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Insulin Resistance, the Metabolic Syndrome, Diabetes, and Cardiovascular Disease Risk in Women with PCOS

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Polycystic ovary syndrome is the most common endocrinopathy of reproductive aged women affecting 6–10% of the population. Traditionally considered a reproductive disorder manifesting as chronic anovulation, infertility, and hyperandrogenism, management has primarily focused on short-term reproductive outcomes. Recently, however, significant metabolic aspects in conjunction with longer-term health sequealae of PCOS have been recognized. The metabolic features are primarily related to underlying insulin resistance (IR), which is now understood to play an important role in both the pathogenesis and long-term sequelae of PCOS.

Key Words: Polycystic ovary syndrome; cardiovascular disease; arterial stiffness; insulin resistance.

Introduction

Polycystic ovary syndrome is the most common endocrinopathy of reproductive aged women affecting 6–10% of the population *(1)*. Traditionally considered a reproductive disorder manifesting as chronic anovulation, infertility, and hyperandrogenism, management has primarily focused on short-term reproductive outcomes. Recently, however, significant metabolic aspects in conjunction with longer-term health sequealae of PCOS have been recognized *(2)*. The metabolic features are primarily related to underlying insulin resistance (IR), which is now understood to play an important role in both the pathogenesis and long-term sequelae of PCOS.

In nonselected populations, IR is an independent predictor of cardiovascular disease *(3–5)*. This risk is further amplified by the coexistence of additional cardiovascular risk factors. The clustering of these cardiovascular disease risk factors with IR has been called the metabolic syndrome (MS) or Syndrome X *(6)*. The prevalence of this syndrome is increasing because of the "obesity epidemic." While there is some contention as to which criteria should be included in the MS, the essential components include IR with at least two of hypertension, dyslipidemia, central obesity, microalbuminuria, or elevated fasting glucose *(7)*. The National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) defined practical criteria for the diagnosis of the metabolic syndrome and established the basic principles for its management (Table 1) *(6,8)*. While controversy exists over the prevalence of metabolic syndrome in PCOS, in general, PCOS is now accepted as associated with this condition (Table 2) *(6)*. In a recent study comparing 100 overweight women with PCOS vs BMI-matched controls, features of the metabolic syndrome were more prevalent in the PCOS women than in overweight controls (Table 3) *(9)*.

There is strong epidemiological evidence linking the presence of IR-related MS with detrimental cardiovascular disease outcomes. In adults aged >50 yr, the prevalence of cardiovascular disease is 13.9% in those with the MS, whereas in those without it is 8.7% *(10)*. Furthermore, interventional studies unequivocally demonstrate that modulation of these independent cardiovascular risk factors, which constitute the MS, reduces the risk of cardiovascular disease.

In the setting of IR, the MS and obesity, theoretically the majority of women with PCOS would appear to be at increased risk. Existing data clearly demonstrate up to a sevenfold increase in type II diabetes in women with PCOS. Yet data on cardiovascular disease risk in this population is limited, although it is suggestive of an increased cardiovascular disease risk. Pending further clarification, conventional approaches to monitoring and treating individual cardiovascular risk factors in this theoretically high group would appear a reasonable approach.

Yet, in this apparently high-risk group of women with PCOS, treatment has conventionally targeted reproductive and hormonal abnormalities, not metabolic features. The authors propose that metabolic features should be considered when recommending therapy. Current medical therapies including the oral contraceptive pill (OCP) used in PCOS may exacerbate IR and the MS. PCOS treatment often begins in late adolescence and may extend through until menopause. The impact of long-term OCP therapy on IR, the MS, and its cardiovascular sequealae may be important and yet has received little consideration to date. Con-

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Table 1
NCEP ATP III Clinical Criteria
for the Diagnosis of the Metabolic Syndrome

Criteria	Defining level
Abdominal obesity (waist circumference)	>102 cm in men >88 cm in women
Triglycerides	≥1.7 mmol/L^{-1}
HDL cholesterol	In men <1.0 mmol/L^{-1} In women <1.3 mmol/L^{-1}
Blood pressure	≥130/85 mmHg
Fasting glucose	≥6.1 mmol/L^{-1}

The diagnosis is based on three or more out of the five criteria, NCEP ATP III, National cholesterol education program adult treatment panel III *(8)*.

Table 2
Features Associated with the Metabolic Syndrome *(6)*

1. Abdominal obesity (↑ waist circumference)
2. Dyslipidaemia (↑TG's, ↓HDL-C, ↑small LDL)
3. Hypertension
4. Insulin resistance ± glucose intolerance
5. Increased inflammation
6. Prothrombotic state
7. Additional features: microalbuminuria, PCOS, fatty liver, hyperandrogenism and endothelial dysfunction.

Table 3
Physical, Metabolic, and Endocrine Characteristics
of PCOS and Overweight Control Subjects (± SE) *(6)*

Variable	Control	PCOS	*p* value
n	20	100	
Age (yr)	33.2 ± 2.3	32.7 ± 1.8	NS
BMI (kg/m^2)	36.7 ± 1.28	37.3 ± 2.43	NS
WHR	0.84 ± 0.02	0.86 ± 0.01	NS
Fasting insulin (μU/mL)	6.6 ± 0.8	19.6 ± 1.42*	<0.001
Fasting Glucose (mg/dL)	81.1 ± 1.44	82.8 ± 1.08	NS
HOMA	1.34 ± 0.17	4.08 ± 0.32*	<0.001
Cholesterol(mmol/L)	4.6 ± 0.2	5.1 ± 0.1*	0.04
LDL(mmol/)	3.2 ± 0.2	3.3 ± 0.1	NS
HDL(mmol/L)	1.2 ± 0.03	1.0 ± 0.08	NS
Triglyceride(mmol/L)	0.87 ± 0.08	1.41 ± 0.06*	<0.001
CRP	4.6 ± 0.9	6.4 ± 0.6	NS

Conversion factors for SI units are insulin = 7.175 pmol/L, glucose = 0.05551 mmol/L, testosterone = 3.467nmol/L.
*$p < 0.05$ difference between groups.

versely, the use of insulin sensitizers may not only provide effective therapy for reproductive problems but may also ameliorate metabolic features of PCOS.

The aim of this review is to summarize the current literature on IR, the MS, and the long-term sequealae including the risk of diabetes and cardiovascular disease in PCOS. On this background we will also consider the potential cardiovascular implications of therapies currently used in PCOS.

