviello et al., 2006, Rossi et al., 2008, as cited in Kandaraki et al., 2009). In an Indian study, MS (defined by IDF criteria) was found in 46.2% of females with PCOS, with both adolescent and adults being similarly affected. Regarding the individual component of the MS, dyslipidaemia and elevated BP were more common than fasting glucose abnormalities in both the adolescents and adults groups (Bhattacharya, 2008). Furthermore, the first study from India utilizing the 2009 "joint interim criteria" reported recently that the adolescents with PCOS were reported to have 4.26 times more chances of developing MS compared to those without (Bhattacharya, 2011). The prevalence of MS, diagnosed by IDF criteria, was 4.7% in the studied Chinese adolescents with PCOS. Metabolic disorders were common in these adolescents as more than one third of them exhibited at least one component of MS. Central obesity and dyslipidemia were the two most common metabolic features, which was also the case in adult PCOS patients according to a previous study (Huang et al., 2010). And finally, compared with Caucasians and Chinese women in Westernized societies, mainland Chinese women with PCOS were found to have a low risk of MS, as, overall, the prevalence of MS was 6.4% and its presence does not vary across the specific four PCOS phenotypes, according to the 2003 Rotterdam consensus criteria (range of 2.3-12.2%) (Guo et al., 2010).

However, there are some important limitations that should be mentioned. Most of these studies have not included patients and controls properly matched for age and BMI. Thus, not only the presence of PCOS but also BMI differences may have contributed to the above findings. The ethnic variations in the rates of MS reported by different studies may be attributable not only to anthropometric differences between diverse ethnicity, but also to differences in the criteria used for PCOS diagnosis. The selection of PCOS patients was based on NIH criteria in studies from the United States, but mostly on Rotterdam criteria in European or other non-US countries. The patients fulfilling the NIH definition of PCOS are expected to be more severely metabolically affected than the patients selected by Rotterdam criteria. Consequently, the MS rates were lower in women with hyperandrogenism and polycystic ovarian morphology, but normal ovulation, as well as in those with anovulation and PCO, but normal androgen levels when compared with women with classic PCOS (Kandaraki et al., 2009). The NCEP-ATP III criteria have been implemented for the diagnosis of MS by the majority of worldwide PCOS studies. However, there was one study that has employed both the WHO (gives more importance to the presence of insulin resistance or glucose intolerance) and the NCEP ATP III criteria, and has proposed the former set as more discriminating and more appropriate for the evaluation of MS in PCOS women with lower degrees of obesity (Carmina et al., 2006a). Accordingly, another study reported a significantly higher MS prevalence in PCOS women than in controls when using the WHO criteria, while the NCEP criteria identified a considerably lower MS in PCOS women, and thus could not show any difference between PCOS women and controls (Vural et al., 2005).

However, in a more recent study carried on a mixed population of obese and non-obese Caucasian PCOS women, comparable rates of MS have been reported, irrespective of the criteria used for MS diagnosis (NCEP, the WHO or the IDF criteria) (Cussons et al., 2008). Last but not least, the use of the 2009 "joint interim criteria" has just begun. The retrospective design is another weakness of the majority of the available studies. Differences in the exclusion criteria of each study should be also considered in the interpretation of their varying results (Kandaraki et al., 2009).

5. Markers of sub-clinical cardiovascular disease

It has been shown that women with PCOS have high CV risk factors. Whether this risk means an increased clinical disease is still debatable (Sukalich & Guzick, 2003).

- Functional studies
  - Ventricular function
  - Arterial stiffness
  - Endothelial function
    - Vascular tone
      - Vascular reactivity
      - Vasoconstrictors: ET-1
      - Vadsodilators: products of nitric oxide
    - Markers of endothelial activation
      - Adhesion molecules (sVCAM, sICAM, E- and P-selectin), ADMA
    - Markers of coagulation/fibrinolysis
      - PAI-1, tPA, fibrinogen, trombomodulin, vWF
    - Markers of inflammation
      - hs-CRP, TNF-α , IL-1, IL-6, ferritine, homocysteine
    - Hormones and substrates with known vascular effects
      - Adiponectin, resistin, visfatin, FFA, leptin, ghrelin
- Morphological studies
  - Carotid wall thickness
  - Arterial calcification

Note: ET-1 = endothelin-1, sVCAM = soluble vascular cell adhesion molecules; sICAM = soluble intercellular adhesion molecule; ADMA = asymmetric dimethylarginine; PAI = plasminogen activator inhibitor-1; tPA = tissue plasminogen activator, vWF = vonWillebrand factor; hs-CRP = highly sensitive C reactive protein; TNF-α = tumor necrosis factor alpha; IL-1 = interleukin 1; IL-6 = interleukin 6; FFA = free fatty acid

Table 1. Studies of markers of subclinical cardiovascular disease

Atherosclerosis is a systemic process the main characteristics of which is chronic inflammation, a disorder that affects all vascular territories and modifies their structure and function, ultimately leading to arterial thrombosis. Due to the fact that there is a long latency phase before clinical symptoms manifest during the progression of this disorder, the possibility of assessing arterial function before angiographically detectable atherosclerotic plaques appear could have a significant role in the early detection and evaluation of the risk for CVD (Soares et al., 2009).

This early detection of CVD risk can be performed with the help of noninvasive methods that permit the assessment of arterial structure and function. Currently, several such approaches exist; echographic measurements of arterial elasticity, IMT and endothelial function are clinically useful techniques for assessing arterial structure and function (Table 1). As a first approximation to studying the translation of risk factors into disease, data are accumulating on "preclinical" CVD of the carotid and coronary arteries as well as changes in vascular function (Sukalich & Guzick, 2003).

5.1 Functional studies

Vascular damage plays a key role in the development and progression of atherosclerosis and has been evaluated as an early risk marker of atherosclerosis and an independent predictor of future cardiac events (Sasaki et al., 2011).

5.1.1 Ventricular function

Left ventricular (LV) diastolic dysfunction is an early manifestation of diabetic cardiomyopathy and atherosclerotic CVD and has been shown to identify hypertensive patients at increased risk of CV events (Cussons et al., 2006). Its aetiology is multifactorial and relates to coronary artery disease, hypertension, autonomic neuropathy, microangiopathy, dyslipidaemia, insulin resistance, endothelial dysfunction and oxidative stress (Aurigemma et al., 2004, as cited in Cussons et al., 2006). Specific myocardial mechanisms include altered cardiomyocyte substrate metabolism and bioenergetics, altered collagen metabolism, inflammation and fibrosis (Watts et al., 2003, as cited in Cussons et al., 2006). Ventricular systolic and diastolic dysfunction may be an early finding for coronary heart disease in patients with PCOS (Kosmala et al., 2008).

In a case-control, echocardiographic study, women with PCOS were found to have an increased isovolumetric relaxation time (IVRT), an index of early LV diastolic dysfunction, and lower ejection fraction (EF) compared with weight matched controls (Tiras et al., 1999, as cited in Cussons et al., 2006). These changes were linked to the presence of a non-restrictive type of diastolic dysfunction and LV stiffness in patients with PCOS. However, E/A ratio, and DT were not significantly different between patients with PCOS and control. A significant direct relationship between plasma insulin levels and IVRT was demonstrated in the PCOS group. These findings were consistent with another report showing an independent correlation between hyperinsulinaemia and LV mass (Orio et al., 2004, as cited in Cussons et al., 2006). The existing studies sustain the hypothesis that insulin resistance may contribute to myocardial dysfunction in PCOS. Moreover, one study also predicted impaired coronary flow reserve and decreased myocardial glucose utilisation in PCOS related to insulin resistance and type 2 DM (Iozzo et al., as cited in Cussons et al., 2006).

