Cardiovascular risk in young women with Polycystic Ovary Syndrome

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CONTENTS

SUMMARY

Background: Young women with Polycystic Ovary Syndrome (PCOS) may have increased measures of cardiovascular risk. It is difficult to determine how much of this risk is due to PCOS itself and how much is due to obesity and insulin resistance, which are common in PCOS and are themselves associated with greater cardiovascular risk.

Aims and Methods: The study aimed to determine if arterial stiffness, carotid intima-media thickness and diastolic dysfunction were increased in young women with PCOS independently of the effects of obesity. A cross-sectional study of women with PCOS and healthy volunteers aged 16-45 years was undertaken. Subjects had a comprehensive assessment of body composition (including computed tomography assessment of visceral fat), measurements of arterial stiffness (aortic pulse wave velocity; aPWV), common carotid intima-media thickness (ccIMT), diastolic function (longitudinal tissue velocity; e':a') and metabolic measures including an oral glucose tolerance test to assess insulin area under the curve (IAUC), a marker of insulin resistance.

Results: After adjustment for age and body mass index, PCOS subjects had greater insulin response (IAUC) following glucose challenge (adjusted difference [AD] 35900 pmol min/l, P<0.001), higher testosterone (AD 0.57 nmol/l, P<0.001) and high molecular weight adiponectin (AD 3.01µg/ml, P=0.02) than controls. There were no significant differences in aPWV (AD -0.13m/s, P=0.33), ccIMT (AD -0.01mm, P=0.13) or e':a' (AD -0.01, P=0.86). After adjustment for age, height and central pulse pressure, aPWV and e':a' were associated with log visceral fat and IAUC. After adjusting for log visceral fat, the relationships between aPWV or e':a' and IAUC were only party attenuated. There was no relationship between cardiovascular measures and adiponectin or testosterone.

Conclusions: Insulin resistance and central obesity are associated with subclinical dysfunction in young women, but a diagnosis of PCOS does not appear to confer additional risk at this age.

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Hocking R, Rees DA. Screening for glucose intolerance in young women with polycystic ovary syndrome: what is the optimum strategy? (Poster) British Endocrine Society, Manchester 2010

ABBREVIATIONS

17-β	17 β-hydroxysteroid dehydrogenase
B	Beta
a’	Late diastolic (atrial) myocardial velocity
ADA	American Diabetes Association
AE-PCOS	Androgen Excess and Polycystic Ovary Syndrome society
AGE	Advanced glycation end products
Ai	Augmentation Index
Akt/PKB	Akt/Protein kinase B
AMH	Anti mullerian hormone
ASAA	Acute-phase serum amyloid A
AUC	Area under the curve
BMI	Body mass index
CAC	Coronary artery calcification
ccIMT	Common carotid intima-media thickness
CI	Confidence interval
CIMT	Carotid intima-media thickness
cm	centimetre
CRF	Clinical Research Facility
CRP	C reactive protein

CT	Computed Tomography
CU	Cardiff University
DENND1A	DENN/MADD domain containing 1A
DEXA	dual energy x ray absorptiometry
DI	Disposition index
DNA	Deoxyribonucleic acid
e’	Early diastolic myocardial velocity
e’:a’	Early: late diastolic myocardial velocity ratio
EBCT	Electron beam computed topography
FFM	Fat free mass
FPG	Fasting plasma glucose
FMD	Flow-mediated vasodilatation
FSH	Follicle stimulating hormone
GDR	Glucose disposal rate
GLUT-4	Glucose transporter 4
GnRH	Gonadotrophin releasing hormone
GWAS	Genome-wide association studies
HbA1c	Glycated haemoglobin
HDL	High density lipoprotein
HMW	High molecular weight

HOMA	Homeostatic model assessment
HOMA-IR	Homeostatic model assessment- insulin resistance
hsCRP	High sensitivity C reactive protein
HV	Healthy Volunteer
ICAM-1	intercellular adhesion molecule-1
ICD-9-CM	International Classification of Diseases, Ninth Revision, Clinical Modification
IGT	Impaired glucose tolerance
IL-6	Interleukin-6
IR	Insulin resistance
IRS	Insulin receptor substrate
l	litre
LDL	Low density lipoprotein
LH	Luteinising hormone
LHCGR	Luteinising hormone/choroidogonadotrophin receptor
m	metre
MAPK	Mitogen-activated protein kinase
MI	Myocardial infarction
ml	Millilitre
mmol	Milimoles

mTOR	Rapamycin
NAD(P)H	Nicotinamide adenine dinucleotide phosphate oxidase
NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute for Health and Care Excellence
NIH	National Institute of Health
NO	Nitric Oxide
OGTT	Oral glucose tolerance test
OR	Odds ratio
OSA	Obstructive sleep apnoea
PCOS	Polycystic Ovary Syndrome
PI3K	Phosphoinositide 3-kinase
PKC	Protein Kinase C
PP	Pulse pressure
PWV	Pulse wave velocity
QUICKI	Quantitative insulin sensitivity check index
RAGE	Receptor for advanced glycation end product
RNA	Ribonucleic acid
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
s’	Longitudinal systolic function

SC	Subcutaneous
T2DM	Type 2 Diabetes
THADA	Thyroid associated protein
TNFα	Tumour necrosis factor alpha
UHW	University Hospital of Wales
USA	United States of America
VCAM-1	Vascular cell adhesion molecule-1
WHO	World Health Organisation
WISE	Women's Ischaemia Syndrome Evaluation
XO	Xanthine Oxidase

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CHAPTER 1: INTRODUCTION

1.1 Polycystic Ovary Syndrome

1.1.1 Introduction

Polycystic ovary syndrome (PCOS) is characterised by hyperandrogenism, ovarian dysfunction (oligo-anovulation and subfertility) and polycystic ovaries. The pathogenesis remains to be fully elucidated although insulin resistance is thought to play a role. Briefly reviewing the historical and scientific literature provides an insight into the difficulties of defining the syndrome and the evolution of the current diagnostic criteria.

1.1.2 Historical Overview and Diagnosis

Hippocrates (460-377 BC) noted 'women whose menstruation is less than three days or is meagre, are robust, with a healthy complexion and masculine appearance; yet they are not concerned about bearing children nor do they become pregnant'[1]. This could be one of the first documented accounts of PCOS. However, reviewing historical documentation and applying diagnoses retrospectively must be done with caution. The paper, 'Le virilisme pilaire et son association a l'insuffisance glycolytique (Diabète des femmes à barbe) by Achard and Thiers in 1921 [2] associates insulin resistance with hirsutism and is sometimes referred to as an early description of PCOS. However, a review of this case report in 2000 concluded that there was little evidence for PCOS and that non-classical adrenal hyperplasia was a more likely diagnosis [3].

In 1935 Stein and Leventhal reported a case series of seven women aged between 20 and 33 years with infertility, amenorrhoea and bilateral polycystic ovaries at

laparotomy. Four of the women were noted to have hirsutism, one woman had acne and three were obese [4]. This is thought to be the first definitive description of the syndrome which bore the name 'Stein-Leventhal syndrome' until the middle of the twentieth century when it became known as PCOS.

During the 20th century, it became possible to assay hormone levels and in 1990 The National Institute of Health (NIH) consensus group included biochemical hyperandrogenism as a diagnostic criterion for PCOS [5]. However, polycystic ovarian morphology was not considered to be important for diagnosis at this time. With the ability to non-invasively assess the ovaries using ultrasound, this was revised and included in the 2003 Rotterdam criteria for diagnosis of PCOS [6]. The diagnostic criteria were updated in 2006 by the Androgen Excess and PCOS (AE-PCOS) Society [7]. Outlined in Table 1.1 are the main consensus groups and the definitions of PCOS that were agreed upon. The Rotterdam criteria include a broader spectrum of PCOS than the NIH and AE-PCOS society criteria, as hyperandrogenism does not need to be present for the diagnosis. In December 2013, the Endocrine Society endorsed the Rotterdam criteria for the diagnosis of PCOS in pre-menopausal women [8].

Recently it has been suggested that Anti Mullerian Hormone (AMH) measurement could itself become a diagnostic criterion for PCOS [9] or as a substitute for polycystic ovarian morphology on ultrasound [10]. However, others have found very high levels of AMH to be specific but not sensitive for PCOS and do not support its use in the diagnosis [11]. The diagnosis of PCOS continues to be debated and is likely to evolve with time.