PCOS and Vascular Risk Factors

Insulin Resistance (IR) and Diabetes

While a full discussion of the mechanisms involved in hyperinsulinemia and cardiovascular disease is beyond the scope of this review, mechanisms are likely to be complex. IR appears to affect 50–70% of women with PCOS *(11)*. The cause of IR is complex and multifactorial with genetic and environmental contributors. While these include obesity, lean women with PCOS also have abnormalities of insulin secretion and action compared to control subjects *(12)*. Specific abnormalities of insulin metabolism identified in PCOS subjects include reductions in secretion *(13, 14)*, reduced hepatic extraction *(15)*, impaired suppression of hepatic gluconeogenesis *(12)*, and abnormalities in insulin receptor signaling *(16)*.

IR appears to contribute to the reproductive manifestations of PCOS. Interestingly, there is a paradoxical expression of IR in PCOS whereby insulin-stimulated androgen production persists, while its role in glucose metabolism is impaired *(16)*. Therefore, IR in PCOS results in hyperinsulinemia with its associated diverse and complex effects on regulating lipid metabolism, protein synthesis, and modulation of androgen production. There is strong epidemiological evidence that IR and hyperinsulinemia, independent of other cardiovascular risk factors, confer a significantly increased risk of cardiovascular disease *(3–5)*. In both diabetic and nondiabetic women there is a linear increase in risk for cardiovascular disease across quintiles of fasting insulin *(17,18)*.

In those women with PCOS who have IR, a susceptible subgroup also develop insufficient pancreatic insulin output or β cell failure. In this setting, insulin output cannot overcome IR and hyperglycemia develops. Indeed 30% of obese women have been noted to have impaired glucose tolerance *(19)* and 15% of postmenopausal women with a history of PCOS have type II diabetes *(20)*. Both impaired glucose tolerance (IGT) and diabetes are very significant cardiovascular risk factors in women. With the diagnosis of diabetes, the relative risk of cardiovascular disease in women increases four to sevenfold, with an even greater risk of cardiovascular disease and heart failure compared to men with diabetes *(21)*.

Hyperinsulinemia contributes to cardiovascular disease both through direct mechanisms such as increasing sympathetic activity *(22)* and inducing abnormalities in endothelial function and vascular reactivity *(23)* and via indirect mechanisms such as its association with impaired fibrinolysis *(24)*, altered insulin-dependent suppression of lipolysis, and induction of hypertension *(25)*.

The Effect of PCOS Therapy on IR

For the majority of women with PCOS who are overweight, lifestyle change and weight loss are integral in their management and effectively improve IR. Even a small loss of between 3–5% can reduce insulin resistance and improve many features of the MS *(26,27)*. Lifestyle change is reviewed in the article by Norman et al. and will not be covered here in detail. Although, because long-term weight loss and maintenance are often difficult or the benefits may not be adequate, alternative medical therapies for PCOS, which target IR, including metformin, are increasingly utilized.

Studies have consistently demonstrated improvements in IR of 20–30% following treatment with metformin *(28–30)*. While there are no long-term data on the role of metformin in the prevention of diabetes in women with PCOS, metformin has been shown to reduce the risk of progression to diabetes by 31% compared to placebo in high-risk nondiabetic individuals *(31)*. Further research is required in this area to clarify the long-term effects of metformin on both DM2 and cardiovascular disease in women with PCOS.

The OCP is often considered first-line medical therapy to control the hormonal manifestations of PCOS, yet there is evidence to suggest that its use is associated with increased IR in both PCOS and non-PCOS populations *(29,32–36)*. It is unclear whether the estrogen or progestin components of the OCP are primarily responsible for the increased IR and if the effect is dose related *(35,37–39)*, although there is some evidence to suggest that higher doses of estrogen may adversely affect IR. In a recent randomized, controlled trial, our group has demonstrated that 6 mo of 35 mcg ethinylestradiol (EE) + 2.5 mg cyproterone acetate combined OCP increased IR, while a 20 mcg EE and levonorgesterol OCP had no effect on IR (unpublished data). This increased IR may have adverse effects on the risk of diabetes and cardiovascular disease; however, confirmatory long-term studies that define these risks and dissect out the effects of estrogens and progestins are needed.

Lipid Metabolism

Dyslipidemia is a critical component of the MS; it is characterized by increased VLDL and LDL cholesterol, hypertriglyceridemia, and reduced HDL *(40)* and is strongly associated with the development of cardiovascular disease *(41,42)*. Both primary and secondary prevention trials targeting lipid abnormalities have demonstrated clear benefits for cardiovascular disease across a wide spectrum of cardiovascular risk in non-PCOS populations *(43,44)*.

In this setting, several studies have demonstrated dyslipidemia in women with PCOS compared with weight-matched controls *(9,25,45,46)*. Compared with control subjects, women with PCOS have higher triglycerides (TG) (122 vs 63 mg/dL), and very-low-density lipoprotein cholesterol (VLDL) (24 vs 13 mg/dL) with lower high-density lipoprotein cholesterol (LDL) (43 vs 58 mg/dL) *(25)*. In addition, women with PCOS have a smaller LDL particle size *(47)*. The dyslipidemia occurs independent of BMI *(25,48)*; however, there is a synergistic deleterious effect of obesity and IR in PCOS, analogous to that seen in DM2. The causes of dyslipidemia in PCOS are again multifactorial. IR appears to have a pivotal role with multiple studies demonstrating significant associations between altered lipid metabolism and insulin levels *(49,50)*. This may be induced in part by the insulin-medicated stimulation of lipolysis and altered expression of lipoprotein lipase and hepatic lipase *(25)*.

The Effects of PCOS Therapies on Lipid Profiles

The limited studies which have focused on the effect of the OCP on lipid metabolism in PCOS demonstrate increases in total serum cholesterol, LDL, and TG *(34,51–53)*. Variable OCP formulations appear to impact differently on lipid metabolism; this may be dependent on type and dose of both progestin and estrogen in the formulation. In a study in 24 adolescents with PCOS, contraceptives containing cyproterone acetate induced a greater rise in TGs compared to a desogestrel containing OCP *(34)*. Desogestrel has been shown to increase total cholesterol (163 vs 185 mg/dL), LDL (90 vs 109 mg/dL), and HDL (51 vs 60 mg/dL) *(54)*. A further study has noted that cyproterone acetate significantly increased all of these cholesterol subfractions *(52)*. Limited data currently suggest that metformin lowers LDL cholesterol and total cholesterol and raises HDL *(28,55–57)*, consistent with improvements in insulin sensitivity. Potentially these metabolic effects of metformin may be preferable to those of the OCP, yet more research is needed.