Orio et al. studied the prevalence of LV hypertrophy and diastolic filling and systolic performance by echocardiography in a selected cohort of young women with PCOS. These authors found that patients with PCOS had significantly higher interventricular septum, left ventricle posterior wall thickness, end-systolic volume and lower LV EF, E/A ratio than controls. Nevertheless, all patients had normal LV EF and only two out of 30 patients with PCOS had abnormal E/A ratio (Orio et al., 2004, as cited in Tekin et al., 2009). Yarali et al. assessed systolic and diastolic function in 30 women with PCOS and 30 controls with

echocardiography (Yarali et al., 2001, as cited in Sukalich & Guzick, 2003). They reported that patients with PCOS had slower mitral E velocity, a lower mitral E/A ratio and shorter IVRT than a group of healthy controls, suggesting that LV diastolic function was impaired in PCOS. However, in this study, patients with PCOS were older and had higher LDL cholesterol and BMI than those found in other patients (Tekin et al., 2009). Of note, they did not report BPs measures of the study population. Thus, it is unclear whether the impaired diastolic function could be attributed to presence of PCOS, per se, or to the inclusion of sicker subjects in PCOS group. On the contrary, Tekin et al. (2009) reported that there were no significant differences between patients with PCOS and control subject with respect to EF, mitral E/A, DT, IVRT and pulmonary velocity. They showed that the tissue doppler profiles of patients with PCOS and controls were also not significantly different. Topcu et al. assessed coronary flow reserve in patients with PCOS, similar in terms of age, BMI, total cholesterol, smoking status and BP with the women in the study of Tekin et al. (2009), by using echocardiographically determined colour Doppler flow mapping of left anterior descending artery. They ended up with normal coronary flow reserve in patients with PCOS who had no associated CV risk factors. They also noticed that mitral E velocity, mitral A velocity and E/A were not significantly different between patients with PCOS and controls (Topcu et al., 2006, as cited in Tekin et al., 2009). Selcoki et al.(2010) also suggest that there are no significant differences in certain conventional and tissue Doppler echocardiographic measures of cardiac function between patients with PCOS and control groups. However, their PCOS populations have low BMI, normal BP, low TG, LDL and total cholesterol. Furthermore, no significant differences were found in insulin, HOMA-IR, HDL, LDL and total cholesterol, and TG levels between the two groups. Hence, these echocardiographic findings support previous reports (Topcu et al., 2006, as cited in Tekin et al., 2009) Tekin et al., (2009) indicating that diastolic function is preserved in young patients with PCOS who have no associated CV risk factors. Risk reduction regimens may be particularly important to those with other recognized risk factors for CVD to herald the progression of the disease. Further studies with obese, older and dyslipidemic subjects are needed.

5.1.2 Arterial stiffness

One of the major contributing factors to CV morbidity and mortality in patients with hypertension is arterial stiffness. The reduced elasticity of central arteries may play an important role in the early changes that predispose to the development of major vascular disease (Sasaki et al., 2011). Reduced arterial compliance and increased arterial stiffness represent independent risk factors for CVD and may favour the development and progression of hypertension, LV hypertrophy, MI and heart failure (Safar &London, 2000, as cited in Soares et al., 2008).

The ankle–brachial index (ABI) – the ratio of ankle-to-brachial systolic blood pressure (SBP) – is a simple, non-invasive, reliable method to estimate the presence of peripheral arterial occlusive disease. The brachial–ankle pulse wave velocity (baPWV) is known to be a marker for both the severity of vascular damage and the prognosis of atherosclerotic vascular diseases in patients with hypertension, end-stage renal failure and DM. The carotid augmentation index (cAI), which is augmentation expressed as a percentage of the pulse pressure and influenced by the vascular tone of the small muscular arteries, is associated with the presence and severity of coronary artery disease, particularly in younger and

middle-aged male patients. Recently, these indices can be measured easily by simultaneous oscillometric measurement of pulse waves and they are widely used as arterial stiffness indices (Sasaki et al., 2011).

In a small case-control study, Kelly et al. reported increased pulse wave velocity of the brachial artery, but not of the aorta, in a young obese PCOS group (Kelly et al., 2002, as cited in Cussons et al., 2006). Similarly, Lakhani et al. demonstrated increased stiffness of both internal and external carotid arteries in woman with both PCOS and PCO (ultrasonographic polycystic ovaries alone) compared with controls (Lakhani et al., 2000, as cited in Cussons et al., 2006).), even if BP and BMI were taken into account. However, this study did not show an independent relationship between insulin resistance or other CV risk factors and arterial stiffness and, as the patients with PCOS studied by these investigators had higher BMIs and arterial pressures and basal insulinaemia than the remaining participants, it was unclear whether the presence of PCOS *per se*, as opposed to the comorbidities of this syndrome, was responsible for this difference. Pulse wave velocity over the carotid-femoral tract, another method for the assessment of arterial distensibility, also demonstrated unfavourable results for young obese women with PCOS in a previous study. A significant relationship between PWV and BP and between PWV and both insulin and glucose has been detected during a glucose tolerance test (Meyer et al., 2005a). However, some possible confounding factors, such as smoking, insulin resistance and hypertriglyceridaemia, were not excluded in this research and no adjustments were made for BP or BMI.

Moreover, in the study by Sasaki et al. (2011), women with PCOS had a significantly higher brachial–ankle pulse wave velocity (baPWV) than that of the controls whereas there was no significant difference in the carotid augmentation index (cAI) as well as ABI between the two groups. There was no significant difference in age or BMI between the controls and the women with PCOS. These women with PCOS had a significantly higher serum testosterone and CRP levels and showed insulin resistance and dyslipidemia. The mean BP in women with PCOS was within the normal range, but still significantly higher than those in the controls. Stepwise multiple regression analysis revealed that BP influences the baPWV in women with PCOS. Arterial stiffness evaluated using the baPWV in mildly-hypertensive women (SBP ≥120 mmHg or DBP ≥90 mmHg) with PCOS was significantly higher than that in the controls or normotensive women with PCOS, suggesting early changes in vascular function in mildly-hypertensive women with PCOS. Furthermore, among the women with PCOS, obese women had significantly higher baPWV as compared to normal-weight women although no significant difference was observed in the ABI and cAI. Hence, it appears that obesity and elevation of BP within subclinical levels in women with PCOS may have adverse effects on vascular functions (Sasaki et al., 2011).

In view of these results, PCOS has been suggested to be associated with arterial stiffening. The question remains whether the risks conferred by obesity and other CV risk factors, commonly found in PCOS subjects and PCOS, are additive. The drawback of other studies that have evaluated nonobese women with PCOS (Arikan et al., 2009, Orio et al., 2004) is the fact that they did not analyse these markers simultaneously so they did not identify those linked specifically with the presence of PCOS and not simply triggered by the metabolic dysfunctions so common in these patients. Thus, Soares et al. (2008) evaluated the subclinical markers of CVD (echographic and serum markers of chronic inflammation) in nonobese women with PCOS but without comorbidities. Common carotid artery stiffness

index (β) (CCA β) was higher in PCOS than in control women and CCA distensibility was lower, indicating that young women with PCOS exhibit changes in vascular elasticity even in the absence of classical risk factors for CVD, such as hypertension, obesity and type 2 DM. These data suggest that patients with PCOS may be at higher risk for CVD, because their arterial distensibility is already reduced during atheroma formation and thus represents a subclinical sign of atherosclerosis (Mattsson et al., 2008, as cited in Soares et al., 2008). However, as the women from this study did not present with hyperglycaemia or hyperinsulinaemia, the increased stiffness index and the reduced distensibility of the carotid artery detected in PCOS women might be attributed to the hyperandrogenism inherent to PCOS and not to comorbid conditions associated with the syndrome (Soares et al., 2008).

On the contrary, and sustaining the results of Muneyyirci-Delate et al., Ketel et al. (2010) demonstrated in a small study that (central) obesity, but not PCOS, is associated with greater arterial stiffness (Muneyyirci-Delate et al., 2007, as cited in Ketel et al., 2010). Beside the fact that most of the studies have not distinguished the effects of obesity from those of PCOS, they also have not taken into consideration the fact that stiffness of elastic (e.g. the carotid) and muscular (e.g. the femoral) arteries may differ in their association with obesity and potentially PCOS. The study of Ketel et al. (2010) is the first comprehensive study taking into account stiffness in both elastic and muscular arteries in both lean and obese women with and without PCOS. Previous investigations, which investigated one type of artery, pulse wave velocity over elastic and (or) muscular vascular regions, or small and large arterial compliance, showed somehow discordant results. Nevertheless, in their research, Ketel et al. (2010) demonstrated that PCOS was not associated with stiffening of either muscular or elastic arteries.

In conclusion, current studies suggest that differences in vascular function between normal women and women with PCOS may be very small if both are young, of normal weight and have normal BP. However, they showed that slightly increased BP, which weakly correlated with BMI, can be rightfully considered a risk factor for arterial stiffness in young women with PCOS. It has not been yet established whether these adverse effects of mild hypertension on arterial stiffness are characteristic to women with PCOS. We thus need further research on arterial stiffness in young women with mild hypertension caused by the other diseases (Sasaki et al., 2011). The mechanism for increased arterial stiffness reported in some studies of PCOS have not been cleared but, as in the metabolic syndrome and insulin resistance, may involve endothelial dysfunction and altered artery wall collagen metabolism (Cussons et al., 2006).

5.1.3 Endothelial dysfunction

Endothelial dysfunction can be defined as the partial or complete loss of balance between vasoconstrictors and vasodilators, growth promoting and inhibiting factors, pro-atherogenic and anti-atherogenic factors, and pro-coagulant and anti-coagulant factors. Endothelial dysfunction is the initiating event in the development of atherogenesis and has been shown to precede the development of clinically detectable atherosclerotic plaques in the coronary arteries, being also implicated in the development of microvascular complications in diabetes (Caballero, 2005). Therefore, the assessment of endothelial function by different methods has emerged as a tool for detection of preclinical CVD. A common approach to the evaluation of endothelial function is the assessment of blood flow and vascular reactivity which allows the investigator to evaluate the status of the endothelial cells as well as that of

the underlying vascular smooth muscle cells. A lot of invasive and noninvasive techniques are available for evaluating these functions such as catheterization, ultrasound, positron emission tomography, laser Doppler flowmetry, and plethysmography (Caballero, 2005).