Table 1.1 Diagnosis of Polycystic Ovary Syndrome

Consensus Group	Year	Criteria
National Institute of Health (NIH) Bethesda, USA[5]	1990	Chronic anovulation **and** Clinical and/or biochemical signs of hyperandrogenism **and** Exclusion of other aetiologies*
European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine Rotterdam[6]	2003	Oligo- and/or anovulation **and/or** Clinical and/or biochemical signs of hyperandrogenism **and/or** Polycystic Ovaries **and** Exclusion of other aetiologies* (2 or more of the first three criteria must be present and the exclusion of other aetiologies)
The Androgen Excess and PCOS Society (AE-PCOS)[7]	2006	Hyperandrogenism (clinical and/or biochemical) **and** Ovarian dysfunction (oligo-anovulation and/or polycystic ovaries) **and** Exclusion of other aetiologies* and **

*Congenital Adrenal Hyperplasia, Androgen secreting neoplasms, Cushing's syndrome, Thyroid dysfunction, Hyperprolactinaemia, Idiopathic Hirsutism and **Syndromes of severe insulin resistance

1.1.3 Limitations of Research

PCOS is a syndrome and not a disease entity; it is a collection of clinical features for which there is no single diagnostic test. The disorder is heterogeneous and different phenotypes exist depending on the diagnostic criteria used. There are no internationally agreed assays for measuring androgens in women, nor which androgens should be measured. The Endocrine Society have stated that biochemical hyperandrogenism refers to an elevated serum androgen level and includes an elevated total, bioavailable or free serum testosterone level [8]. It is acknowledged

in the guideline that there is no absolute level which is diagnostic of PCOS. The ultrasound criterion for diagnosis of PCOS is also debated, with one group recently suggesting that the follicle number should be increased from to 12 to 19 [9]. These factors must be taken into account when comparing research studies as the groups studied may not be comparable.

1.1.4 Pathogenesis

The aetiology of PCOS is yet to be determined and is likely to be multifactorial. Hypotheses have focused on the following observed physiological abnormalities: hypersecretion of Luteinising Hormone (LH), increased ovarian androgen production and insulin resistance. Other hypotheses include prenatal androgen exposure, low birth weight and premature pubarche, and adipose tissue expandability.

1.1.4.1 Abnormal Hypothalamic Pituitary Function

Hypersecretion of LH relative to follicle stimulating hormone (FSH) from the pituitary was one of the earliest findings in women with PCOS. This has been attributed to an increased gonadotropin-releasing hormone (GnRH) pulse frequency from the hypothalamus which increases production of LH relative to FSH [12]. The higher LH levels result in an increase in ovarian theca cells androgen production [13] as explained below.

The disordered GnRH secretion could be secondary to an intrinsic defect in the GnRH pulse generator. An alternative explanation is that GnRH is less sensitive to and less inhibited by oestradiol and progesterone in women with PCOS [14]. Treatment with anti-androgens restores the GnRH pulse sensitivity to oestradiol and progesterone inhibition feedback [15]. This suggests that the hypothalamus and pituitary have a role in the pathogenesis of PCOS but hyperandrogenism precedes

the disordered GnRH secretion. A hypothalamic-pituitary defect is unlikely to explain the pathogenesis of PCOS alone, as not all women with PCOS have elevated LH levels.

1.1.4.2 Ovarian Androgen Production

In the ovary, theca cells synthesise androgens and granulosa cells synthesise oestrogen. LH stimulates the theca cells to produce androstenedione mediated by cytochrome P-450c17. The androstenedione is then converted to testosterone by 17 β-hydroxysteroid dehydrogenase (17β) or aromatized by cytochrome P-450arom to form oestrone. Oestrone is then converted to oestradiol also within the granulosa cell. FSH regulates the aromatase activity of the granulosa cells. In normal women androstenedione is preferentially converted to oestradiol.

In PCOS the theca cells are more efficient at converting androstenedione to testosterone than normal theca cells [16]. There is also evidence to suggest increased theca cell proliferation [13] and increased cytochrome-P17 transcription within the PCOS theca cells [17]. The ovaries preferentially synthesise androgens when there is a higher LH relative to FSH concentration which is another contributing factor to the higher ovarian androgen production in PCOS.

1.1.4.3 Insulin Resistance

Insulin resistance is defined as a decreased ability of insulin to stimulate glucose uptake in adipocytes, skeletal and cardiac muscle cells, suppress hepatic glucose production and suppress lipolysis. In people with insulin resistance, higher levels of insulin are required to achieve the metabolic effects of insulin [18]. In 1980 basal- and glucose-stimulated hyperinsulinaemia was reported in women with PCOS compared to weight-matched controls [19]. The positive correlation of insulin and

androgen levels in women with PCOS suggests that insulin might contribute to the hyperandrogenism [19]. Interestingly, insulin action on steroidogenesis in the PCOS ovary is preserved but insulin action on glucose metabolism is decreased [20]. Theca cells from women with PCOS are more responsive to the androgenic effects of insulin than controls [21]. Insulin also acts synergistically with LH in the ovary, to increase androgen production [22].

In the liver, insulin decreases the synthesis of sex hormone binding globulin, resulting in an increase in free testosterone in the circulation [23].

Insulin may also have an effect on the hypothalamic-pituitary axis. In rat pituitary cells, it has been shown that insulin enhances GnRH-mediated LH and FSH secretion [24].

The hyperinsulinaemia in PCOS could potentially explain the LH hypersecretion and the increased ovarian androgen production.

1.1.4.4 Prenatal Androgen Exposure

In 1982 it was demonstrated that female rhesus monkeys exposed to exogenous androgens prenatally had ambiguous genitalia at birth [25]. Long-term follow up of the monkeys found the development of a PCOS-like phenotype, in particular those that were overweight, with hyperinsulinaemic androgen excess, elevated LH levels, polycystic ovaries, dyslipidaemia and increased visceral adiposity [26]. However, this finding has yet to be confirmed in humans. A longitudinal study of 244 unselected girls recruited prenatally, failed to demonstrate an association between diagnosis of PCOS at age 15 and maternal hyperandrogenism throughout pregnancy or foetal hyperandrogenism at birth [27]. The diagnosis of PCOS in adolescents is challenging and a longer follow up and larger study group may have yielded

different results. It is also possible that the sampling of androgens during pregnancy missed a window of maternal or foetal androgen excess [28].

1.1.4.5 Low-birth Weight and Premature Pubarche

Girls with low birth weight and premature pubarche, experience menarche before 12 years of age and develop hyperinsulinaemic androgen excess [29]. Ibanez *et al* have treated this group with metformin pre and during puberty and have found this prevents the development of features of PCOS [30]. The adipose tissue expandability hypothesis, explained below, could explain why low birth weight girls are predisposed to developing PCOS.

1.1.4.6 Adipose Tissue Expandability

The adipose tissue expandability theory, proposed by Virtue and Vidal-Puig [31], is based on the idea that subcutaneous (SC) adipose tissue has a limited capacity to expand safely. Once the individual capacity (determined by genetic and environmental factors) has been exceeded, a lipotoxic state develops. This is characterised by dyslipidaemia, an unfavourable adipocytokine profile and lipid deposition in non-subcutaneous adipose tissue and organs such as the liver or muscle, resulting in insulin resistance. This theory could explain the hyperinsulinaemic androgen excess seen in PCOS. Some patients develop symptoms after a period of weight gain. Other women with PCOS are of normal weight and these women may have exceeded their individual capacity of adipose tissue expansion.

The prenatal and early years is an essential time when the adult number of adipocytes is determined. Low birth-weight babies have their growth restricted in utero and it is proposed that the number of pre-adipocyte precursors are reduced,

reducing the amount of adipose tissue development. Some will catch-up during the post natal period but development of fat free mass is prioritised, resulting in little subcutaneous fat mass accumulation. These girls will therefore have a lower set point for exceeding their individual capacity for adipose tissue expansion and therefore may be predisposed to developing PCOS [32].

1.1.4.7 Genetic Factors

There is often a clustering of PCOS within families [33, 34] and twin studies have shown a heritability of 75% for PCOS with a correlation of 0.71 between monozygotic twins and 0.38 between dizygotic twins [35]. This suggests that there is a genetic predisposition/factor involved in the aetiology of PCOS. There have been numerous studies looking for a genetic defect in PCOS using a candidate gene approach, but the results of these are inconclusive [36]. The candidate gene approach is limited by the lack of understanding into PCOS and it is hoped that using the genome-wide association study (GWAS) approach will overcome this. An initial GWAS in China found association of PCOS (Rotterdam criteria) with the following three loci: 2p16.3 (luteinising hormone/choroidogonadotrophin receptor; LHCGR), 2p21 (thyroid associated protein; THADA) and 9q33.3 (DENN/MADD domain containing 1A; DENND1A) [37]. Two of these loci, THADA and DENND1A have been confirmed to be present in a European PCOS cohort (NIH criteria) but there was insufficient power to confirm LHCGR as a risk locus [38]. McAllister *et al* have demonstrated overexpression of DENND1A in polycystic ovary theca cells and concluded that the gene plays a key role in the hyperandrogenaemia seen in PCOS [39]. A GWAS has identified THADA as a novel Type 2 diabetes (T2DM) gene associated with pancreatic beta cell dysfunction [40]; this might explain in part the increased risk of T2DM observed in PCOS.