Hypertension

Hypertension is a well-recognized risk factor for cardiovascular disease, highlighted in an extensive recent meta-analysis. Hypertension was identified as a leading cause of primary cardiovascular mortality *(58,59)*, with reductions in blood pressure (BP) significantly improving cardiovascular outcomes *(60,61)*.

Studies of blood pressure in PCOS have yielded inconsistent results. Two studies comparing 38 women in total with PCOS to age- and sex-matched controls, found no difference in BP even on 24 h ambulatory readings *(62,63)*. Consistently, our work has demonstrated no differences in 24 h BP between overweight women with PCOS and overweight controls. In contrast, a study of 36 women with PCOS compared to 55 BMI-matched controls examined both clinic and 24 h ambulatory BP readings. They found that women with PCOS had higher mean ambulatory BP and higher daytime systolic BP. These differences persisted after adjustment for body fat distribution and IR *(64)*. Potential mechanisms for hypertension in PCOS may include hyperinsulinemia, which directly activates the sympathetic nervous system, alters vascular smooth muscle *(65)*, and increases

renal sodium retention *(66)*. Also, IR blunts the normal vasodilating effect of insulin on arterial smooth muscle *(67)*. We have found that obese women with PCOS have increased arterial stiffness compared to obese controls *(9)*. In later life this would be expected to translate to increased BP. Further research is needed to clarify if BP profiles are truly increased in PCOS. It may be that this only becomes evident in older women with PCOS.

The Effect of PCOS Therapies on Blood Pressure

The effect of medical therapies for PCOS on BP is not well established. In non-PCOS populations weight loss and exercise improve BP, whereas the OCP has been shown to increase systolic BP by around 5 mmHg after adjustment for age and BMI *(68,69)*. The effect of metformin on BP appears favorable with a 10 mmHg reduction noted in mean systolic BP in obese women with PCOS *(70)*. However, these results are not consistent and, in a recently completed randomized, controlled study by our group, metformin therapy over 6 mo did not affect 24 h BP profiles in obese women with PCOS (unpublished data).

Obesity

The cardiovascular risk associated with abdominal obesity is well recognized with the Nurses Health study demonstrating a relative risk of 3.4 for cardiovascular disease in those with a BMI > 30 *(71)*. Central obesity is an independent predictor of cardiovascular events *(72)* and has an important role in the pathogenesis of the MS compounding the problems of IR, dyslipidemia, and hypertension *(73)*. IR increases with a BMI > 27 potentially via elevated free fatty acid (FFA) levels *(74)*. The increased FFAs may contribute to IR by increasing hepatic gluconeogenesis and curtailing both glucose oxidation and uptake in skeletal muscle *(75)*.

Effects of PCOS Therapies on Obesity

Overweight or obesity is a feature in up to 70% of women with PCOS *(76)*; furthermore, increased central obesity with elevated waist–hip ratios are seen independent of BMI *(77)*. As in non-PCOS populations, central obesity compounds the MS, and weight loss can improve this. A meta-analysis of randomized trials in non-PCOS populations has confirmed that weight loss is difficult to achieve and generally not maintained with 50% relapse in 12 mo and 90% relapse in 5 yr *(78)*. In PCOS, although weight loss and lifestyle change are integral parts in management, this is again difficult to achieve. Even in the setting of an intensive trial aimed at weight loss, women with PCOS averaged only 4 kg loss over 6 mo and literature suggests that caloric restriction in PCOS needs to be greater than in non-PCOS populations *(30)*. It has been hypothesized this is because of the hyperinsulemia; however, mechanisms are not adequately understood.

The effect of medical therapy on body fat in PCOS remains unclear. Metformin in non-PCOS populations leads to small weight loss, although the Cochrane review on metformin in PCOS reported that, in nine studies involving 140 women, metformin did not affect weight, BMI, or waist circumference *(57)*. The OCP in non-PCOS populations has been associated with some weight gain (majority gain 2–3 kg) *(79)*; however, a Cochrane review in non-PCOS populations concluded that the modern combined OCP is likely to have little if any effect on weight *(80)*. Any effect of the OCP may be dose-related, as lower-dose OCPs do not appear to induce weight gain *(81)*. Given the impact of obesity in PCOS, further research into the effect of hyperinsulemia on ability to loose weight, on effective lifestyle interventions, and on the effects of medical therapy on weight in women with PCOS is important.

Coagulation and Fibrinolysis

In healthy individuals there is an equilibrium between the hemostatic coagulation and fibrinolytic systems, with thrombosis resulting from an imbalance between the complex systems *(82)*. The hemostatic system plays an important role in cardiovascular disease with acute events precipitated by thrombosis developing on a ruptured arterial plaque. Furthermore, activation of coagulation and impairment of fibrinolysis are associated with increased cardiovascular risk. Proenzymes and inhibitors can be accurately measured to reflect hemostatic activity, with several markers noted to be predictive of cardiovascular events. Impaired fibrinolysis, reflected by increased PAI-1 levels (an inhibitor of fibrinolysis), is associated with increased thrombotic risk in the arterial and venous systems *(24,83)*. In subjects with cardiovascular disease, elevated PAI-1 is associated with increased acute myocardial infarctions *(24)*. IR appears to be linked to elevated PAI-1 with several studies demonstrating strong associations between insulin levels and PAI-1 in subjects with IGT *(84)*, types I *(85)*, and II diabetes *(86,87)*.

Given these observations, it is not surprising that IR PCOS subjects have increased PAI-1 levels *(62,88,89)*. Other hemostatic markers have not been systematically studied in PCOS. Random isolated measurements of factor VII, antithrombin III, and fibrinogen have yielded inconclusive results *(88,90)*.

The Effects of PCOS Therapies on Coagulation and Fibrinolysis

The effect of medical therapies on the hemostatic system is complex. Estrogen in the OCP activates coagulation in a dose-dependent manner as well as increasing fibrolysis (reduces PAI-1), yet the net effect is adverse with an increased the risk of venous and probably arterial thrombosis. Estrogen in hormone therapy in postmenopausal women also increases coagulation and fibrinolysis and increases arterial and venous thromboembolic events *(91)*. The effects of metformin on many markers of hemostatic activity have not been studied; however, Velasquez et al. have shown that metformin reduces PAI-1 levels in obese women with PCOS *(92)*. In non-PCOS individuals with IR and type II diabe-

tes, metformin does not increase the risk of venous thrombosis and reduces the risk of cardiovascular events *(93)*.