Measurement of post-ischemic flow mediated dilatation (FMD) of the brachial artery with high resolution ultrasonography is an established method to assess endothelial function of conduit arteries and correlates with measures of coronary endothelial dysfunction (Meyer et al., 2005b). Most of the studies performed in women with PCOS showed altered endothelial function in women with this disorder either using non-invasive methods (endothelium-dependent FMD or endothelium-independent, glyceroltrinitrate (GTN)-mediated dilatation vascular responses of the brachial artery) or invasive methods (Orio et al., 2004; Kravariti et al., 2005; Diamanti-Kandarakis et al., 2005, 2006a; Tarkun et al., 2004; Sorensen et al., 2006; Dagre et al., 2006) as compared to the control group. Impaired FMD is associated with insulin resistance, total cholesterol and CRP levels, while relationship to testosterone levels is controversial (Cussons et al., 2006; Kravariti et al.; Tarkun et al., 2004). However, it is not clear yet if the endothelial dysfunction found in women with PCOS is independent of obesity and insulin resistance that characterize so often this disorder. Therefore, Cussons et al. (2009) performed a study in nonobese, noninsulin resistant women with PCOS, assessing endothelial function (with FMD of the brachial artery) and arterial stiffness (with PWV and augmentation index (AI)). Although there were no significant differences between PCOS and control subjects in terms of BMI, BP, HOMA-IR, lipids, oestradiol and markers of arterial stiffness, the PCOS subjects had significantly lower FMD of the brachial artery compared with the controls. On the contrary, others suggested that middle-aged patients with PCOS display signs of endothelial dysfunction in comparison to age-matched controls, but that this is largely due to the increased prevalence of independent risk factors for CVD found in this group (Hudecova et al., 2010). On the other hand, there are also reports which do not sustain an association between PCOS and impaired endothelial function (assessed by FMD of the brachial artery) (Arikan et al., 2009; Brinckworth et al., 2006; Mather et al., 2000). Hence, Arikan et al. (2009) could not find a deteriorated endothelial function in young nonobese women with PCOS in spite of a significant insulin resistance in PCOS patients. They suggest that the existence of insulin resistance alone may not be an adequate factor for deterioration of endothelial function and carotid IMT in young, nonobese patients with PCOS. Other factors such as duration of insulin resistance, older age, presence of obesity, and inflammatory markers may play an important role in this process.

Endothelial dysfunction of the microcirculation and resistance vessels in PCOS has also been proved by invasive studies employing intra-arterial infusion of vasoactive agents with thermodilutional or plethysmographic assessment of limb blood flow. In a study by Paradisi et al., leg blood flow (LBF) was measured by means of an intravenous thermodilution catheter in a group of obese women with PCOS showing impaired LBF responses to methacoline and to hyperinsulinemia during an euglycaemic clamp compared with the control group. However, it should be mentioned that LBF response to sodium nitroprusside, an endothelium independent vasodilator, was not assessed, meaning that the demonstrated defect could not necessarily be localized to the endothelial cell as opposed to smooth muscle. Impaired endothelium-dependent vasodilatation was related to both androgen levels and insulin resistance (Paradisi et al., 2001). Using the same techniques, an improvement in the endothelial dysfunction was noted after 3 months of treatment with rosiglitazone in PCOS subjects compared with controls and this improvement was

associated with a decrease in insulin resistance, testosterone and PAI-1 level (Paradisi et al., 2003). In an ex vivo study, the contractile response of gluteal resistance arteries to norepinephrine before and after incubation with insulin was measured, thus confirming the above results, namely that there is a resistance to the vasodilator effects of insulin in PCOS (Kelly et al., 2002), which proved to be present even in the absence of other risk factors. The defect in the resistance arterioles revealed in this study appears to be specific to the action of insulin, as constriction to norepinephrine and relaxation to Ach were not different between the PCOS group and the control group. In contrast, Bickerton et al.(2005), using venous occlusion plethysmography to assess differences in reactive hyperemia of the forearm microcirculation, found no evidence of endothelial dysfunction in women with PCOS compared with age- and weight-matched controls without PCOS. Carmassi et al. (2005), who studied PCOS women with and without insulin resistance, demonstrated a blunted vasodilatory response to insulin and an abolished expression of tissue plasminogen activator (t-PA) exclusively in insulin-resistant patients, suggesting that insulin resistance, rather than PCOS status, confers endothelial dysfunction in this vascular bed.

The measurement of plasma levels of several markers of endothelial activation (e.g. soluble vascular cell adhesion molecules (sVCAM), soluble intercellular adhesion molecule (sICAM), E- and P-selectin), coagulation and/or fibrinolysis (e.g. PAI-1, tPA, fibrinogen, von Willebrand factor), vascular tone (e.g. ET-1, products of nitric oxide), inflammation and other products, cytokines and hormones associated with vascular function also represent common approaches to evaluate endothelial function in humans (Caballero, 2005) (Table 1).

Endothelin-1 has endothelial mitogenic effects (Dubin et al., 1989, as cited in Ilie et al., 2008) and seems to play a role in the early events of endothelial dysfunction. Therefore, it can be used as a marker of abnormal vascular reactivity. Diamanti-Kandarakis et al. were the first ones to demonstrate elevated ET-1 levels in PCOS compared with controls. A positive correlation of ET-1 with free testosterone levels was shown, as well as a negative correlation of ET-1 with glucose utilization, which might indicate an involvement of hyperinsulinemia and insulin resistance as well as of hyperandrogenemia in the abnormal endothelial status (Diamanti-Kandarakis et al., 2001). It appears that early impairment of endothelial structure and function is present even in young women with PCOS as suggested by significantly higher ET-1 levels in normal-weight, non-dyslipidemic and non-hypertensive women with PCOS than in BMI-matched healthy controls (Orio et al., 2004). The metformin therapy appeared to have beneficial effects, decreasing ET-1 levels (Diamanti-Kandarakis et al., 2001; Orio et al., 2005, as cited in Ilie et al., 2008). Consistent with others results, we also clearly demonstrated in a previous report that young, euglycaemic, eulipidemic and normotensive women with PCOS have evidence of endothelial dysfunction as assessed by both hemodynamic (FMD) and biochemical methods (ET-1). Impaired vascular reactivity and increased level of ET-1 were linked to markers of hyperandrogenemia in our study (Ilie et al., 2011). Interestingly, visfatin, an adipokine predominantly expressed in, and secreted from visceral adipose tissue, has also been shown to play a role in the pathogenesis of endothelial dysfunction in PCOS, independent of additional risk factors, since circulating visfatin was significantly related to brachial artery FMD and free testosterone (Pepene, 2011).

In healthy vessels, an infusion of an endothelium-dependent vasodilatator, such as acetylcholine, induces a nitric oxide (NO)-mediated vasodilatory response; however, in patients with endothelial dysfunction, this effect is blunted or paradoxical vasoconstriction

may occur (Verma et al., 2003, as cited in Ilie et al., 2008). Nacul et al. are the first who evaluated the concentration of nitrite/nitrate (as index of endothelium-derived NO) in a PCOS group compared to an age-matched control group and found similar levels of nitrite/nitrate and fibrinogen in the two groups. A negative, BMI-independent correlation between NO and insulin resistance was seen in PCOS patients only, but no association was observed between NO and BMI, WC, WHR or androgens suggesting that in PCOS endothelial dysfunction is related to the presence of insulin resistance (Nacul et al., 2007). Moreover, in a very recent study, Battaglia et al. (2010) demonstrated that young women with PCOS displayed levels of nitrite/nitrate within normal range and similar with those found in controls, which preserved the endothelium-dependent vasodilatation, which was also similar in both groups. There are also reports which, on the contrary, found lower levels of nitrites/nitrates in PCOS and subjects with polycystic ovaries (Battaglia et al., 2008) as well as in normal weight, eumenorrheic, nonhirsute daughters of patients with PCOS (Battaglia et al., 2009) compared with controls, which suggests that PCOS is a condition associated with an increased vascular risk.

Overall, data from the literature support the presence of endothelial dysfunction in women with PCOS, including those who are young and apparently not displaying CV risk factors. So far, insulin resistance appears to be the major responsible factor related to endothelial dysfunction in PCOS subjects. Additionally, there are also reports indicating an association of endothelial dysfunction with obesity, hsCRP and, less consistently, with hyperandrogenemia. The slight differences among the obtained results might be explained either through the different methodology used for the investigation of vascular reactivity and endothelial dysfunction, or through the different metabolic status of the recruited patients. Additionally, small sample sizes, bias in case-control designs, and the use of different criteria to define PCOS might also contribute to discrepancies amongst some studies.