The FTO gene has been associated with obesity and a meta-analysis of European data [41] and Chinese data [42] have confirmed the association of the FTO gene with PCOS. In the Chinese data, this positive association was observed in both obese and non-obese women.

The Chinese have demonstrated different genotype-phenotype correlations of PCOS [43] which suggests it is a heterogeneous condition with different genes contributing to the different phenotypes seen.

1.1.5 Prevalence

PCOS is the commonest endocrinopathy in pre-menopausal women. During the 1990s, studies in the United States of America (USA) [44, 45], Spain [46] and Greece [47] using the NIH criteria for diagnosis, found prevalence rates of PCOS between 4-6.8%. However, an Oxford study conducted at the same time found prevalence rates of between 8% (using NIH criteria) and 26% (using polycystic ovaries on ultrasound and an additional feature of PCOS as diagnostic criteria) [48]. In a study of unselected obese, Spanish women a PCOS prevalence rate of 28.3% was demonstrated using the NIH criteria [49]. More recent studies [50-52] have found similar prevalence rates of PCOS using all three consensus group definitions; these are summarised in Table 2. However, an analysis of a USA commercial medical database estimated the prevalence of PCOS at 1.6% [53]. This much lower prevalence rate in a large population group of 12,171,830 may be attributed to the analysis relying on International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes and none of the group were assessed clinically. March *et al* [50] in their community Australian study found that 68% of women with

PCOS were undiagnosed and this could also contribute to the difference in prevalence rates seen between the studies in Table 1.2 and the American study.

Table 1.2 Prevalence of Polycystic Ovary Syndrome by Different Diagnostic Criteria

Study Group	Population number	NIH	Rotterdam	AE-PCOS
Australia[50]	728	8.7%	11.9%	10.2%
Iran[51]	1126	7.1%	14.6%	11.7%
Turkey[52]	392	6.1%	19.9%	15.3%

1.1.6 Clinical Features

Traditionally, PCOS has been seen as a syndrome with reproductive and dermatological features but more recently metabolic components have been recognised.

1.1.6.1 Reproductive

Approximately 70% of women with PCOS have oligomenorrhoea or amenorrhoea [54]. Menstrual cycles are irregular and can be anovulatory resulting in infertility in up to 30% [54]. Women with PCOS are also more likely to develop gestational diabetes in pregnancy and have a higher risk of pregnancy complications than controls [55].

1.1.6.2 Dermatological

Hirsutism is present in 66% of women with PCOS, 25% have male pattern balding or acne, and acanthosis nigricans (a sign of insulin resistance) is seen in 2.5% [54].

1.1.6.3 Metabolic

Women with PCOS are commonly overweight or obese. Although prevalence rates vary greatly (6-100%) depending on population studied and diagnostic criteria used, a recent systematic review and meta-analysis estimated the pooled prevalence to be 61% [56]. Insulin resistance is common in PCOS with prevalence rates reported from 44 to 70% [57] and has been shown to be independent of obesity [58]. Dyslipidaemia is present in up to 70% of women with PCOS [59]. There is an adverse lipid profile with high triglycerides, low high-density lipoprotein cholesterol (HDL-cholesterol), increased low density lipoprotein cholesterol (LDL-cholesterol) and increased non HDL-cholesterol [60]. A meta-analysis has found that this adverse profile remained after body mass index (BMI) matching [60]. Hypertension has been reported to be up to three times higher in PCOS women compared to controls [61] however, obesity could be an important confounding factor [62]. Impaired glucose tolerance (IGT) and T2DM are elevated in women with PCOS in both BMI and non-BMI matched studies [63] and will be discussed in more detail later. With many of the individual components of the metabolic syndrome more prevalent in the PCOS population, it is not surprising that the prevalence of metabolic syndrome is itself increased [63]. Obstructive sleep apnoea (OSA) has been found to be five- to thirty-times higher in women with PCOS compared to BMI-matched controls [64]. Non-alcoholic fatty liver disease (NAFLD) has been found in 0-100% of women with PCOS depending on the criteria used for diagnosis of both PCOS and NAFLD and the presence of obesity [65].

1.1.7 Natural History

The clinical features of PCOS could result in long term health consequences for women with the syndrome.

1.1.7.1 Cancer

Oligomenorrhoea or amenorrhoea in the presence of pre-menopausal oestrogen levels could in theory lead to endometrial hyperplasia and an increased risk of endometrial cancer. A recent meta-analysis has found that women with PCOS have an increased risk of endometrial cancer (Odds Ratio (OR) 2.79; 95% Confidence Interval (CI) 1.31-5.95, $p<0.008$) and in those under 54 years of age this was increased further (OR 4.05; 95% CI 2.42-6.76, $P<0.00001$) [66]. The meta-analysis also evaluated breast and ovarian cancers. There was no significant increased risk in either breast or ovarian cancer, except in women under 54 years of age where there was an increased risk of ovarian cancer (OR 2.52; 95% CI 1.08-5.89, $p<0.03$) [66]. However, no adjustment was made for obesity,which is common in PCOS and is itself a risk factor for endometrial cancer. Work conducted in our own group has used a large, community-based database to show that women with PCOS did not have an increased risk of cancer, including endometrial, compared to age- and BMI-matched controls [67].

1.1.7.2 Cardiovascular Disease

Obesity, hypertension, dyslipidaemia and T2DM are all risk factors for cardiovascular disease. In 1992 a metabolic risk factor model predicted a 7 fold-increase in myocardial infarction (MI) in women with PCOS [61]. However, early retrospective cohort studies of women with PCOS did not reveal a significant excess of cardiovascular death or morbidity [68, 69] compared to the general population.

The nurses' health study, a prospective cohort study of 82,439 nurses with 14 years of follow-up, found a significantly increased risk of non-fatal or fatal coronary heart disease in women who had reported very irregular menstrual cycles (age-adjusted relative risk 1.67, 95% CI 1.35-2.06) which remained after adjustment for body mass index and other confounding factors (multivariate risk ratio 1.53, 95% CI 1.24-1.90) [70]. Although there was no confirmed diagnosis of PCOS in these women, the commonest cause for irregular menstrual cycles is PCOS. A second prospective cohort study with follow-up for 40 years found an increased risk of cardiovascular mortality in women who had a past history of irregular menstrual cycles (age adjusted hazard ratio 1.42, 95% confidence interval 1.03-1.94), but this did not remain significant after adjusting for BMI (age adjusted hazard ratio 1.35, 95% CI 0.97-1.52) [71]. A subgroup analysis of The Women's Ischemia Syndrome Evaluation (WISE) study found that postmenopausal women with features of PCOS had a significantly worse cumulative 5-year cardiovascular event-free survival than women without clinical features of PCOS, 78.9% versus 88.7% p=0.006 [72]. A meta-analysis in 2011 found the relative risk for coronary heart disease or stroke to be 2.02 comparing women with PCOS to women without PCOS. This increased relative risk was reduced to 1.55 following adjustments for body mass index (BMI) [73]. However, the research group who predicted a 7-fold increase in myocardial infarction in women with PCOS did not confirm this in their own 21 year controlled follow-up study. There was no difference in MI, stroke or mortality between the two groups, although the PCOS group had a higher prevalence of hypertension and elevated triglyceride levels [74]. Work conducted in our own group has used a large, community-based database to show that women with PCOS did not have an increased risk of large vessel disease but there was an increased risk of T2DM

compared to age- and BMI-matched controls [67]. The studies to date are therefore conflicting with respect to clinical cardiovascular disease. Further, large prospective cohort studies are thus needed with clearly defined PCOS phenotype groups and a longer term follow-up, in order to clarify this association.

1.1.7.3 Type 2 Diabetes

As mentioned above, there is an increased risk of developing T2DM in PCOS. A number of research groups have studied this and the prevalence rates for T2DM in women with PCOS vary from 1.6%-10% depending on the criteria used for the diagnosis and the age, ethnicity and BMI of the population. This is summarised in Table 1.3.