Novel Cardiovascular Markers

Highly Sensitive C-Reactive Protein (HsCRP)

Inflammation within arterial plaque progresses atheroma development from initial leukocyte recruitment, through to unstable plaque rupture, precipitating acute ischemic events *(94)*. CRP has a direct role in the vascular inflammatory process stimulating release of inflammatory cytokines and increasing endothelial expression of cellular adhesion molecules, which mediate leukocyte migration *(94)*. HsCRP levels are a strong independent predictor of cardiovascular disease and death in healthy subjects *(95–97)*, those with unstable angina *(98)*, and subjects undergoing revascularization procedures *(99)*.

Elevated HsCRP levels have been associated with IR states in healthy women *(100)*, those with DM2 and IGT *(101)*, as well as with obesity *(102)*. Weight loss decreases HsCRP in non-PCOS populations *(103)*.

Elevated levels of HsCRP have been noted in PCOS *(104)*; however, it is unclear whether this is primarily BMI related *(104,105)*. In PCOS, 6 mo of metformin reduced HsCRP levels by 31% in non-obese subjects and 56% in obese subjects *(105)*. In accordance with other studies, the fall in HsCRP with metformin was associated with improvements in central obesity. Increases in HsCRP have been noted in women on the OCP, independent of changes in BMI. This may be mediated by estrogen effects on the liver (the primary site of CRP production), and/or may reflect the deterioration in IR noted with the OCP. The clinical relevance of these changes in HsCRP still requires clarifications.

Adiponectin

Adiponectin is an abundant adipose tissue-specific adipocytokine involved in the regulation of both carbohydrate and lipid metabolism; low levels have been proposed to contribute to IR. Adiponectin is negatively correlated to markers of insulin resistance such as fasting insulin, HOMA, and clamp studies *(106,107)*. It has potential protective effects against cardiovascular disease. Based on animal studies low adiponectin levels have been associated with an increased inflammatory response to vascular injury *(108)*.

In PCOS, adiponectin correlates with obesity and IR; however, there is no difference between subjects with PCOS and weight-matched controls suggesting adiponectin primarily tracks with obesity and not PCOS per se *(109)*. The effects of medical therapies for PCOS on adiponectin are unknown.

Homocysteine

Numerous clinical and epidemiological trials have shown that elevated homocysteine is a marker of increased risk of coronary, cerebral, and peripheral atherosclerosis. Hyperhomocysteinemia is associated with insulin across the spectrum of IR. In PCOS, homocysteine is correlated with IR regardless of body weight *(110–112)*. IR women with PCOS have higher homocysteine than noninsulin-resistant PCOS subjects (12.4 ± 8.4 micromol/L vs 9.6 ± 4.4 micromol/L), independent of BMI *(110)*. Exercise decreases homocysteine levels in overweight young women with PCOS independent of changes in BMI or insulin levels *(113)*. There are no studies examining the effect of relevant medical therapies (OCP and metformin) on homocysteine, although the effects of medical therapies on IR may be expected to impact on homocysteine levels.

Noninvasive Markers of Vascular Disease

Given the prevalence of cardiovascular risk factors in women with PCOS, it is perhaps expected that these women will have increased cardiovascular disease. However, this critical issue has not been resolved. There are major study limitations when focusing on clinical end-points (covered below). Given these difficulties, another feasible approach is to study reproducible accurate non-invasive surrogate markers of cardiovascular disease in this population *(114)*. These include measures of arterial structure and function encompassing large artery biomechanical properties, endothelial function, and assessment of atherosclerosis. These markers are associated with known cardiovascular risk factors and are predictive of clinical cardiovascular events in non-PCOS populations *(115–118)*. However, they remain surrogate markers and cannot replace ultimate longitudinal data on clinical events.

Endothelial Function

The vascular endothelium is a dynamic endocrine gland regulating vascular tone and reactivity through the release of vasodilating substances such as nitric oxide (NO), platelet adhesion, and thrombosis. NO is a potent vasodilator, inhibits inflammation, oxidation, and smooth muscle proliferation. Damage to the endothelium promotes the development of atheromatous disease. Traditional cardiovascular risk factors such as hypertension and dyslipidemia are associated with endothelial dysfunction *(119)*. In addition, IR has been associated with endothelial dysfunction *(120–122)*. Endothelial function can be assessed noninvasively based on flow-mediated vasodilation (FMD). This noninvasive assessment of endothelial function has been shown to be predictive of clinical coronary events *(123)*.

Noninvasive studies of endothelial function in women with PCOS have produced varied results. Mather et al., found that in 18 otherwise healthy women with PCOS, there was no correlation between IR or hyperandrogenism and endothelium-dependent and -independent vasodilation *(124)*. Compared with age-matched controls, those with PCOS were more obese, but endothelium-dependent and independent vasodilation was not different.

In contrast to this, Paradisi et al. have found that endothelium-dependent and insulin-mediated flow responses

of the femoral artery in 12 obese women with PCOS were impaired compared to age- and weight-matched controls. In this study glucose disposal rates measured during a euglycemic hyperinsulinemic clamp study, directly related to insulin-mediated flow responses of the femoral artery *(125)*. Our group has demonstrated that compared to BMI-matched controls, PCOS subjects have impaired FMD with IR being an independent predictor of FMD *(9)*. In support of these findings, treatment of women with PCOS with the insulin-sensitizing PPARγ agonist troglitazone has been shown to restore endothelium-dependent vasodilation to control levels *(126)*. Data are inconsistent, though, with a recent study by our group in 105 women with PCOS showing no change in FMD with either metformin or the OCP, despite dichotomous effects on IR (unpublished data).

Pulse Wave Velocity (PWV)

PWV is a robust vascular parameter reflecting arterial stiffness that correlates with cardiovascular risk factors including lipids, age, and hypertension. It is predictive of cardiovascular events in subjects with hypertension and renal disease and has been correlated to the extent of coronary artery disease *(114,127)*. IR has been correlated with arterial stiffness as has fasting glucose levels in non-diabetic subjects: an 80% higher fasting insulin (approx 1 SD) was associated with 5.1% and 7.5% greater arterial stiffness index in men and women, respectively *(128)*.

In a study of 19 women with PCOS, Kelly et al. demonstrated increased PWV compared to controls and concluded that IR may have an important causative role *(129)*. These findings are supported by a study of overweight women with PCOS and controls, by our group, where PWV was increased in PCOS compared to control subjects. Furthermore, IR independently contributed to the variance in PWV *(9)*.