5.2 Morphological studies

The two major anatomic markers for sub-clinical cardiovascular disease are carotid IMT, assessed by ultrasonography or angiography and coronary artery calcifications (CAC) evaluated by electron beam tomography (Essah et al., 2007). Increased carotid IMT has been reported to occur relatively early in the atherosclerotic process and has been linked with traditional CV risk factors, such as increasing age, obesity and adverse lipid profile, as commonly observed in PCOS (Talbott et al., 2004a). Several studies demonstrated the role of carotid IMT in predicting coronary and cerebrovascular events, with increasing carotid IMT associated with an elevated age-adjusted cardiovascular risk (Cussons et al., 2006). In particular, Talbott et al. demonstrated a difference in carotid IMT between middle-aged women with PCOS (≥45 years) and age-matched controls, but not in younger women (group aged 30 to 45 years) (Talbott et al., 2000a). However, there are also reports showing significantly higher carotid IMT values also in young, normal weight PCOS women compared with controls (Carmina et al., 2006b; Orio et al., 2004, Luque-Ramirez et al., 2007). Increased carotid IMT was found to be related to insulin resistance and lower adiponectin levels (Carmina et al., 2006b) or correlate with free androgen index or TT (Costa et al., 2008; Orio et al., 2004; Luque-Ramirez et al., 2007) and DHEAS (Pamuk et al., 2008), thus suggesting a contribution of hyperandrogenemia to the progression of atherosclerosis in PCOS (Ilie et al., 2008). By contrast, other studies showed that increased carotid IMT was inversely correlated with plasma DHEAS and androstendion levels suggesting an intriguing

vasculoprotective effect of hyperandrogenemia in PCOS. However, further researches are needed to confirm whether elevated DHEAS actually protects against atherogenesis in PCOS (Cussons et al., 2006). As inflammation has been implicated as a novel risk factor in the development and progression of atherosclerosis, the same Talbott et al. investigated the elevated CRP, as a marker of inflammation, as a possible determinant of increased IMT in middle-aged women with PCOS. They noted, however, that although CRP was significantly higher in PCOS patients than in controls, it did not appear to appreciably mediate the influence of PCOS on IMT (Talbott et al., 2004b). On the contrary, in another study, elevated serum IL-18 levels were associated with greater carotid IMT in patients with PCOS, suggesting a link between IL-18 and carotid atherosclerosis in patients with this disorder (Kaya et al., 2009). In line with other studies (Arikan et al., 2009; Costa et al., 2008; Meyer et al., 2005a), we could not find evidence of altered arterial structure in our group of young subjects with PCOS, as compared to controls (Ilie et al., 2011).

Coronary artery calcifications, both radiographic markers for coronary atherosclerotic plaque and predictors of clinical events were found more prevalent in premenopausal obese women with PCOS aged 30-45 years in comparison to controls (Christian et al., 2003, as cited in Ilie et al., 2008). In a nine year follow-up study, an increased incidence of CAC was described in middle-aged women with PCOS; the degree of calcification was dependent on central obesity, elevated BP, dyslipidemia and thus insulin resistance (Talbott et al., 2004a). Furthermore, the prevalence of coronary artery calcium (CAC) was significantly higher also in young, obese women with PCOS, as compared with age and weight matched controls, even though the majority of subjects with detectable coronary artery calcification did not have traditional CV risk factors. The finding supports the idea that the presence of PCOS status per se appeared to contribute to this increased risk of CAC (Shroff et al., 2007). These reports suggest that women with PCOS have morphological evidence of coronary atherosclerosis and that insulin resistance, in particular, is the major causal factor. While the morphological changes in the vascular wall seem to be more evident in middle-aged and older women with PCOS, endothelial dysfunction is more likely a feature of the vasculopathy of young women with PCOS. This is not surprising, as endothelial dysfunction has been shown to occur earlier in the atherosclerosis development and progression, preceding the onset of increased carotid IMT (Meyer et al., 2005a, b).

5.3 Low-grade chronic inflammation

Recent evidence has been focused on a state of low-grade chronic inflammation, which has been described in PCOS as a potential link between hyperandrogenism, insulin resistance or abdominal adiposity and the metabolic and CV long-term complications of the syndrome.

Low-grade inflammation can be defined as a condition characterized by increased circulation levels of several mediators of inflammation triggered by a noxious stimulus, such as classic molecules (TNF-α, IL-1, IL-6, CRP), or white blood cell (WBC) count (Repaci et al., 2011). Obesity, particularly the visceral phenotype, has been defined as a state of low-grade inflammation because visceral adipose tissue is able to produce cytokines (TNF-α, IL-6, and IL-1), chemokines (interferon-inducible protein-10 (IP-10), IL-8, IL-18, monocyte chemotactic protein-1 (MCP-1)) and other adipokines (FFA, PAI-1, leptin, resistin, visfatin, and adiponectin) that act, directly or indirectly, as mediators of systemic inflammation. Interestingly, the adipo-cytokines and, overall, the chronic inflammatory state associated

with obesity are also related to the insulin resistance state (Sell & Eckel, 2009, as cited in Repaci et al., 2011), type 2 DM or CVD due to the development of hypertension, dyslipidemia, and endothelial dysfunction (Yudkin et al., 1999, as cited in Repaci et al., 2011) (Fig. 2). Furthermore, accumulating evidence suggests that atherosclerosis represents a chronic inflammatory process and that the inflammation at the level of the vessel wall plays a pivotal role in atherosclerotic lesion formation, progression, and eventual rupture.

There is much evidence sustaining that high levels of IL-6, TNF-α, and leptin, and reduced levels of adiponectin inhibit the expression and activity of nitric oxide synthase (eNOS), leading to decreased NO synthesis and reduced vasodilation and consequently promoting endothelial dysfunction (Rizvi, 2007, 2009, as cited in Repaci et al., 2011) (Fig. 2). The release of cytokines and chemokines, such as TNF-α, MCP-1, and IL-18 has been demonstrated to promote the recruitment of macrophages from the bloodstream which, through the production of adhesion molecules, in turn accelerate adhesion, arrest, and diapedesis of inflammatory cells through the endothelium with the consequent infiltration of the lipid core. These inflammation mediators also promote the proliferation of smooth muscle cells and their migration from the tunica media to the tunica intima (Sengenès et al., 2007, as cited in Reapaci et al., 2011), leading to the formation of atherosclerotic plaque. Hence, chronic inflammation is responsible of early and late atherosclerotic processes.

5.3.1 Classic and non-classic markers of low-grade inflammation in PCOS

A classic and widely studied marker of low-grade inflammation is CRP, which has been shown to significantly predict the incidence of peripheral vascular diseases, endothelial dysfunction, MI, stroke, and sudden death (Ridker, 2003, as cited in Repaci et al., 2011). Recent data suggests that CRP is not only an inflammatory marker of atherosclerosis, but it may have some direct deleterious effects in the vascular wall, contributing to the pathogenesis of lesion formation, plaque rupture, and coronary thrombosis by interacting with and altering the endothelial cell phenotype (Verma et al., 2003, as cited in Ilie et al., 2008). CRP may directly promote endothelial dysfunction by increasing the expression of endothelial cell adhesion molecules (CAMs), the MCP-1 secretion and macrophage LDL-uptake. Also it appears that CRP directly influences the production of endothelium-derived vasoactive factors: it decreases the NO production while augmenting production of the potent endothelium-derived vasoconstrictor ET-1 as well as of PAI-1 (Devaraj et al., 2003, as cited in Ilie et al., 2008). Additionally, CRP facilitates endothelial cell apoptosis and attenuates angiogenesis. The most published data, but not all (Capoglu et al., 2009; Mohlig et al., 2005; Shroff et al., 2007), demonstrate increased levels of hsCRP in women with PCOS (Diamanti-Kandarakis et al., 2006a, b; Talbott et al., 2004b; Tarkun et al., 2004). However, many studies, including our previous report, have demonstrated that in women with PCOS the CRP values are primarily dependent to upon co-existent obesity and fatty mass or insulin resistance (Kelly et al., 2001, as cited in Ilie et al., 2008; Benson et al., 2008, as cited in Repaci et al., 2011; Ilie et al., 2011), rather than to PCOS status per se. Hence, increased serum CRP levels were found in obese and non-obese PCOS compared to controls (Tarkun et al., 2004; Talbott et al., 2004b; Kelly et al., 2001, Orio et al., 2005, as cited in Ilie et al., 2008) with significantly higher values in obese than in non-obese PCOS subjects (Morin-Papunen et al., 2003), decreasing after metformin (Morin-Papunen et al., 2003). Regarding hyperandrogenemia, in PCOS subjects, either no correlation (Tarkun et al., 2004; Puder et al., 2005) or a positive association between increased androgen levels and indices of chronic

inflammation (Diamanti-Kandarakis et al., 2006a, b) were described. Diamanti-Kandarakis et al. (2006a) demonstrated that serum CRP concentrations were positively related to BMI and negatively related to FMD values and insulin sensitivity indices. Furthermore, FMD was statistically higher in PCOS population with CRP≤1mg $^{-1}$ when compared with PCOS population with CRP≥1mg $^{-1}$. The finding that the increased levels of CRP are consistent with the severity of endothelial dysfunction supports the hypothesis that chronic inflammation contributes to endothelial dysfunction (Diamanti-Kandarakis et al., 2006a).