Table 1.3 Prevalence of Type 2 Diabetes in Polycystic Ovary Syndrome

Author	Population	Type 2 Diabetes
Ehrmann et al 1999[75]	122 USA PCOS Oligo/amenorrhoea+hisutism, acne Infertility+hyperandrogenism Mean age 25.5	ADA criteria 10% T2DM
Legro et al 1999[76]	254 US PCOS, 80 controls Anovulation and biochemical hyperandrogenism Age 14-44,	WHO criteria 7.5% T2DM
Palmert et al 2002[77]	27 USA adolescents Oligomenorrhoea + biochemical hyperandrogenism Mean age 16.7 and BMI 38.4	WHO criteria 3.7% T2DM
Ehrmann et al 2005[78]	408 USA women Oligo/amenorrhoea + biochem hyperandrogenism Mean age 28.7 BMI 36.2	ADA criteria 4% T2DM
Trolle et al 2005[79]	91 Danish women (27 OGTT) Oligo/amenorrhoea + elevated testosterone (NIH) Mean age 30.3 BMI 32.9	WHO criteria 1.1%T2DM (FPG)
Chen et al 2006[80]	102 Chinese women Rotterdam criteria 14-41 years, mean BMI 21.74	WHO criteria 1.9% T2DM
Vrbikova et al 2007[81]	244 Czech PCOS, 57 controls Rotterdam criteria Mean age 27.4 BMI 27.5	 1.6% T2DM
Weerakiet et al 2008[82]	170 Thai women Rotterdam criteria Mean age 28.6 and BMI 27.1	ADA criteria 10% T2DM

ADA, American Diabetes Association; WHO, World Health Organisation; T2DM, Type 2 diabetes; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test

1.2 PCOS and Glucose Tolerance

As outlined above, insulin resistance contributes to the pathogenesis of PCOS, and T2DM is seen in up to 10% of women with the syndrome. Insulin resistance and beta cell dysfunction contribute to glucose intolerance which can progress to diabetes. This will be discussed in more detail along with the relationship to obesity and inflammation in the following section.

1.2.1 Insulin Resistance

Insulin resistance (IR) has previously been defined and its contribution to the pathogenesis of PCOS outlined.

The prevalence of IR in PCOS varies greatly in published studies, from 44-95% [57, 83, 84] compared to 10-25% of the general population [83]. The wide variation could be attributed to the use of different diagnostic criteria for PCOS, different methods to assess and define IR, and differences in BMI and ethnicity. For example, a group investigating IR in different phenotypes of PCOS, found that 71.4% of the study population were IR. However, 80.4% of the classic phenotype, 65% of the ovulatory phenotype and 38.1% of the normoandrogenic phenotype were IR [85]. An Australian group found that 95% of obese women with PCOS had IR compared to 75% of lean women with PCOS [84].

Insulin binds to its cell membrane receptor and activates intracellular cascades through the phosphoinositide 3-kinase (PI3K) pathway and the mitogen-activated protein kinase (MAPK) signal transduction pathway [86]. The PI3K pathway mediates glucose uptake by increasing the translocation of the insulin-responsive glucose transporter, GLUT 4, from intracellular vesicles to the cell surface; increases glycogen synthesis via activation of Akt/protein kinase B (Akt/PKB) and regulates protein synthesis and degradation via rapamycin (mTOR) [87]. The MAPK pathway controls cell growth and differentiation [87]. In women with PCOS, abnormalities in cell responsiveness to insulin and insulin signalling defects have been described. The major defect is in the early stages of insulin signal transduction, post receptor binding. This has been demonstrated in adipocytes [88, 89] and skeletal muscle [90, 91]. The defect appears to be secondary to increased inhibitory serine

phosphorylation of the insulin receptor and insulin receptor substrate (IRS)-1 [57]. In adipocytes there is also reduced expression of GLUT-4 [92]. As mentioned previously, in ovarian granulosa-lutein cells insulin resistance is selective, affecting metabolic but not other actions of insulin [20]. However, in skeletal muscle it is thought that both metabolic and mitogenic pathways are affected [93].

1.2.2 Beta Cell Dysfunction

Insulin is secreted by pancreatic β-cells in response to a glucose stimulus. More insulin is secreted in an environment of insulin resistance, resulting in hyperinsulinaemia [94]. When the β-cells are not able to secrete enough insulin to meet the body's requirements, glucose intolerance develops [95]. Therefore, insulin secretion should be investigated in the context of insulin sensitivity.

Increased fasting insulinaemia and increased insulinaemia at 2 hours after an oral glucose tolerance test (OGTT) have been demonstrated in women with PCOS (regardless of diagnostic group) in comparison to age-matched controls [96]. However, the control women had a significantly lower body mass index than the PCOS subjects and therefore obesity could be a confounding factor. Other studies have found reduced insulin secretory responses to intravenous glucose and meals when expressed relative to the degree of insulin resistance, in women with PCOS [97, 98]. The disposition index (DI), the product of insulin sensitivity and insulin secretion, is reduced in both obese and lean women with PCOS when compared to age- and weight-matched controls [99]. These studies suggest that there is a defect in insulin secretion in response to glucose in women with PCOS.

1.2.3 Glucose Intolerance

When the body is no longer able to maintain blood glucose levels in the normal range, glucose intolerance develops. This occurs when there is a defect in the secretion of insulin and/or the pancreatic cells are unable to compensate for the body's insulin resistance. The World Health Organisation definitions of dysglycaemia and diabetes are in Table 1.4.

Table 1.4 World Health Organisation: Thresholds for Diagnosis of Diabetes and Dysglycaemia.

	Venous plasma glucose (mmol/L)
Normal	Fasting <6.1 **and** 2hr glucose during OGTT <7.8
Diabetes	Fasting ≥ 7.0 **or** 2 hour glucose during OGTT ≥ 11.1 **or** HbA1c ≥48mmol/mol
Impaired Glucose Tolerance	Fasting <7.0 **and** 2 hour glucose during OGTT ≥ 7.8 and <11.1
Impaired Fasting Glycaemia	Fasting ≥ 6.1 **and** <7.0

OGTT, Oral glucose tolerance test 75 grams of glucose. An HbA1c 42-47mmol/mol identifies people with 'pre diabetes'. Adapted from the World Health Organisation Website

As insulin resistance and beta cell dysfunction are present in women with PCOS, IGT would be expected. A recent meta-analysis found an increased prevalence of IGT in women with PCOS (OR 2.48; 95% CI 1.63-3.77) and this remained in studies who had BMI-matched women (OR 2.54; 95% CI 1.44, 4.47) [100]. The prevalence of IGT in PCOS ranges from 9.4-35% [75-82] depending on diagnostic criteria used for PCOS and IGT and the age, BMI and ethnicity of the subjects. The highest prevalence was in an American population; in a similar aged, unscreened female US population the prevalence of IGT was 1.6% (Third National Health and Nutrition

Survey). In women with PCOS, increasing age, a first degree relative with diabetes and a higher BMI increases the risk of developing IGT and T2DM [75, 76, 78, 101].

There is an excess of IGT in women with PCOS. This is important as IGT identifies individuals at high risk of developing T2DM and is itself a risk factor for cardiovascular disease [102, 103]. With intervention it is possible to reduce the risk of patients with IGT developing T2DM [104, 105]. Therefore, it would be important to identify women with PCOS who have IGT as it possible to intervene and improve their cardiovascular risk profile. It is debated how and when women with PCOS should be screened for glucose intolerance. The Androgen Excess Society recommends that all PCOS patients should be screened with an OGTT on diagnosis; women with IGT should have an annual OGTT to look for the development of T2DM and women with normal glucose tolerance should have a biannual OGTT [106]. The National Institute for Health and Care Excellence (NICE) have also recommended an OGTT at diagnosis and an annual OGTT to women with IGT. They suggest an annual OGTT should be considered in women at high risk of T2DM; strong family history of T2DM, BMI more than 30 (more than 25 in Asians) and in women who have had gestational diabetes. All women who do not have an annual OGTT should have a biannual OGTT or a fasting glucose annually and an OGTT if the fasting glucose is more than 5.6 mmol [107]. An OGTT is costly for the patient and healthcare provider. Research groups have attempted to identify the highest risk individuals but these have often used biochemical tests not routinely available in clinical practice [80-82]. One group used decision tree modelling based on medical history and clinical data only and were able to identify all women with normal glucose tolerance and allowed a 22% reduction in the number of OGTTs [108]. Measurement of glycated haemoglobin (HbA1c) is now accepted as a

diagnostic criterion for diabetes but it has not been shown to be effective in identifying IGT in women with PCOS [109].