To date, there is only one recent study examining the effects of medical therapies on PWV in women with PCOS. Six months of a 35 μg estradiol OCP preparation in overweight women with PCOS increased arterial stiffness, an effect primarily related to increased IR. This study did not demonstrate any effect of metformin on arterial stiffness (unpublished data).

Vascular Structure:

Carotid Intimal Medial Thickness (IMT)

IMT is an established marker for early structural atherosclerotic disease, representing the combined intima, media, and adventitia of the carotid artery *(130)*. It correlates with cardiovascular risk factors including hypertension, hyperlipidemia, diabetes, and obesity *(131–133)*, is a reliable indicator of atheroma progression and predicts cardiovascular events in longitudinal studies *(134)*. Increased carotid IMT has been associated with IR in healthy subjects independent of conventional risk factors for atherosclerosis *(135,136)*. Insulin may exert its effect on atherosclerosis both directly affecting the arterial wall and indirectly via effects on lipids and blood pressure *(137)*.

Limited studies to date have examined carotid IMT in women with PCOS *(138)*. One study in 100 subjects with PCOS demonstrated a higher IMT compared to controls but only in those older than 45 yr *(139)*. In 19 young women (<35 yr), subjects with PCOS had increased carotid IMT compared to controls, after adjustment for other cardiovascular risk factors *(140)*. However, a recent study of 100 overweight younger women with PCOS compared to weight- and age-matched controls, IMT was similar in the two groups *(9)*. It could be hypothesised that IR in PCOS increases arterial stiffness and endothelial dysfunction, which when combined with other features of the metabolic syndrome results in structural atherosclerosis (IMT) with advancing age and ultimately in increased cardiovascular events. Yet, this theoretical progression of events requires long-term longitudinal studies to establish.

Coronary Artery Disease

Coronary artery calcification is also assessed non-invasively using computed tomography and is a marker for atherosclerosis, which correlates with histological coronary plaque *(141)*. In 36 overweight young women with PCOS, coronary calcification was increased compared to controls with the major predictors being abdominal obesity and dyslipidemia *(142)*. In 143 women, aged <60 yr of age, referred for coronary angiography, those with ultrasound-diagnosed PCO had greater stenosis compared to women with normal ovaries. Logistic regression revealed the extent of coronary disease was an independent predictor of PCO *(143)*.

Epidemiological Studies

Despite numerous studies demonstrating the prevalence of cardiovascular risk factors in women with PCOS, data on actual cardiovascular events are less readily available. The largest retrospective study of 786 women diagnosed with PCOS (1930–1979) followed for around 30 yr, with an average age of 56.4 yr at follow up, found that standardized mortality ratios (SMR) based on 59 deaths were the same as national rates for circulatory disease (SMR 0.83; 95% CI, 0.46–1.37). Ischemic heart disease ratios were similar (SMR 1.40; 95% CI, 0.75–2.40), but there was an increase in deaths from diabetes (odds ratio 3.6; 95% CI 1.5–8.4) *(144)*. A subsequent follow up study of this same cohort found that although there was an increase in dyslipidemia, hypertension, and diabetes, the SMR for all cause mortality and cardiovascular disease was the same as the general population. There was, however, a statistically significant increase in the odds ratio for cerebrovascular disease (OR 2.8; 95% CI 1.1–7.1) *(145)*. These studies are limited by ambiguities in prior PCOS diagnostic criteria (women were diagnosed based on surgical appearance of the ovaries), the long lag time between diagnosis of PCOS and cardiovascular events, and the young age of the women at follow-up (mean 54 yr). A significant proportion of subjects were also unable to be traced. These factors potentially significantly under-esti-

mate the prevalence of cardiovascular sequale in this population *(123)*.

Conclusion

There is clear evidence that insulin resistance is involved in the pathogenesis of PCOS. Based on case-controlled studies, women with PCOS appear to have a high prevalence of the metabolic syndrome including obesity and dyslipidemia. There is also an increased risk of diabetes. Noninvasive measures of arterial structure and function suggest early atherosclerotic disease in PCOS; however, long-term cardiovascular event rates are yet to be clarified. In this setting, while it is important that the long-term impact of PCOS on the cardiovascular system is clarified with adequately powered prospective clinical studies, there is currently sufficient evidence to necessitate the screening for and treatment of both modifiable cardiovascular risk factors and diabetes in this population.

In the setting of high rates of metabolic syndrome and diabetes, the potential impact of treatment on the metabolic features of the syndrome should be considered. Optimally, lifestyle modification and weight loss should be the aim to treat the condition and to prevent long-term complications. Additional medical therapy including the OCP and insulin sensitisers may be required. The OCP potentially has detrimental effects in this population, while insulin sensitisers have many practical and theoretical advantages in PCOS. These effects of therapy require further clarification, considering the potential long-term implications in women with PCOS.