Other classic markers of low-grade inflammation, which are significantly increased in PCOS, although not constantly (Shroff et al., 2007) are IL-18 (Escobar-Morreale et al., 2004, as cited in Repaci et al., 2011; Kaya et al., 2008), TNF-α and IL-6 (Gonzalez et al., 1999, as cited in Repaci et al., 2011; Tarkun et al., 2006). Like CRP, these cytokines have been related to the risk of developing type 2 DM and CVD and are strongly associated with insulin resistance and body fat amount (Repaci et al., 2011; Tarkun et al., 2006).

Accordingly, an increase of WBCs, particularly lymphocytes and monocytes (Orio et al., 2005a, as cited in) or neutrophils (Ibanez et al., 2005, as cited in Ilie et al., 2008) which have been demonstrated to be strongly associated with insulin resistance (Orio et al., 2007; Ibanez et al., 2005) has also been noted in PCOS. Metformin and the combination flutamide-metformin had a beneficial effect on low-grade inflammation, attenuating WBC (Orio et al., 2007, as cited in Ilie et al., 2008) and hyperneutrophilia (Ibanez et al., 2005, as cited in Ilie et al., 2008), that further supports a link between hyperleukocytosis and insulin resistance (Ilie et al., 2008). A high expression of other classic markers, such as IL-6, MCP-1 and matrix metallopeptidase-2 (MMP2), have also been described in PCOS. Whether this relationship may be attributable to increased adiposity, particularly to the visceral phenotype, or, conversely, may depend on other factors is, however, still debatable (Vgontzas et al., 2006; Hu et al., 2006; Lewandowki et al., 2006; Orio et al., 2004b; Gonzalez et al., 2006a,b, 2009; Glintborg et al., 2009, as cited in Repaci et al., 2011).

Endothelial dysfunction triggers the expression of cell adhesion molecules on the surface of endothelial cells and leukocytes to handle the intricate process of leukocyte rolling, adhesion, and transmigration into the sub-intimal space. Last but not least, in several studies, PCOS has been shown to associate with high levels of adhesion molecules, such as sICAM-1, sVCAM-1, ET-1, and sE-selectin, (Ley and Huo, 2001; Pai et al., 2004; Meigs et al., 2004; Roldan et al., 2003; Corti et al., 2004; Kowalska et al., 2002; Kadoand Nagata, 1999; Diamanti-Kandarakis et al., 2006b, as cited in Repaci et al., 2011). An interesting fact reported was that all these adhesion molecules were positively associated not only with insulin resistance (Diamanti-Kandarakis et al., 2006b), but also with the degree of hyperandrogenemia in PCOS (Diamanti-Kandarakis et al., 2001, 2006a). This association makes androgen excess a potential factor in the development of endothelial dysfunction. This hypothesis is confirmed by a recent in vitro study which proved that high levels of dihydrotestosterone favored the development of atherosclerotic lesions by inducing the expression of sVCAM on endothelial cell surface through a NF-κB dependent mechanism, which finally induces the adhesion of monocytes to endothelial cells (Death et al., 2004).

Among the non-classic markers of low-grade inflammation, increased levels of ferritin have been described in obesity, type 2 DM and the MS (Fernandez-Real et al., 2002, as cited in Repaci et al., 2011) as well as in PCOS, where it was associated with obesity and insulin excess (Escobar-Morreale et al., 2005, Botella-Carretero et al., 2006; Luque-Ramirez et al.,

2007b, as cited in Repaci et al., 2011). Osteoprotegerin (OPG) has both anti-inflammatory and antiapoptotic effects, particularly at the level of endothelial cells, where it exerts a protective role in the development of atherosclerotic plaque and plaque rupture (Scatena and Giachelli, 2002; Schoppert et al., 2002; Kiechl et al., 2007; Kim et al., 2005, as cited in Repaci et al., 2011). Reduced levels of OPG have been observed in obesity and in the presence of insulin resistance (Ugur-Altun et al., 2005; Holecki et al., 2007, as cited in Repaci et al., 2011), as well as in PCOS (Escobar-Morreale et al., 2008), where they were related to the androgen excess (Escobar-Morreale et al., 2008). Interestingly, we have demonstrated very recently that circulating OPG was negatively and significantly correlated with FMD and, moreover, that PCOS women in the upper OPG quartile group (> 2.65 pmol/l) presented significantly altered endothelial function compared to all the rest. Nevertheless, it is unclear whether the relationship between OPG and FMD found here reflects a compensatory response of the endothelium to injury or if it should rather be regarded as a direct involvement of OPG in the pathogenesis of endothelial dysfunction. In previous studies, elevated plasma OPG concentrations, reported in several states associated with vascular damage, were negatively related to endothelium-dependent arterial dilation in newly diagnosed diabetes and hypothyroidism (Shin et al., 2006, Rasmussen et al., 2006, Guang-da et al., 2008, as cited in Pepene et al., 2011). Coming back to our results, since patients with important insulin resistance and severely impaired FMD presented with the highest OPG levels, we might speculate that in PCOS, OPG may link disturbances in insulin sensitivity to impaired endothelial dysfunction and progression of atherosclerosis, independently from hyperandrogenemia. Moreover, confirming previous results (Escobar-Morreale et al., 2008) that suggest a deleterious effect of androgen excess on OPG, the latter was negatively related to FT in our study (Pepene et al., 2011). Finally, PCOS has been associated with high levels of advanced glycation endproducts (AGEs), which is described to act as oxidants and inflammatory mediators as well as increased levels of platelet mean volume (MPV), an indicator of platelet activation and aggregation (Repaci et al., 2011).

5.3.2 Androgen excess and low-grade chronic inflammation

As far as the relationship between androgen excess and low-grade chronic inflammation is concerned, there are still debates whether molecules involved in the low-grade inflammatory state are involved in the pathogenesis of hyperandrogenemia or, conversely, whether androgen excess might promote the inflammatory state. Either no correlation (Tarkun et al., 2004; Puder et al., 2005; Cascella et al., 2008; Tosi et al., 2009) or a positive association between increased androgen levels and indices of chronic inflammation (Diamanti-Kandarakis et al., 2006a, b) were described in PCOS subjects. The in vitro studies support the hypothesis that the state of low-grade inflammation influences androgens, showing that TNF-α stimulates the proliferation and steroidogenesis of theca cells (Spaczynski et al., 1999, as cited in Repaci et al., 2011), whereas IL-6 is capable of stimulating human adrenal cells, thereby increasing adrenal steroidogenesis (Path et al., 1997, as cited in Repaci et al., 2011).

In spite of the fact that the role of androgens in low-grade inflammation is yet to be demonstrated, vast evidence has accumulated to suggest that androgens could, however, play an indirect role in the development of low-grade inflammation through the impact on the adipose tissue. In fact, androgens seem to have a stimulatory effect on the hypertrophy of adipocytes by influencing the expression of enzymes and proteins involved in lipid and

carbohydrate metabolism, in oxidative stress and in the differentiation of pre-adipocytes into mature adipocytes. Additionally, androgens enhance lipolysis, determining an increased release of FFA (Cortón et al., 2007, Xu et al., 1991, as cited in Repaci et al., 2011). Briefly, there still are aspects that need to be cleared regarding the bidirectional relationship between low-grade inflammation and androgen excess in PCOS, and much more research should, thus, be performed in this exciting area.

Several polymorphisms of cytokines have been studied for the possible association with PCOS. In particular, Escobar-Morreale et al. showed the association between one polymorphism at the level of the promoter of TNF-α [−308 A] and increased androgen circulating levels in both hyperandrogenic and healthy women, independent of the presence of obesity and insulin resistance (Escobar-Morreale et al., 2001).