1.2.4 Type 2 Diabetes

PCOS is recognised by the American Diabetes Association as a non-modifiable risk factor for Type 2 Diabetes. A meta-analysis has found an increased prevalence of Type 2 Diabetes in PCOS, [OR 4.43 (95% CI 4.06, 4.82)] and in BMI matched populations, [OR 4.00 (95% CI 1.97, 8.10)] [100]. The progression to type 2 diabetes is about 2-3% per year and as with IGT, higher BMI and family history of type 2 diabetes are strong predictors [76,101]. Fasting plasma glucose is advocated as a screening test for type 2 diabetes but in women with PCOS this underestimates the prevalence of type 2 diabetes by 50% [76]. An OGTT is the most reliable method to diagnose type 2 diabetes but as mentioned previously is expensive and time consuming. There have been a few studies looking at HbA1C for the diagnosis of type 2 diabetes in PCOS but these have shown HbA1c to be inferior to the OGTT [109-111]. Currently screening for type 2 diabetes in PCOS should be in line with guidance from the Androgen Excess Society and NICE. Identifying type 2 diabetes in women with PCOS is important as there is significant morbidity and mortality attached to the diagnosis and it is itself a risk factor for cardiovascular disease. It allows women to receive appropriate treatment and screening for microvascular disease.

1.2.5 Obesity and Visceral Adiposity

Obesity and in particular visceral adiposity (intra-peritoneal fat, also known as central fat) are independent risk factors for cardiovascular disease [112, 113, 114].

Visceral adiposity is also a risk factor for Type 2 diabetes independent of body mass index [115].

A systematic review and meta-analysis has found that in women with PCOS the prevalence of obesity and overweightness varies from 6-100% with a pooled estimated prevalence of 61% (95% CI 54-68%) [56]. When obesity alone is considered, the prevalence is 12.5-100% with a pooled estimated prevalence of 49% (95% CI 42-55%) and this is significantly higher in studies comparing women with and without PCOS [56]. The prevalence of central obesity ranged from 20-85.5% with a pooled estimate of 54% (95% CI 43-62%) and in studies comparing women with and without PCOS, the prevalence of central obesity was significantly higher [56].

It is not known whether PCOS itself contributes to obesity/central adiposity or whether obesity/central adiposity contributes to PCOS. A Spanish study diagnosed PCOS in 28.3% of overweight/obese women consulting for weight loss compared to an overall prevalence of PCOS in 6.5% pre-menopausal Spanish women [116]. However, not all women who are obese have PCOS and not all women with PCOS are obese, so obesity alone does not cause PCOS but may unmask the phenotype in women predisposed to developing the condition. In support of this, losing 5% of body weight has been shown to result in significant improvements in symptoms of hyperandrogenism and ovulatory function in women with PCOS [117].

As outlined above, women with PCOS [56] and non-obese women with PCOS [118] may have greater central adiposity than BMI matched controls. However, the former study used waist circumference >80 cm and waist to hip ratio >0.85 and the latter used ultrasound to assess central adiposity. These methods are not the gold standard

and one study, using Magnetic Resonance Imaging (the gold standard), did not find any difference in body fat distribution between PCOS patients and BMI-matched controls [119]. Central obesity in PCOS worsens hyperandrogenaemia [120, 121], anovulation [122] and is associated with greater insulin resistance [120, 123].

1.2.6 Inflammation

Central adiposity is associated with insulin resistance and cardiovascular disease, as discussed above, and has also been associated with low-grade inflammation [124]. C-reactive protein (CRP), an acute phase reactant that is a marker of systemic inflammation is elevated in obesity [125] and is itself an independent risk factor for type 2 diabetes and cardiovascular disease [126].

Interleukin-6 (IL-6), secreted by adipocytes and stromovascular cells, and tumour necrosis factor alpha (TNFα), secreted by macrophages infiltrating adipocytes, are both mediators of inflammation. They are associated with obesity [127] and both interfere with insulin signalling in adipose tissue resulting in insulin resistance [128, 129].

Circulating inflammatory markers in PCOS have recently been examined in a meta-analysis. No significant differences were found in TNF-α or IL-6 but CRP was 96% higher in women with PCOS compared to controls, an observation which remained even after studies with mismatches for BMI or prevalence of obesity were excluded [130].

1.3 PCOS and Cardiovascular Risk

Impaired glucose tolerance, type 2 diabetes, obesity, visceral adiposity and chronic inflammation are independent cardiovascular risk factors and were discussed above. This section will examine the presence of other cardiovascular risk factors in patients with PCOS, including hypertension, dyslipidaemia and smoking status.

1.3.1 Hypertension

In a large retrospective, Chinese study, the prevalence of hypertension in PCOS was 19.2% compared to 11.9% in controls [131]. Hypertension has been reported to be up to three times higher in PCOS women compared to controls [61]. After matching for body mass index, the Chinese study found that hypertensive PCOS patients had significantly higher lipid, insulin and glucose levels than those who were normotensive [131] suggesting that hypertension in PCOS could be a marker for a worse metabolic profile, increasing the risk factors for cardiovascular disease or a marker for the metabolic syndrome. One study investigating the effect of obesity and androgens on blood pressure, found that overweight women with PCOS had similar frequencies of undiagnosed hypertension to overweight men and higher than those in non-hyperandrogenic women [132]. They also found that weight excess and hypertension in men and women with PCOS increased left ventricular wall thickness (a marker of target organ damage) [132]. Obesity could be an important confounding factor [62]. However, a Taiwanese study found that higher free androgen index was associated with increased systolic and diastolic blood pressure independent of age, BMI and insulin resistance [133].

1.3.2 Dyslipidaemia

Dyslipidaemia is present in up to 70% of women with PCOS [59]. It can present with different patterns, including low levels of high-density lipoprotein cholesterol (HDL-cholesterol), and increased triglycerides, low density lipoprotein cholesterol (LDL-cholesterol) and non HDL-cholesterol [60]. The most common pattern is the atherogenic lipoprotein phenotype characterised by hypertriglyceridemia and decreased HDL-cholesterol. It is the pattern most commonly seen in type 2 diabetes and thought to be a consequence of insulin resistance. It is seen most commonly in obese PCOS patients and is present in up to 70% of American women with PCOS and only 50% of Italian women with PCOS [134]. The prevalence of increased levels of LDL-cholesterol in women with PCOS is lower and ranges from 24 to 40% [134, 135] and is less dependent on body weight [60]. Non-HDL cholesterol is also significantly higher in women with PCOS, and remains so after adjusting for BMI [60].

1.3.3 Smoking

Work conducted in our group, using a large community-based database, found that 39% of women with PCOS were current or ex-smokers compared to 35.1% of controls [67]. Women with PCOS who smoke have a worse lipid profile than PCOS non-smokers [136, 137]. Increased testosterone and fasting insulin levels have been demonstrated in PCOS smokers compared to non-smokers [138], but another study found no differences in androgen levels [136]. As obesity may be a confounding factor in cardiovascular risk in women with PCOS, an Italian group studied smoking in lean PCOS women and found that smoking increased markers of cardiovascular

risk in this group [139]. After adjustment for BMI, triglyceride levels were still higher in PCOS smokers than non-smokers [136].

1.3.4 Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is the accumulation of fat in more than 5% of hepatocytes (confirmed on imaging or histology) with no significant alcohol history or other cause found for the hepatic steatosis. There is evidence from cross-sectional studies that NAFLD is associated with increased cardiovascular risk and disease [140-142]. However, the evidence for this from cohort studies is conflicting [143]. Potential co-founders are obesity and Type 2 diabetes which are commonly present in patients with NAFLD, and are themselves independent risk factors for cardiovascular disease.

NAFLD is present in 0-100% of women with PCOS depending on the criteria used to diagnose PCOS and NAFLD [65]. Currently, there is little evidence to suggest that NAFLD confers additional cardiovascular risk in women with PCOS.

Dawson *et al* compared the cardiovascular risk profiles in women with PCOS and women with PCOS and NAFLD [144]. They did not find a difference in lipid profile, blood pressure, biomarkers of endothelial function or inflammatory markers between the two groups, despite the PCOS and NAFLD group being heavier. They suggested that no difference was found because insulin resistance is thought to contribute to the pathogenesis of both conditions. Sprung *et al* studied endothelial function and body fat composition in women with PCOS. The hepatic intracellular triaclyglycerol pools did not account for the endothelial dysfunction and the authors concluded that PCOS women with NAFLD do not have additional cardiovascular risk compared to women with PCOS alone [145].