References

1. Carmina, E. and Lobo, R. (1999). *J. Clin. Endocrinol. Metab.* **84,** 1897–1899.
2. Wild, R. (2002). *Am. J. Obstet. Gynecol.* **186(1),** 35–43.
3. Ruige, J., Assendelft, J., Dekker, J., Kostense, P., Heine, R., and Bouter, L. (1998). *Circulation* **97(10),** 996–1001.
4. Haffner, S. (1996). *Diabetes Care* **19,** 275.
5. Koskinen, P. J., Manttari, M., and Manninen, V. (1992). *Diabetes Care* **15,** 820.
6. Liberopoulos, E. N., Mikhailidis, D. P., and Elisaf, M. S. (2005). *Obesity Rev.* **6,** 283–296.
7. Haffner, S. and Taegtmeyer, H. (2003). *Circulation* **108(13),** 1541–1545.
8. Expert panel. Executive summary of the third report of the National Cholesterol Education program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III) Final Report. (2001). *JAMA* **285,** 2486–2497.
9. Meyer, C., McGrath, B., and Teede, H. (2005). *J. Clin. Endocrinol. Metab.* **90(10),** 5711–5716.
10. Alexander, C., Landsman, P., Teutsch, S., et al. (2003). *Diabetes* **52,** 1210–1214.
11. Legro, R., Castracane, D., and Kauffman, R. (2004). *Obstet. Gynecol. Sur.* **59,** 141–154.
12. Dunaif, A., Segal, K., Futterweit, W., and Dobrjansky, A. (1989). *Diabetes* **38,** 1165.
13. Dunaif, A. and Finegood, D. (1996). *J. Clin. Endocrinol. Metab.* **81(3),** 942–948.
14. O'Meara, N., Blackman, J., Ehrmann, D., et al. (1993). *J. Clin. Endocrinol. Metab.* **76(5),** 1241–288.
15. O'Meara, N. M., Blackman, J. D., Ehrmann, D. A., et al. (1993). *J. Clin. Endocrinol. Metab.* **76(5),** 1241–1247.
16. Dunaif, A. (1997). *Endocr. Rev.* **18(6),** 774–800.
17. Folsom, A. R., Szklo, M., Stevens, J., Liao, F., Smith, R., and Eckfeldt, J. (1997). *Diabetes Care* **20(6),** 935–942.
18. Lehto, S., Ronnemaa, T., Pyorala, K., and Laakso, M. (2000). *Diabetologia* **43,** 148–155.
19. Legro, R. S., Kunselman, A. R., Dodson, W. C., and Dunaif, A. (1999). *J. Clin. Endocrinol. Metab.* **84(1),** 165–168.
20. Dahlgren, E., Johansson, S., Lindstedt, G., et al. (1992). *Fertil. Steril.* **57(3),** 505–513.
21. Barrett-Connor, E., Giardina, E., Gitt, A., Gudat, U., Steinberg, H., and Tschoepe, D. (2004). *Arch. Intern. Med.* **164,** 934–942.
22. Reaven, G., Lithell, H., and Landsberg, L. (1996). *N. Engl. J. Med.* **334,** 374.
23. Westerbacka, J., Vehkavaara, S., Bergholm, R., Wilkinson, I., Cockcroft, J., and Yki-Jarvinen, H. (1999). *Diabetes* **48(4),** 821–827.
24. Hamsten, A., de Faire, U., and Waldius, G. (1987). *Lancet* **2,** 3–9.
25. Wild, R., Painter, P., Coulson, P., Carruth, K., and Ranney, G. (1985). *J. Clin. Endocrinol. Metab.* **61,** 946–951.
26. Valsamakis, G., McTernan, P., Chetty, R., et al. (2004). *Metabolism* **53(4),** 430–434.
27. Brook, R., Bard, R., Glazewski, L., et al. (2004). *Am. J. Cardiol.* **93,** 1012–1016.
28. Moghetti, P., Castello, R., Negri, C., et al. (2000). *J. Clin. Endocrinol. Metab.* **85(1),** 139–146.
29. Morin-Papunen, L. C., Vauhkonen, I., Koivunen, R. M., Ruokonen, A., Martikainen, H. K., and Tapanainen, J. S. (2000). *J. Clin. Endocrinol. Metab.* **85(9),** 3161–3168.
30. Pasquali, R., Gambineri, A., Biscotti, D., et al. (2000). *J. Clin. Endocrinol. Metab.* **85(8),** 2767–2774.
31. Group DPPR. (2002). *N. Engl. J. Med.* **346(6),** 393–403.
32. Nader, S., Riad-Gabriel, M. G., and Saad, M. F. (1997). *J. Clin. Endocrinol. Metab.* **82(9),** 3074–3077.
33. Korytkowski, M., Mokan, M., Horwitz, M., and Berga, S. (1995). *J. Clin. Endocrinol. Metab.* **80(11),** 3327–3334.
34. Creatas, G., Koliopoulos, C., and Mastorakos, G. (2000). *Ann. NY Acad. Sci.* **900,** 245–252.
35. Godsland, I. F., Crook, D., Simpson, R., et al. (1990). *N. Engl. J. Med.* **323,** 1375–1380.
36. Gossain, V., Sherma, N., Michelakis, A., and Rovner, D. (1983). *Am. J. Obstet. Gynecol.* **147,** 618–623.
37. Spellacy, W. (1982). *Am. J. Obstet. Gynecol.* **142,** 732–734.
38. Godsland, I., Walton, C., Felton, C., Proudler, A., Patel, A., and Wynn, V. (1992). *J. Clin. Endocrinol. Metab.* **74(1),** 64–70.
39. Kojima, T., Lindheim, S., Duffy, D., Vijod, M., Stanczyk, F., and Lobo, R. (1993). *Am. J. Obstet. Gynecol.* **169(6),** 1540–1544.
40. Wilson, P. W. F. and Grundy, S. M. (2003). *Circulation* **108 (13),** 1537–1540.
41. Durrington, P. (2003). *Lancet* **362,** 717–731.
42. Girman, C., Rhodes, T., Mercuri, M., et al. (2004). *Am. J. Cardiol.* **93(2),** 136–141.
43. LaRosa, J. (2003). *Am. J. Cardiovasc. Drugs* **3(4),** 271–281.
44. Gresser, U. and Gathof, B. (2004). *Eur. J. Med. Res.* **9(1),** 1–17.
45. Wild, R. A. (1995). *Am. J. Med.* **98(1A),** 27S–32S.
46. Talbot, E., Clerici, A., and Berga, S. (1998). *J. Clin. Epidemiol.* **51,** 415–422.
47. Austin, M., Breslow, J., and Hennekens, C. (1988). *JAMA* **260,** 1917–1921.
48. Wild, R. and Bartholomew, M. (1988). *Am. J. Obstet. Gynecol.* **159,** 423–427.