Additionally, some studies have demonstrated the association between adipokines and androgens. One of these adipokines is visfatin, whose mRNA levels are increased in the adipose tissue of women with PCOS (Panidis et al., 2008, as cited in Repci et al., 2011; Carmina et al., 2009) and who has recently been shown to be positively associated with LH, androstenedione, and free testosterone levels, and negatively with SHBG (Panidis et al., 2008; Kowalska et al., 2007; Gen et al., 2009, as cited in Repaci et al., 2011; Pepene CE, 2011). Adiponectin and leptin are another two adipokines involved in the mediation of systemic inflammation, whose relationship with androgens is the subject of conflicting results (Page et al., 2005; Seftel, 2005; Yilmaz et al., 2009, as cited in Repaci et al., 2011). Regarding leptin in particular, some studies failed to demonstrate any relationship with androgens (Vicennati et al., 1998; Pehlivanov and Mitkov, 2009; El Orabi et al., 1999, as cited in Repaci et al., 2011), whereas others showed a positive relationship between leptin circulating levels and androgens (Hislop et al., 1999; Castrogiovanni et al., 2003; Pardo et al., 2004, as cited in Repaci et al., 2011). More convincing results have been obtained by the analysis of the ratio adiponectin/leptin, considered a good marker of a state of inflammation in PCOS (Xita et al., 2007), that was found to be positively related with SHBG. Reduced adiponectin (Carmina et al., 2006b), and increased leptin (Gomez-Ambrosi et al., 2002, as cited in Repaci et al., 2011) may also contribute to the development of endothelial dysfunction in PCOS, as shown by some reports. Finally, circulating levels of ghrelin and androgens, particularly androstenedione, have been found to be negatively connected in women with PCOS. These results have been further confirmed by the observation that a short-term treatment with flutamide, a drug with anti-androgenic properties, triggered a significant increase in ghrelin circulating levels (Glintborg et al., 2006; Pagotto et al., 2002; Panidis et al., 2005; Gambineri et al., 2003, as cited in Repaci et al., 2011).

In conclusion, PCOS is associated with a low-grade of chronic inflammation mainly attributable to the accumulation of visceral fat, although an effect of insulin resistance cannot be excluded. The impact of hyperandrogenemia can be restricted so far to the influence of androgens on the development and distribution of the adipose tissue, which is, in fact, a true endocrine gland. A vicious circle is established with a continuous release of inflammatory mediators that are responsible for the development of insulin resistance, dyslipidemia, endothelial dysfunction, and metabolic and CV long-term complications. In addition, through the impact on the regulation of the synthesis and secretion of androgens in the ovary and in the adrenal, the state of low-grade inflammation also seems able to contribute to the maintenance of the syndrome (Repaci et al., 2011).

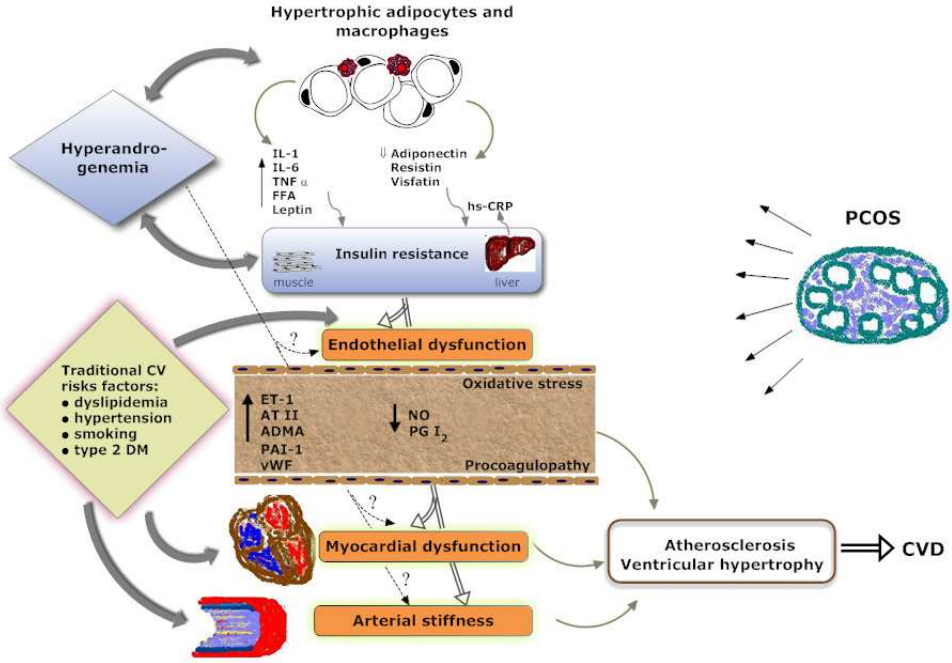

Fig. 2. Some hypothetical pathways for the development of cardiovascular disease in women with PCOS

Several traditional and non-traditional cardiovascular risk factors commonly met in PCOS play a role either directly or indirectly by developing insulin resistance (in the liver and the muscles). The latter definitely has a major influence, beside other mechanisms, in the development of subclinic cardiovascular disease, eventually leading to cardiovascular disease. The hypertrophic adipocytes secrete various molecules, including inflammatory markers and adipokines, which may, in turn, determine the development of insulin resistance, an increase of the hepatic production of hsCRP, an increase of ovarian and adrenal androgens production which may further aggravate central adiposity distribution and may even directly act to determine vascular injury. It is not, yet clear if androgen excess (the marker of PCOS) has a direct influence in the development of atherosclerosis and preclinic cardiovascular disease. TNF-α = tumor necrosis factor alpha; IL-1 = interleukin 1; IL-6 = interleukin 6; PAI-1 = plasminogen activator inhibitor-1; IL-18 = interleukin 18; FFA = free fatty acids; hsCRP = highly sensitive C reactive protein; ET-1=endothelin-1; AT II= angiotensin II; ADMA= asymmetric dimethylarginine; vWF= vonWillebrand factor; NO=nitric oxide; PG I2 = prostacyclin; CVD=cardiovascular disease.

6. Oral contraceptives may further exacerbate the metabolic profile of women with PCOS

Since its introduction in 1960, the combined oral contraceptive (COC) pill has become one of the most widely and frequently used methods of contraception worldwide. COC also have a long history of use in patients with PCOS, where they are prescribed for obtaining a regular

menstrual cycle and for improving the clinical signs of hyperandrogenism. Although highly effective, COC, in particular early formulations, were associated with significant adverse effects and unacceptable CV risk. In addition, a decade after the advent of oral hormonal contraception, epidemiologic research confirmed an increased risk for cardiovascular events, particularly venous thromboembolism (VTE), in women using the high-dose COCs or COCs containing the newer gonane progestins desogestrel and gestodene (commonly referred to as "third-generation" progestins) (Burkmana et al., 2011). Research indicates also that COC use is associated with an increased risk of MI, particularly in women who smoke and are older than 35 years and in those who have underlying risk factors for coronary artery disease, such as hypertension (Burkman et al., 2011). Different estrogens and progestins exert different metabolic and CV effects. Estrogen component is almost always ethinyl estradiol (EE) in doses ranging from 15 to 50 μg. The progestin component is of variable potency and androgenicity. Hence, while in combined COCs the EE component seems to be responsible for the slight changes in the procoagulation and fibrinolytic balance, most progestins appear not to affect the coagulation factors and liver proteins when given alone (Sitruk-Ware & Nath, 2011). However, the metabolic effects of pills are extremely variable; for example estrogens impair insulin action dose-dependently and the associated progestins may modify these effects (Nader & Diamanti-Kandarakis, 2007). It was suggested that when a dose of EE < 50 μg/day is used, the effects of the COCs on the lipid and glycoinsulinemic metabolism is related to the progestin used in the combination (Mancini et al., 2010). Hence, the estrogen component of hormonal contraceptives when administered orally tends to increase the production of lipoproteins such as, the very low density lipoprotein (VLDL) and HDL levels while decreasing LDL levels (Sitruk-Ware & Nath, 2011). Non-androgenic or anti-androgenic progestins exert minimal influence on the lipid profile and carbohydrate metabolism, which lessens the risk of developing coronary heart disease while androgenic progestins have shown an adverse effect over total and HDL cholesterol (Nath & Sitruk-Ware, 2009, as cited in Sitruk-Ware & Nath, 2011). Over the past half century, COCs have undergone a number of evolutionary steps, involving modifications of hormone doses and types, dosage regimens and administration schedules (Burkmana et al., 2011) in order to improve the safety profile, particularly the metabolic and cardiovascular safety. In young women, the risk of CVD is very low but can be strongly influenced by smoking and the presence of other risks factors, such as hypertension, obesity, and DM or PCOS, specially for women over 35 (Farley et al., 1998, 1999, as cited in Sitruk-Ware & Nath, 2011).