1.4 PCOS and Cardiovascular Disease

With the increased prevalence of cardiovascular risk factors in women with PCOS, it would be reasonable to expect an increase in cardiovascular events such as myocardial infarction or stroke. Three large prospective cohort studies have found an increased risk of cardiovascular events in women reporting clinical features of PCOS [70-72] and this was discussed in more detail in section 1.1.7.2.

A meta-analysis in 2011 found the relative risk for coronary heart disease or stroke was 2.02 comparing women with PCOS to women without PCOS. This increased relative risk was reduced to 1.55 following adjustments for BMI [73]. However, a large community-based, controlled study of young women with PCOS failed to find an increased risk of large vessel disease [67]. This may have been because the population was young and yet to develop cardiovascular events. A further meta-analysis in 2014 examined non-fatal cardiovascular events in women with PCOS. In women over 45 years, the risk of non-fatal stroke was significantly increased but this became non-significant after matching for BMI [146]. In the group as a whole there was no increased risk of non-fatal stoke or non-fatal coronary heart disease [146].

Two studies with 21 and 23 years of follow-up found no difference in cardiovascular events between PCOS women and controls [74,147]. However, a 20 year retrospective cohort study found (age-group-specific) odds ratios for the prevalence of MI and angina in women with PCOS compared to the local female population ranged between 2.6 (95%CI 1.0, 6.3) and 12.9 (95% CI 3.4, 48.6) with the highest ratio being for MI in the over 65 year age group [148]. In this study there were significant correlations with age, history of hypertension and smoking.

There is thus no evidence presently of increased cardiovascular events in young women with PCOS. There may be an excess of cardiovascular events in older women with PCOS but it is not possible to ascertain the mechanism from these studies.

1.5 PCOS and Subclinical Cardiovascular Disease

Clinical cardiovascular events, such as MI and stroke, do not appear to be increased in young women with PCOS but are possibly increased in older women with the syndrome. It would be preferable to determine whether women with PCOS are at increased risk of developing cardiovascular events before they happen. Coronary atherosclerosis was found in 50% of young men (mean age 22 years) killed in the Vietnam War [149]. Is it therefore possible to identify cardiovascular disease in young asymptomatic women with PCOS?

1.5.1 Subclinical Cardiovascular Disease

Risk factors for cardiovascular disease identify people at higher risk of cardiovascular events but do not assess their individual burden of disease. They are useful therapeutic targets but do not identify an individual's risk of sustaining an event. Cardiovascular events are preceded by the development of atherosclerosis, endothelial and myocardial dysfunction, which may serve as useful surrogate measures of disease risk.

Atherogenesis begins at sites of injury to the endothelium (the inner layer of the arterial vessel wall), often caused by local shear forces. This is exacerbated by high levels of LDL-cholesterol, low levels of HDL-cholesterol, smoking, diabetes, hypertension, oxidative stress and systemic inflammatory states. These factors

decrease the endothelial cell production of nitric oxide (NO). This vasodilatory molecule inhibits key events in the development of atherosclerosis such as leukocyte adhesion and migration, smooth muscle cell proliferation, platelet adhesion and aggregation. When NO production is decreased, the vasodilatory capacity of the blood vessel is reduced and the normal protective function of the vascular endothelium is lost. LDL-cholesterol can then infiltrate into the subendothelial space where it is oxidised. The dysfunctional endothelial cells also express adhesion molecules (such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1)) that promote the binding of circulating monocytes to the endothelium. The monocytes migrate into the carotid intima where they differentiate into macrophages. The macrophages internalise the oxidised LDL-cholesterol and become lipid-laden foam cells. Inflammatory mediators invade this lesion and promote the formation of a fibrous cap. Ongoing inflammatory response in the vascular wall promotes the accumulation of more LDL-cholesterol and the growth of the lesion. The fibrous cap can rupture, resulting in platelet aggregation and coagulation leading to the formation of thrombus. The thrombus can partially or totally occlude a vessel resulting in a cardiovascular event [150-152].

Subclinical cardiovascular disease is the identification of these early changes in asymptomatic individuals, before the progression to significant narrowing of the blood vessels and symptomatic disease. This is important to improve risk stratification and prevent clinical disease. For example, one study has demonstrated that identifying subclinical cardiovascular disease improves medication adherence in asymptomatic individuals [153].

1.5.2 Assessment of Subclinical Cardiovascular Disease

There are several non-invasive and invasive methods to identify subclinical cardiovascular disease. These include assessment of endothelial function and measuring the burden of arterial calcification and plaques, carotid artery intima-media thickness, arterial stiffness and diastolic function of the heart.

Peripheral endothelial function can be measured using plethysmography of the forearm circulation, flow-mediated vasodilatation (FMD) of the brachial artery and finger plethysmography [152]. The former is limited by the need for arterial puncture and the latter is a relatively new method. The most commonly used method is the FMD technique. This involves placing an occluding cuff over an artery immediately proximal or distal to the site to be imaged, for 5 minutes. In response to the ischaemia, endothelial nitric oxide is released during the reactive hyperaemia (flow-mediated) causing dilatation of the artery. Measurements of the artery diameter are taken pre and post cuff inflation. When endothelial dysfunction is present, FMD is reduced. Biochemical indices of endothelial function can also be measured including total nitric oxide, nitrite and nitrate.

Coronary artery calcification (CAC) can be assessed using electron-beam computed tomography (EBCT). Coronary calcium reflects the atherosclerotic plaque burden, because calcium deposits are related to the lipid and apoptotic remnants of the plaque [154]. The advantage of this technique is that it is non-invasive, however there is exposure to a small amount of radiation and no information on the susceptibility of plaques to rupture is gained [152].

Carotid intima-media thickness (CIMT) is measured in the common carotid artery, using B-mode ultrasonography. It reflects the diffuse thickening of the intimal layer

during atherosclerosis. This non-invasive technique does not expose a subject to radiation and is highly reproducible. However, as with EBCT CAC measurement, it does not give an indication of susceptibility of plaques to rupture [152]. High resolution magnetic resonance imaging is a non-invasive method which does not expose an individual to radiation and is able to measure plaque volume, composition and susceptibility to rupture [152]. However, this is not widely available and is expensive. Coronary angiography allows direct visualisation of the plaque burden and degree of luminal narrowing of the coronary arteries but is invasive.

Arterial stiffening occurs when the arterial wall becomes less elastic or distensible resulting in a reduction of the capacity of the vessel to accommodate volume changes throughout the cardiac cycle. Atherosclerosis is thought to contribute to arterial stiffening [155,156]. Possible mechanisms of arterial stiffening include derangement of the elastic laminae with increasing age [157]; hypertension induced structural changes such as hypertrophy and changes in the extracellular matrix [158]; disruption of the nitric oxide pathway in the endothelium affecting the vascular tone and elastic properties of the arterial wall [159] and a direct effect of insulin on the vessel wall or elevated glucose levels resulting in collagen cross linking due to non-enzymatic glycation [160]. Arterial stiffness can be measured non-invasively using applanation tonometry and this technique is discussed in more detail in Chapter 2. The principal measurement recorded is the pulse wave velocity (PWV); the stiffer the arteries the higher the PWV.

There are two aspects of diastolic heart function; relaxation and stiffness, which describe different properties of the myocardium. The relaxation phase (a dynamic and energy consuming phase) begins at the end of systolic contraction and continues throughout isovolumic relaxation and early diastolic filling. Impairment of this

phase is due to regional dyssynchrony or a reduction in energy supply such as myocardial hypertrophy or ischaemia. Diastolic stiffness is measured at the end of diastole and reflects passive ventricular motion; it is dependent on left atrial contractility and the viscoelastic properties of the left ventricle. Changes in stiffness result from changes in the composition of the myocardium such as interstitial fibrosis or left ventricular hypertrophy [161]. Diastolic function may by assessed by pulsed myocardial velocity imaging of the lateral and septal mitral annulus averaged over three consecutive cycles. Early diastolic (e') and atrial (a') myocardial velocities are recorded. The e' reflects passive filling and a' reflects filling related to atrial contraction. In a compliant ventricle, most of the filling occurs passively and e' is larger than a'. In impaired relaxation, diastolic dysfunction, e' declines and a' increases as atrial contraction contributes more to diastolic filling.

1.5.3 Endothelial Dysfunction

Endothelial dysfunction measured by FMD has been shown to correlate with coronary artery endothelial function [162] and be an independent predictor of coronary artery disease [163]. Endothelial dysfunction in women with PCOS, assessed by reduced FMD, has been demonstrated in some [164,165] but not all [166,167] studies. A recent meta-analysis has found that the pooled mean FMD was 3.4% lower in women with PCOS compared to controls and that the difference was not influenced by BMI or age [168].