49. Wild, R., Alaupovic, P., and Parker, I. (1992). *Am. J. Obstet. Gynecol.* **166,** 1191–1197.
50. Goodarzi, M., Erickson, S., Port, S., Jenrich, R., and Korenman, S. (2003). *Metabolism* **52(6),** 713–719.
51. Elter, K., Imir, G., and Durmusoglu, F. (2002). *Hum. Reprod.* **17(7),** 1729–1737.
52. Prelevic, G., Wurzburger, M., Trpkovic, D., and Balint-Peric, L. (1990). *Gynecol. Endocrinol.* **4(3),** 157–168.
53. Mastorakos, G., Koliopoulos, C., and Creatas, G. (2002). *Fert. Steril.* **77(5),** 919–927.
54. Escobar-Morreale, H. F., Miguel, L., and Jose, S. (2000). *Fert. Steril.* **74(4),** 816–819.
55. Fleming, R., Hopkinson, Z. E., Wallace, A. M., Greer, I. A., and Sattar, N. (2002). *J. Clin. Endocrinol. Metab.* **87(2),** 569–574.
56. Ng, E., Wat, N., and Ho, P. (2001). *Hum. Reprod.* **16,** 1625–1631.
57. Lord, J., Flight, I., and Norman, R. (2003). *Cochrane Database Syst. Rev.* **3,** CD003053.
58. Ezzati, M., Lopez, A., Rodgers, A., Vander Hoorn, S., and Murray, C. (2002). *Lancet* **360,** 1347–1360.
59. Murray, C., Lauer, J., Hutubessy, R., et al. (2003). *Lancet* **361,** 717–725.
60. Group SCR. (1991). *JAMA* **265,** 3255–3264.
61. Dahlof, B., Lindholm, L., Hansson, L., Schersten, B., Ekbom, T., and Wester, P. (1991). *Lancet* **338,** 1281–1285.
62. Sampson, M., Kong, C., Patel, A., Unwin, R., and Jacobs, H. S. (1996). *Clin. Endocrinol. (Oxf.)* **45(5),** 623–629.
63. Zimmermann, S., Phillips, R., Dunaif, A., et al. (1992). *J. Clin. Endocrinol. Metab.* **75(2),** 508–513.
64. Holte, J., Gennarelli, G., Berne, C., Bergh, T., and Lithell, H. (1996). *Hum. Reprod.* **11,** 23–28.
65. Ferrari, P. and Weidmann, P. (1990). *J. Hyperten.* **8,** 491–500.
66. Finch, D., Davis, G., Bower, J., and Kircher, K. (1990). *Hypertension* **15,** 514–518.
67. Westerbacka, J., Wilkinson, I., Cockcroft, J., Utriainen, T., Vehkavaara, S., and Yki-Jarvinen, H. (1999). *Hypertension* **33,** 1118–1122.
68. Nawrot, T., Den Hond, E., Fagard, R., and Hoppenbrouwers, K. (2003). *Eur. J. Cardiovasc. Prev. Rehabil.* **10(6),** 438–42.
69. Murayama, N., Matsunaga, A., Tangbanluekal, L., Tantawiwat, S., and Ohtsu, R. (2003). *J. Biosoc. Sci.* **35(2),** 243–261.
70. Velazquez, E., Mendoza, S., Hamer, T., et al. (1994). *Metabolism* **43,** 647–654.
71. Manson, J., Colditz, G., and Stampfer, M. (1996). *N. Engl. J. Med.* **322,** 882.
72. Despres, J. (1992). *Can. J. Cardiol.* **8,** 561–562.
73. Wilson, P. W. F. and Grundy, S. M. (2003). *Circulation* **108(12),** 1422–1424.
74. Bray, G. A. (2004). *J. Clin. Endocrinol. Metab.* **89(6),** 2583–2589.
75. Le Roith, D. and Zick, Y. (2001). *Diabetes Care* **24,** 588–597.
76. Azziz, R., Woods, K., Reyna, R., Key, T., Knochenhauer, E., and Yildiz, B. (2004). *J. Clin. Endocrinol. Metab.* **89,** 2745–2749.
77. Talbott, E., Guzick, D., and Clerici, A. (1995). *Atherosclerosis Thromb. Vasc. Biol.* **15,** 821.
78. NHMRC. Clinical Practise Guidelines for the management of overweight and obesity in adults. NHMRC Clinical practise guidelines, 2003, p. xiii.
79. Le, M., Laveissiere, M., and Pelissier, C. (2003). *Gynecol. Obstet. Fertil.* **31(3),** 230–239.
80. Gallo, M., Grimes, D., Schulz, K., and Helmerhorst, F. (2003). *Cochrane Database Syst. Rev.* 2(CD003987).
81. Reisman, H., Martin, D., and Gast, M. (1999). *Am. J. Obstet. Gynecol.* **181,** S45–52.
82. Bauer, K. (1993). *Arch. Pathol. Lab. Med.* **117,** 71–77.
83. Lowe, G., Danesh, J., Rumley, A., et al. (2001). *Blood Coag. Fibrinolysis* **12,** A1–A2.
84. Meigs, J., Mittleman, M., Nathan, D., et al. (2000). *JAMA* **283(2),** 221–228.
85. Carmassi, F., Morale, M., Puccetti, R., et al. (1992). *Thromb. Res.* **67(6),** 643–654.
86. Aso, Y., Matsumoto, N., Fujiwara, Y., Tayama, K., Inukai, T., and Takemura, Y. (2002). *Metabolism* **51(4),** 471–476.
87. Hu, J., Wei, W., Din, G., Yuan, L., and Liu, Z. (1998). *J. Tongji Med. Univ.* **18(4),** 233–235.
88. Dahlgren, E., Janson, P. O., Johansson, S., Lapidus, L., Lindstedt, G., and Tengborn, L. (1994). *Fertil. Steril.* **61(3),** 455–460.
89. Sills, E., Drews, C., Perloe, M., Tucker, M., Kaplan, C., and Palermo, G. (2003). *Gynecol. Endocrinol.* **17,** 231–237.
90. Tsanadis, G., Vartholomatos, G., Korkontzelos, I., et al. (2002). *Hum. Reprod.* **17(2),** 314–319.
91. Committee WsHiS. (2004). *JAMA* **291(14),** 1701–1712.
92. Velazquez, E., Mendoza, S., Wang, P., and Glueck, C. (1997). *Metabolism* **46(4),** 454–457.
93. Group UPDS. (1998). *Lancet* **352,** 854–865.
94. Blake, G. and Ridker, P. (2001). *Circ. Res.* **89**, 763–771.
95. Ridker, P., Hennekens, C., Buring, J., and Rifai, N. (2000). *N. Engl. J. Med.* **342,** 836–843.
96. Ridker, P., Buring, J., Shih, J., Matias, M., and Hennekens, C. (1998). *Circulation* **98,** 731–733.
97. Danesh, J., Whincup, P., Walker, M., et al. (2000). *BMJ* **321,** 199–204.
98. Luizzo, G., Biasucci, L., Gallimore, J., et al. (1994). *N. Engl. J. Med.* **331,** 417–424.
99. Chew, D., Bhatt, D., Robbins, M., et al. (2001). *Circulation* **104,** 992–997.
100. Pradhan, A., Cook, N., Buring, J., Manson, J., and Ridker, P. (2003). *Arterioscler. Thromb. Vasc. Biol.* **23(4),** 650–655.
101. Temelkova-Kurktschiev, T., Henkel, E., Koehler, C., Karrei, K., and Hanefeld, M. (2002). *Diabetologia* **45,** 151.
102. Ford, E. (1999). *Diabetes Care* **22,** 1971–1977.
103. Tchernof, A., Nolan, A., Sites, C., Ades, P., and Poehlman, E. (2002). *Circulation* **105,** 564–569.
104. Kelly, C., Lyall, H., Petrie, J., Gould, G., Connell, J., and Sattar, N. (2001). *J. Clin. Endocrinol. Metab.* **86,** 2453–2455.
105. Morin-Papunen, L., Rautio, K., Ruokonen, A., Hedberg, P., Puukka, M., and Tapanainen, J. S. (2003). *J. Clin. Endocrinol. Metab.* **88,** 4649–4654.
106. Tschritter, O., Fritsche, A., Thamer, C., et al. (2003). *Diabetes* **52,** 239–243.
107. Weyer, C., Funahashi, T., Tanaka, S., et al. (2001). *J. Clin. Endocrinol. Metab.* **86,** 1930–1935.
108. Havel, P. J. (2004). *Diabetes* **53,** S143–151.
109. Orio, F., Palomba, S., Cascella, T., et al. (2003). *J. Clin. Endocrinol. Metab.* **88(6),** 2619–2623.
110. Schachter, M., Raziel, A., Friedler, S., Straaburger, D., Bern, O., and Ron-El, R. (2003). *Hum. Reprod.* **18(4),** 721–727.
111. Loverro, G., Lorusso, F., Mei, L., Depalo, R., Cormio, G., and Selvaggi, L. (2002). *Gynecol. Obstet. Invest.* **53,** 157–162.
112. Wijeyaratne, C., Nirantharakumar, K., Balen, A., Barth, J., Sheriff, R., and Belchetz, P. (2004). *Clin. Endocrinol.* **60,** 560–567.
113. Randeva, H., Lewandowski, K., Drzewoski, J., et al. (2002). *J. Clin. Endocrinol. Metab.* **87(10),** 4496–4501.
114. Liang, Y., Teede, H., Kotsopoulos, D., Shiel, L., Cameron, J., and McGrath, B. (1998). *Clin. Sci. (Lond.)* **95,** 669–679.
115. Wofford, J., Kahl, F., Howard, G., McKinney, W., Toole, J., and Crouse, J. (1991). *Arterioscler. Thromb. Vasc. Biol.* **11,** 1786–1794.
116. Safar, M. E., Blacher, J., Pannier, B., et al. (2002). *Hypertension* **39(3),** 735–738.