As a special subgroup of COCs users, women with PCOS have an increased risk for IGT and as COCs are often prescribed as first-line treatment, a worsening of the metabolic outcomes in this population (e.g. insulin resistance, glucose tolerance, lipid profile or chronic inflammation) would be of concern. In the literature, there are conflicting reports about the CV effects of OCPs on PCOS patients (Battaglia et al., 2010; Meyer et al., 2007). Some of these reports support the beneficial effects of OCPs on the CV system in PCOS patients (Luque-Ramirez et al., 2009; Mancini et al., 2010). However, a recent meta-analysis suggested that OCPs may have negative effects on CV events in healthy individuals and these effects may be aggravated in patients displaying increased CV risk, such as PCOS (Baillargeon et al., 2005, as cited in Gode et al., 2010; Nader & Diamanti-Kandarakis, 2007). The outcome of the studies evaluating the effects of OCPs on carbohydrate metabolism in PCOS has been extremely variable, their results covering all possibilities: improvement, no change,

deterioration (Nader & Diamanti-Kandarakis, 2007). Different OCPs used with different dosage of estrogen, type of progestin and formulation of the pill, different laboratory methods used to assess these effects represent, however, few but important limitations of these studies which should be mentioned.

Hence, Nader & Diamanti-Kandarkis (2007) concluded that the effects of OCPs on carbohydrate metabolism in PCOS will be determined by the degree of androgenicity of the woman and the androgen-lowering effect of the pill, genetically determined endogenous insulin sensitivity of the individual, anthropometric differences that can affect insulin action and by the natural history of PCOS or environmental influences and by other factors, such as puberty, which is associated with decreased insulin sensitivity (Moran et al., 1999, as cited in Nader & Diamanti-Kandarakis, 2007).

These concepts are represented in a few studies. Thus, Cagnacci et al. compared a monophasic 35-μg EE/cyproterone acetate (CPA) pill, widely used in PCOS with a biphasic 40/30 EE/desogestrel pill. The results were contradictory: insulin sensitivity improved with the CPA pill (lowering of androgens with a pill containing the most potent antiandrogenic progesterone) and deteriorated with the desogestrel pill (a more androgenic pill and/or a different formulation with a higher initial dose of estrogen) (Cagnacci et al., 2003, as cited in Nader & Diamanti-Kandarakis, 2007). Ibanez & de Zegher (2004a) also demonstrated the effect of different progestins of potentially different androgenicity. They included post-adolescent PCOS subjects who were already taking flutamide and metformin along with a 20-μg EE-/gestodene-containing OCP in their study and, after some months of this treatment, they randomized subjects to replacement of the gestodene OCP with a drospirenone (DRP) OCP containing 30 μg of EE. The patients who were switched DRP presented a reduced total and abdominal fat and an increased lean body mass (a less androgenic OCP reduced the abdominal fat and potentially improved the metabolic profile in spite of the higher dose of estrogen in the drospirenone pill) as compared with those who remained on gestodene.

As far as lipid metabolism is concerned, HDL -C and TG generally increase after OCP treatment in PCOS and this effect varies with the progestin. Higher TG concentrations are seen with less androgenic progestins (Mastorakos et al., 2002, as cited in Nader & Diamanti-Kandarakis, 2007). Ibanez & de Zegher (2004b) demonstrated that abnormal adipocytokines (increased IL-6 levels and decreased adiponectin levels), hypertriglyceridaemia and body adiposity deteriorated in a group of adolescents and young women with PCOS who were administered EE/DRP, and improved towards the norm after flutamide and metformin were added. They also showed that flutamide and metformin have beneficial effects on these adverse factors either taken individually or together (Ibanez et al., 2004; Ibanez & de Zegher, 2005, as cited in Nader & Diamanti-Kandarakis, 2007).

Furthermore, Halperin et al. (2011) undertook a meta-analysis of published observational studies to investigate the association between COC use and dysglycemia, dyslipidemia and insulin resistance in women with PCOS. COC use was significantly associated with an increase in HDL-C and TG, but was not associated with changes in fasting glucose or fasting insulin. The authors concluded that the use of COCs in women with PCOS was not associated with clinically significant adverse metabolic consequences (Halperin et al., 2011). Additionally, according to the findings in the literature, OCPs seem to have neutral or negative effects on endothelial function among healthy individuals (Gode et al., 2010).

Regarding different OCPs components, FMD appears to increase when circulating levels of estrogens augment naturally or synthetically and this beneficial vasodilator effect of estrogens on the arterial function might be antagonized by some certain types of progestins e.g. levonorgestrel, desogestrel (Torgrimson et al., 2007), but not by DRP, a progestin with antiandrogenic and antimineralocorticoid activity (Meendering et al., 2010). Consistent with these findings, it has been demonstrated that endothelial function was less in women using either a levonorgestrel combined OCP or medroxyprogesterone acetate (MPA) (a highly androgenic progestin) compared to nonusers (Meendering et al., 2010). However, controversial results have been reported in PCOS, suggesting that OCPs may have deteriorating, neutral or improving effects on endothelial function (Battaglia et al., 2010; Mancini et al., 2010; Meyer et al., 2007). In the study of Mancini et al. (2010), hypocaloric diet was prescribed to the obese patients in addition to OCPs and a significant weight reduction was observed after the treatment. In lean patients with PCOS, the DRP/EE30μg did not seem to affect endothelial function, whereas in overweight PCOS women it did not seem to counteract the loss of weight due to healthier lifestyle changes, which was associated with an improvement of insulin sensitivity and FMD. Therefore, it may be hypothesized that life style implementation may hinder the negative effects of treatment or natural progression of PCOS. In another recent study, there was a tendency of reduction in FMD in PCOS women during the 6-month treatment with EE and CPA combination. In addition, the significant alteration, which was observed particularly in the overweight group, supports the previous reports, which emphasize the independent role of obesity in CV risk (Vural et al., 2005, Mancini et al., 2009, as cited in Gode et al., 2010). Raised plasma concentrations of the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine (ADMA) have been associated with a higher prevalence of CV risk factors and CVD in different populations, including PCOS women (Dahlgren et al., 1992). Interestingly, ADMA levels were significantly decreased in PCOS subjects, after the administration for a longer period of time-12 months -of natural or synthetic estrogens, combined with anti-androgens (CPA or desogestrel), to levels comparable with those in the control group, in accordance with previous reports on ADMA concentrations in young and healthy women. A borderline but interesting decrease in ET-1 was also observed, possibly further indicating the beneficiary effect estrogens, combined with antiandrogens, have on endothelial function in women with this syndrome. The results of the present study are in agreement with previously published evidence regarding an inhibitory effect of estrogenic compounds on ADMA production (Charitidou et al., 2008).

Regarding carotid IMT, a non-significant tendency toward a decrease in carotid IMT was reported with EE/CPA treatment in PCOS subjects (Luque-Ramirez et al., 2009). The remarkable aspect of the study was that a diet and physical activity were prescribed to all patients in addition to the ongoing treatment. The results of Gode et al. (2010) were contrary to the above mentioned study, and reported a significant increase in both right and left carotid IMTs in both the normal weight and the overweight groups after the 6-month follow-up of PCOS patients given OCP containing CPA. It is possible that the significant change in carotid IMT was triggered by increased lipid taking into account the fact that, with the exception of HDL, all lipid profile components were significantly augmented in the normal-weight patients while only TG levels were significantly increased in the overweight patients and endothelial dysfunction and increased IMT coexist with abnormal lipid profile (Karasek et al., 2006, as cited in Gode et al., 2010). Moreover, a recent study reported a CIMT

progression rate of 0.015 ± 0.024 mm per year among healthy young women (Johnson et al., 2007, as cited in Gode et al., 2010). However, the increase in carotid IMT in PCOS patients was 0.03 ± 0.01 mm at the end of the 6 month-period, a value which is four times higher than that reported in the healthy young women of that study. The quick advance of these CV risk parameters draws the attention to the importance of close follow-up in PCOS patients. However, due to the fact that the study included only one type of pill, to explain the deterioration of arterial structure through CPA would be a rushed conclusion. Another possibility is that EE plays a role in this fast progression of atherosclerosis. Therefore, we need additional studies to include different types of OCPs to provide clear and accurate results.

In previous studies, a CRP greater than 3 mg/L was accepted to be correlated with CVD (Boulman et al., 2004, as cited in Gode et al., 2010). In the same study performed by Gode et al. (2010), there was also a tendency toward increment in hCRP after 6 months treatment with EE/CPA. Sustaining these observations, the use of OCPs (EE−Desogestrel combination) in obese and nonobese patients with PCOS with impaired glucose tolerance resulted in significantly higher ADMA and hs-CRP levels compared to pretreatment values, thus creating an increase in the metabolic risk (Kilic et al., 2010). The hsPCR levels increased after a 6 month-treatment with COC in young, obese PCOS patients even though CO included progestatives with antiandrogenic activity (Morin-Papunen et al., 2003; Teede et al., 2010b). Both estrogens and progestin content and dosage appear to be implicated in CRP regulation, even though the role of oestrogen might be more important than that of progestin (Haarala et al., 2009). It was noted that COCs stimulate hepatocytes directly to synthesize CRP, and not via IL-6-mediated inflammation. Furthermore, there are studies that demonstrate that DRP/EE30μg monotherapy additionally deteriorated the already increased levels of IL-6 and the decreased levels of adiponectin as well as the adiposity of young PCOS female patients, whereas the treatment with flutamide-metformin reduced all studied indices towards normal levels (Ibanez L & de Zegher, 2006).