A few studies have measured total nitric oxide in women with PCOS and controls and not found any differences between the groups [169, 170]. Work in our own group has confirmed this using more sensitive methods to assess nitric oxide and its metabolites [171].

1.5.4 Coronary Artery Calcification

A meta-analysis of 30 prospective studies in asymptomatic subjects found that the presence of CAC was associated with an increased risk of cardiovascular and all-cause mortality [172]. The American College of Cardiology Foundation and the American Heart Association have provided pooled data for outcome with CAC scores [173]. In the absence of CAC, there was a low risk of cardiovascular events. However, the relative risk of cardiovascular events in those with intermediate and high levels of CAC scores compared to those with low levels were 4.3 and 7.2 respectively.

The Dallas heart study did not find an increase in CAC in pre-menopausal women with PCOS compared to controls despite the PCOS group having a greater prevalence of cardiovascular risk factors and higher BMI and blood pressure [174]. However, other studies have found an increased prevalence of CAC in pre-menopausal women with PCOS [175-177] and post-menopausal women with PCOS [178].

1.5.4 Carotid Intima Media Thickness

A systematic review and meta-analysis of CIMT and cardiovascular events found an increase in CIMT thickness of 0.10mm predicted a 15% increased risk of myocardial infarction and an 18% increased risk of stroke [179].

Some studies have demonstrated an increase in CIMT in young women with PCOS compared to BMI and age matched controls [180-185] but not others [166, 186]. One study demonstrated a difference in CIMT in older PCOS subjects only [187]. The mean difference in CIMT among women with PCOS compared to controls was found to be 0.072 mm (95% CI 0.04-0.105, $P<0.0001$) in a meta-analysis of high

quality studies, however there was no significant difference in studies with fair or lower quality [188].

1.5.5 Arterial Stiffness

Augmentation Index and PWV, both measures of arterial stiffness, independently predict cardiovascular events and/or mortality in the general population [189, 190], the elderly [191, 192] and in disease states including end-stage renal disease [193, 194], hypertension [195, 196] and impaired glucose tolerance/diabetes [197]. Arterial stiffness has also been associated with cardiovascular risk factors including hypercholesterolaemia [198] and smoking [199].

In women with PCOS some studies have found evidence of increased arterial stiffness [201-203]. One study found evidence of increased arterial stiffness in the brachial artery but not the aorta of women with PCOS [204].

1.5.6 Diastolic Dysfunction

Risk factors for the development of preclinical diastolic dysfunction include coronary artery disease, diabetes, hypertension, hyperlipidaemia and the metabolic syndrome [205]. Diastolic dysfunction has been shown to progress to heart failure and is an independent risk for all-cause mortality [206,207].

Impaired diastolic function has been reported in some studies comparing PCOS women with age and BMI matched controls [180, 208] but not all [209].

1.6 Mechanisms of Cardiovascular Disease in PCOS

The potential mechanisms of cardiovascular disease in PCOS are discussed below.

1.6.1 Obesity

Traditionally, adipose tissue has been seen as an inert store of energy but has recently been identified as a metabolically active endocrine organ which secretes adipose-tissue derived substances such as adipocytokines affecting a range of body functions. Excess adipose tissue results in obesity, a chronic, low-grade pro-inflammatory state in which the hyperplasia and hypertrophy of adipose cells causes a disturbance of adipokine secretion [210]. The systemic inflammation and abnormal production of adipocytokines have been identified as important factors in the formation and progression of atherosclerosis [211].

1.6.1.1 Adipose Tissue Distribution

The distribution of adipose tissue is important as visceral adiposity is an independent risk factor for cardiovascular disease [112, 113, 114] and there are obese people who appear to be metabolically healthy and not at increased risk for cardiovascular events [212, 213]. Waist circumference is a stronger risk factor than BMI for cardiovascular disease [113, 214, 215] and waist circumference correlates well with visceral adipose tissue volume [216]. This has been proposed as a reason for men having higher rates of cardiovascular disease than women as they are more likely to accumulate fat around the abdomen (android obesity) than women who are more likely to accumulate fat around the lower body (gynoid obesity) [217]. The Framingham study found that 40% of men and women had increased visceral fat despite modestly elevated BMI of 27 kg/m^2 in women and 28 kg/m^2 in men [218]. The adipose tissue expandability theory, mentioned previously, could explain this

finding. In a state of positive energy balance, once an individual has reached the limit of their capacity to store fat in the subcutaneous tissues a lipotoxic state develops and one of the consequences is accumulation of fat in ectopic deposits. Not all individuals increase visceral fat when overfed, only those with a defective regulation of lipid-storing genes in subcutaneous fat [219].

Women with PCOS may have greater central adiposity than BMI-matched controls in both non-obese and obese subjects [56, 118]. Whether obesity or central adiposity are independent risk factors for cardiovascular risk/disease in PCOS is yet to be established.

As part of the adipose tissue expandability hypothesis, an unfavourable adipocytokine profile is suggested and this may provide a link between visceral fat and cardiovascular disease and will be discussed further below.

1.6.1.2 Adipocytokines

Adipocytokines are peptides secreted by adipose tissue. Adipokines (adiponectin, leptin, visfatin, apelin, chemerin and acute-phase serum amyloid A) are secreted from the adipocytes whereas cytokines (TNFα and interleukins) are secreted from the stromovascular cells. Resistin is an adipocytokine secreted from both adipocytes and stromovascular cells.

Adiponectin has anti-inflammatory, anti-atherogenic and anti-diabetic effects, and promotes good endothelial function [220]. Weight loss and insulin-sensitising drugs increase plasma adiponectin levels [221]; pro-inflammatory cytokines reduce the secretion of adiponectin from cultured adipocytes [222] which provides evidence to support the link between inflammation and insulin resistance and obesity. Plasma adiponectin levels appear to be reduced in people with insulin resistance and

coronary artery disease [223]. A systematic review and meta-analysis has found that adiponectin levels are significantly lower in PCOS women compared to BMI-matched controls [224]. Low levels of adiponectin were associated with insulin resistance and PCOS severity but not with testosterone in the PCOS group [224]. However, a recent randomised controlled trial in women with PCOS found no effect of metformin on adiponectin levels despite weight loss and an improvement in insulin resistance [225].

Leptin promotes insulin resistance, hypertension, atherosclerosis, vascular inflammation, oxidative stress and endothelial dysfunction but may reduce infarct size and decrease hypoxic damage in cardiomyocytes [220]. Leptin levels rise with increasing body fat and may be a link between obesity and cardiovascular disease. In the general population leptin is associated with cardiovascular disease but this is dependent on BMI [226]. In women with PCOS, some studies have found no association of leptin with PCOS after accounting for BMI [227, 228] but others have noted increased leptin levels independent of obesity [229, 230]. One study has found that secretion of leptin is altered in women with PCOS compared to normal women [231].

Visfatin is mainly produced in visceral fat and may reduce infarct size and reduce hypoxic-induced damage. However, high levels of visfatin are associated with endothelial inflammation, plaque destabilisation, increased oxidative stress and increased pro-inflammatory cytokine levels [220]. One study has suggested that visfatin may be an important link between intra-abdominal obesity and diabetes [232] but others have disputed this [233, 234]. Visfatin increases insulin secretion and insulin receptor phosphorylation in mouse pancreatic β-cells [235] and increases with progressive β-cell deterioration in Type 2 Diabetes [236]. In women with

PCOS, circulating visfatin levels are positively associated with BMI [237-239], fasting insulin levels [239] and blood pressure [238].

Apelin reduces atherogenesis, macrophage inflammation, cardiomyocyte contractility and heart failure and increases serum adiponectin levels and decreases leptin levels [220]. Significantly lower levels of apelin have been demonstrated in women with PCOS compared to BMI matched controls [240, 241].

Chemerin is thought to reduce endothelial cell inflammation and promote angiogenesis [220]. It also plays a role in adipocyte differentiation, glucose homeostasis and lipolysis in adipose tissue [242]. Plasma levels are positively correlated with BMI, blood pressure and circulating triglycerides [243]. It has been found to be associated with arterial stiffness, after adjustment for other cardiovascular risk factors [244] but does not predict coronary atherosclerosis [245]. In women with PCOS, chemerin levels are increased and metformin has been shown to reduce levels whereas insulin increases the levels [246].