117. Laurent, S., Boutouyrie, P., Asmar, R., et al. (2001). *Hypertension* **37(5),** 1236–1241.
118. Blacher, J., Asmar, R., Djane, S., London, G. M., and Safar, M. (1999). *Hypertension* **33,** 1111–1117.
119. Hsueh, W. and Quinones, M. (2003). *Am. J. Cardiol.* **92(4A),** 10J–17J.
120. Suzuki, M., Takamisawa, I., Suzuki, K., et al. (2004). *Am. J. Hypertens.* **17(3),** 228–232.
121. Steinberg, H., Chaker, H., Leaming, R., Johnson, A., Brechtel, G., and Baron, A. (1996). *J. Clin. Invest.* **97,** 2601–2610.
122. Celermajer, D., Sorensen, K., Bull, C., Robinson, J. S., and Deanfield. J. (1994). *J. Am. Coll. Cardiol.* **24,** 1468–1474.
123. Suwaidi, J., Hamasaki, S., Hingino, S., Nishimura, R., Holmes, D., and Lerman, A. (2000). *Circulation* **101,** 948–954.
124. Mather, K. J., Verma, S., Corenblum, B., and Anderson, T. J. (2000). *J. Clin. Endocrinol. Metab.* **85(5),** 1851–1856.
125. Paradisi, G., Steinberg, H., Hempfling, A., Cronin, J., Hook, G., and Shepard, M. (2001). *Circulation* **103,** 1410–1415.
126. Paradisi, G., Steinberg, H., Shepard, M., Hook, G., and Baron, A. D. (2003). *J. Clin. Endocrinol. Metabol.* **88,** 576–580.
127. Cameron, J. (1999). *Clin. Exp. Pharmacol. Physiol.* **26,** 285–294.
128. Salomaa, V., Riley, W., Kark, J. D., Nardo, C., and Folsom, A. R. (1995). *Circulation* **91(5),** 1432–1443.
129. Kelly, C., Speirs, A., Gould, G., Petrie, J., Lyall, H., and Connell, J. (2002). *J. Clin. Endocrinol. Metab.* **87(2),** 742–746.
130. Gamble, G., Zorn, J., and Sanders, G. (1994). *Stroke* **25,** 11–16.
131. Crouse, J. (1997). In: Touboul, P. and Crouse, J. (eds.), *Intima-media thickness and atherosclerosis. Predicting the risk?* The Parthenon Publishing Group: New York, p. 105.
132. Bonithon-Kopp, C. (1997). In: Touboul, P. and Crouse, J. (eds.), *Intima-medis thickness and atherosclerosis. Predicting the risk?* Parthenon: New York, pp. 27–44.
133. Howard, G., Sharrett, A., and Heiss, G. (1993). *Stroke* **24,** 1297–1304.
134. Salonen, J. and Salonen, R. (1991). *Arterioscler. Thromb.* **11,** 1245–1249.
135. Bokemark, L., Wikstrand, J., Wedel, H., and Fagerberg, B. (2002). *Diabet. Med.* **19,** 144–151.
136. Agewall, S., Fagerberg, B., Attvall, S., Wendelhag, I., Urbanavicius, V., and Wikstrand, J. (1995). *Stroke* **26,** 956–960.
137. Stout, R. (1990). *Diabetes Care* **13,** 631–654.
138. Guzick, D. S. (1996). *Semin. Reprod. Endocrinol.* **14(1),** 45–49.
139. Talbott, E., Guzick, D., Sutton-Tyrrell, K., et al. (2000). *Arterioscler. Thromb. Vasc. Biol.* **20(11),** 2414–2421.
140. Lakhani, K., Hardiman, P., and Seifalian, A. (2004). *Atherosclerosis* **175(2),** 353–359.
141. Rumberger, J. A., Simons, D. B., Fitzpatrick, L. A., Sheedy, P. F., and Schwartz, R. S. (1995). *Circulation* **92(8),** 2157–2162.
142. Christian, R. C., Dumesic, D. A., Behrenbeck, T., Oberg, A. L., Sheedy, P. F. II, and Fitzpatrick, L. A. (2003). *J. Clin. Endocrinol. Metab.* **88(6),** 2562–2568.
143. Birdsall, M., Farquhar, C., and White, H. (1997). *Ann. Int. Med.* **126,** 32–35.
144. Pierpoint, T., McKeigue, P., Isaacs, A., Wild, S., and Jacobs, H. (1998). *J. Clin. Epidemiol.* **51(7),** 581–586.
145. Wild, S., Pierpoint, T., McKeigue, P., and Jacobs, H. (2000). *Clin. Endocrinol.* **52,** 595–600.