To date combined oral contraceptive pills (OCPs) have been the first-line treatment for PCOS. They induce predictable cyclic menses, reduce luteinizing hormone secretion, lower ovarian androgen production and ameliorate androgenic symptoms, increase sex hormone binding globulin thus reducing free androgens and protecting the endometrium. Taken together, until further definitive results are available, these findings revealed that OCPs may not be protective against the progression of CV risk parameters in PCOS. Thus, in some PCOS patients, such as the obese or pubertal, this additional metabolic risk should be considered when OCPs are prescribed, and appropriate surveillance is advisable, as is concomitant use of agents that modify these effects, such as metformin. Some additional treatment modalities such as life style implementation may be added to the treatment, especially in overweight PCOS patients too. Additionally, it is preferable to evaluate CV risk factors of PCOS patients under treatment with OCP, especially the obese ones. Long-term studies with larger populations are needed to confirm how specific OCP formulations affect vascular function of the arterial system in young women.

7. Do cardiovascular risk factors in PCOS result in more cardiovascular events?

The high prevalence of CV risk factors and signs of early atherosclerosis (e.g. increased IMT or altered endothelial reactivity) in young women with PCOS may determine an increased

rate of CVDs in postmenopausal women affected by PCOS. However, in spite of the fact that risk factors have accumulated, we still need evidence to indicate that the high risk increases events and available studies have not ascertained, yet, a uniform association between PCOS and CVD. This is because of the lack of adequate PCOS characterization, appropriate CV disease measurement, and sufficient duration of follow-up or a true lack of association.

Initial studies on the prevalence of CVDs in postmenopausal women who were probably affected by PCOS indicated an increased risk for developing MI (Dahlgren et al., 1992). It was calculated that postmenopausal women with previous PCOS have a 7.1 higher risk than non-PCOS women of developing MI (Carmina, 2009). In a Pittsburgh cohort of 162 Caucasian women with PCOS who were followed up for CV events for up to 12 years, 5 women reported MI, angina pectoris, and/or coronary bypass or angioplasty, whereas no events were observed among 142 control women who were similarly followed (Talbott et al., 2004c). Additionally, a 4-fold risk in cardiac events among women with PCOS was reported in a cohort from the Czech Republic (Cibula et al., 2000). On the other hand, reviewing death certificates of 786 women in the United Kingdom who were diagnosed with PCOS at an average age of 26.4 years and followed for an average duration of 30 years, Pierpoint et al. (1998) failed to show a statistically significant increase in CV mortality. This study has been criticized because the diagnosis of PCOS was based on historical records over a very long period and was not supported by hormonal studies or ovarian morphology. Additionally, interpretation of their data is constrained by the fact that PCOS cases were diagnosed mainly on the basis of hospital records related to wedge resection and by the absence of a matched control cohort. Wedge resection can correct the anovulation and metabolic changes that are observed in PCOS for long periods of time, and this method of case identification may under-ascertain PCOS cases as defined by clinical characteristics. Probably, a more important criticism is that the study evaluated the causes of death of relatively young women. However, in a later report, the same authors (Wild et al., 2000) noted a higher prevalence of cerebrovascular accidents in women who had PCOS during their fertile age. The history of coronary heart disease was, on the contrary, not significantly more common in women with PCOS. On the other hand, in the Nurses' Health Study, which followed 82 439 women for 14 years, it was noted that the women with very irregular menses, which can be considered a surrogate marker for PCOS, had a significantly increased relative risk of 1.5 (95% confidence interval (CI) 1.3–1.9) for coronary heart disease and 1.9 (95% CI 1.3–2.7) for fatal MI compared with eumenorrheic women (Dawber et al., 1951, as cited in Carmina, 2009). More recently, a sub-study of the Women's Ischemia Evaluation Study (WISE) confirmed that women with PCOS have a larger number of CV events. In this study, 104 postmenopausal women with PCOS and a control group of 286 matched normal postmenopausal women were followed prospectively for close to 10 years; multi-vessel angiographic coronary artery disease was observed in 32% of PCOS women compared to 25% of non-PCOS women (odds ratio 1.7) and correlated with several factors, including increased free testosterone. In addition, the event-free survival (including fatal and non-fatal events) was significantly lower in PCOS compared to non- PCOS women. The difference between the two groups was higher when cerebrovascular accidents were also considered, confirming the association of PCOS with stroke. Moreover, women with clinical features of PCOS with increased hsCRP concentrations had a 12.2-fold higher risk of CV death or nonfatal MI than women without clinical features of PCOS and lower levels of hsCRP, suggesting that the independent adverse association between PCOS status and postmenopause CV events may act vis-à-vis inflammatory pathway (Shaw et al., 2008).

While other studies are needed to confirm and expand the available information, PCOS seems, however, to be associated with an increased risk for cerebrovascular events (stroke) and probably also for fatal and non-fatal coronary heart disease, that is independent of their underlying clinical risk, indicating that PCOS-related protracted hyperandrogenism may be one possible mechanism for their CV risk. Consequently, identification of clinical features of PCOS in postmenopausal women may provide an opportunity for risk factor intervention for the prevention of CV disease and CV events.

Nevertheless, there is a clear need for longitudinal, prospective study of a large cohort of well-characterized premenopausal PCOS, along with an appropriate cohort of matched control women that can be followed into menopause. Studies like these will provide a more categorical analysis of the increased CV risk in PCOS and a more accurate assessment of the risk for CV events and mortality in a way that could apply to the overall population of women with PCOS.

8. PCOS status - A major cardiovascular risk factor in women

Taken all together, there is no doubt that the diagnosis of PCOS implies increased cardiac risk. Moreover, in a previous research, we noted that the regression analysis performed on our subjects (women with PCOS and healthy age-matched controls) revealed a predictive value of PCOS status on both FMD and ET-1 values (Ilie et al., 2011). Therefore, in line with others who have suggested PCOS as a major unrecognized CV risk factor in women (Alexander et al., 2009), we proposed that PCOS status should be regarded as a predictor marker of CV risk, beside other well-known CV risk factors, e.g. insulin resistance, dyslipidemia.

The common clustering of CV risk factors with PCOS in reproductive young women might become a public health concern if proved to trigger an increased risk for the development of CVD. Hence, there is a need for longitudinal, prospective, case-control, more rigorous, large sample size studies in young women with PCOS, all of which should be not only long term so as to go beyond menopause, but also homogeneous in terms of both prevalence and degree of obesity as well as the methodology used for evaluating vascular injury or insulin resistance in order to document the early presence of vascular abnormalities and chronic inflammation and to establish whether these CV risk factors subsequently lead to CV events. These are also especially important because it was hypothesized that differences in risk factors between women with PCOS and controls may diminish with increasing age as a consequence of both spontaneous decrease in androgen secretion after the age of 35 years in normal women and in those with PCOS as well as decrease in the polycystic ovary prevalence with age (Labrie et al., 1997, Koivunen et al., 1999, as cited in Carmina, 2009). This might account for the possible overestimation of CV risk in young women with PCOS or for failure to detect excess risk of CV mortality among women with this disorder. However, these is very unlikely since at least one-third of PCOS patients present with increased levels of non-HDL C (Berneis et al., 2009, as cited in Carmina, 2009) and/or display several other traditional and nontraditional CV risk factors. Moreover, short-term trials of pertinent interventions, for example OCPs, insulin sensitizers, on surrogate CV outcomes are warranted, since their effect on CV risk factors and events are not clearly settled. We need accurate studies to establish CV screening guidelines for women with

PCOS. Early CV screening and treatment of all modifiable CV risk factors may be clinically considered a secondary preventing intervention. Recognition of PCOS as an independent risk factor for subsequent CV events would then justify earlier risk-factor intervention.

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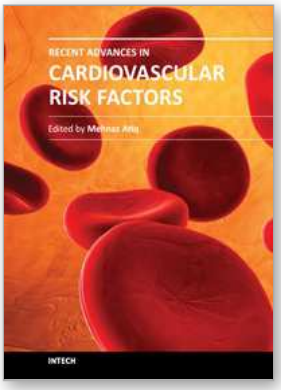

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Among the non-communicable diseases, cardiovascular disorders are the leading cause of morbidity and mortality in both the developed and the developing countries. The spectrum of risk factors is wide and their understanding is imperative to prevent the first and recurrent episodes of myocardial infarction, stroke or peripheral vascular disease which may prove fatal or disabling. This book has tried to present an update on risk factors incorporating new research which has thrown more light on the existing knowledge. It has also tried to highlight regional diversity addressing such issues. It will hopefully be resourceful to the cardiologists, general practitioners, family physicians, researchers, graduate students committed to cardiovascular risk prevention.

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