Acute-phase serum amyloid A (ASAA) is a pro-inflammatory adipokine that is increased in obese and insulin-resistant people. It is also elevated in the plasma and adipose tissue (visceral and subcutaneous depots) in women with PCOS. Treatment with metformin for six months reduces the level of ASAA [247].

Adiponectin and leptin, two of the first adipokines to be discovered, have been more extensively researched than the others. Adiponectin and apelin appear to have a protective role and be anti-inflammatory and anti-atherogenic whereas leptin and ASAA are pro-inflammatory and pro-atherogenic. Chemerin and visfatin are less clear-cut. Adipokines have a role in the metabolic abnormalities seen in PCOS but whether they are a cause or consequence of the syndrome is yet to be determined.

Resistin is secreted by macrophages and is associated with endothelial dysfunction (promotes adhesion molecules and proliferation), insulin resistance and increases secretion of pro-inflammatory cytokines [220]. It has been shown to be predictive of 5 year mortality in people who have had an atherothrombotic stroke [248]. There is no difference in resistin serum levels between PCOS subjects and matched controls but messenger RNA levels were twice as high in omental adipocytes from PCOS women [249].

Interleukins and TNF-α were discussed in section 1.2.6.

1.6.2 Insulin Resistance

Insulin resistance is present in both obese and non-obese women with PCOS [84] and could provide a link between PCOS and cardiovascular disease independently of BMI. Insulin resistance itself increases the risk for coronary artery disease [250].

Endothelial cells, vascular smooth muscle cells and macrophages all involved in the development of atherosclerosis, express insulin receptors which are down-regulated in hyperinsulinaemia [251]. The PI3K and MAPK insulin signalling pathways regulate endothelial NO production and secretion of endothelin-1 (a vasoconstrictor) respectively, and the balance of these determines the response of the vessel to insulin. In insulin resistance the PI3K pathway is down regulated but the MAPK pathway remains intact [252]. This results in a reduction in available NO and therefore a relative increase in endothelin-1 both of which contribute to endothelial dysfunction and the development of atherosclerosis, as demonstrated in mice [251].

In humans, IR has been shown to alter large artery compliance [253] and affect endothelial function [254]. Insulin is not able to reduce aortic stiffness in insulin-resistant subjects and this correlates with whole-body insulin resistance [255]. These

studies provide further evidence to support a link between insulin resistance and subclinical cardiovascular disease.

1.6.3 Hyperglycaemia

Insulin resistance and a failure of pancreatic β cells to secrete enough insulin results in hyperglycaemia. There is evidence that lowering glucose levels results in a reduction in cardiovascular events in Type 1 and Type 2 Diabetes [256-258]. An association between glucose level 2 hours after a 75 gram glucose load and cardiovascular disease has been demonstrated in the general population [259, 260]. These findings suggests that there could be a link between hyperglycaemia and cardiovascular disease.

Hyperglycaemia results in increased leucocyte adhesion to endothelial cells, an initial step in atherogenesis, likely mediated by increased expression of adhesion molecules such as VCAM-1 and ICAM-1 and increased oxidative stress [261]. Hyperglycaemia-induced advanced glycation end products (AGEs) formed by non-enzymatic reaction of glucose with proteins and lipids and can occur intra- or extra-cellularly [262]. These bind to the receptor for AGEs (RAGE) which promotes VCAM-1 expression [263]. Blocking of RAGE has been shown to prevent atherosclerosis in a mouse model [263]. High intracellular glucose concentrations also activate protein kinase C (PKC) which results in generation of reactive oxygen species [264] which are discussed below and are implicated in atherogenesis.

1.6.4 Oxidative Stress

Excessive production of oxidants in the presence of limited antioxidants results in oxidative stress and this has been proposed as a unifying mechanism for the development of endothelial dysfunction resulting from a number of cardiovascular

risk factors such as diabetes, hypertension and smoking [265]. Oxidants include reactive oxygen species (ROS), products of normal cellular metabolism derived from oxygen and reactive nitrogen species (RNS) derived from nitrogen oxide. At low levels, ROS and RNS have a beneficial role including defence against infections. At high levels, they damage DNA and cellular lipids and proteins [266]. Antioxidants such as glutathione peroxidase and paroxanase, dispose of or prevent formation of oxidants.

ROS have been implicated in the formation of the fatty streak in atherosclerosis through to plaque rupture [265]. In humans, ROS production is significantly higher in unstable angina versus stable angina, suggesting a role in modulating plaque stability [267]. Nicotinamide adenine dinucleotide phosphate oxidase (NAD(P)H) and Xanthine Oxidase (XO) are enzymes in vascular cells which generate ROS, and these have been shown to be activated in coronary arteries of patients with coronary artery disease [268].

Oxidant levels are increased in obesity [269, 270] and are decreased in response to weight loss and calorie restriction [271, 272]. Contributors to oxidative stress in obesity include hyperglycaemia, hyperlipidaemia, hyperleptinaemia and chronic inflammation [272].

A meta-analysis has found increased circulating levels of homocysteine and asymmetric dimethylarginine (both increase ROS production) in women with PCOS compared to controls [273]. Circulating glutathione, and paroxonase-1 activity (both anti-oxidants) were decreased in women with PCOS compared to control women [273]. These findings persisted when studies which BMI and age matched women in PCOS and control groups were examined [273]. This is an important finding as

obesity itself is associated with increased oxidative stress and obesity is common in PCOS. Oxidative stress may have a role in vascular dysfunction in women with PCOS independently of weight.

1.6.5 Androgens

Before the age of 75, the incidence of heart disease is lower in women than men. It has been postulated that androgens may have a detrimental effect on cardiovascular health and/or that oestrogens are protective. In support of this, adolescent boys have higher blood pressure than age-matched girls when androgen levels are increasing [274] and in rat models, castrated male rats and males rats treated with flutamide have blood pressures equivalent to female rats and significantly lower than untreated males [275]. It has been speculated that androgens cause an increase in renin activity, resulting in an increase in angiotensin II. This results in hypertension via oxidative stress causing renal vasoconstriction and by increasing sodium reabsorption [276].

However, there is an increasing amount of data suggesting that low testosterone levels in men are associated with an increase in cardiovascular mortality and morbidity [277-281]. Low testosterone may reflect poor health; obesity and Type 2 Diabetes are associated with low testosterone levels, which are risk factors for cardiovascular disease themselves and therefore possibly confounding factors. If testosterone was detrimental to cardiovascular health, it would be expected that female to male transsexual patients treated with testosterone would have significantly increased cardiovascular disease events, but this has not been found [281]. Testosterone replacement to physiological levels has been shown to improve risk factors for atherosclerosis including reduction of central adiposity and insulin

resistance, improvement of lipid and inflammatory profiles and vascular function [282]. Animal studies have shown that castration accelerates aortic plaque development whilst testosterone replacement significantly reduced plaque formation, also suggesting a link between testosterone and atherosclerosis [283]. Testosterone may exert an anti-atherogenic effect by causing vasodilatation; reducing pro-inflammatory cytokine production; reducing adhesion molecule expression and inhibiting the formation of foam cells [284]. It is not clear whether androgens are beneficial or detrimental in health.

In women with PCOS there are also conflicting results regarding cardiovascular disease and androgens. Compared to healthy controls, CIMT was increased in women with PCOS independently of obesity but related directly to androgen excess [185]. In a different study, treatment with spironolactone (an anti-androgen) improved endothelial function to the same extent as healthy controls but without a change in androgen levels [285]. However, one study found that dehydroepiandrosterone sulphate was a strong negative predictor of CIMT in women with PCOS, suggesting a protective effect of androgens [184].

1.7 Thesis Aims

PCOS is a common endocrine disorder of young women associated with many cardiovascular disease risk factors. Whether this collection of risk factors results in an increase in cardiovascular events is debated. Large cohort studies have suggested an increase in non-fatal and fatal cardiovascular events in women who reported irregular menstruation [70-72]. One meta-analysis found that women with PCOS were twice as likely to have a cardiovascular event compared to controls, however the odds ratio was reduced to 1.55 after adjustment for BMI [73]. Other studies have failed to demonstrate an increase in cardiovascular events in pre- or post-menopausal women with PCOS [67, 147, 148].

Markers of subclinical cardiovascular disease may be increased in young and middle aged women with PCOS including endothelial dysfunction [164-166], carotid intima-media thickness [180-185], arterial stiffness [164, 166, 200] and myocardial dysfunction [180, 208]. However, it is difficult to determine how much of this is due to PCOS *per se* or to obesity which is common in PCOS and is itself a risk factor for cardiovascular disease. One study has found no difference in large artery distensibility in women with PCOS compared to controls, but did find that it was inversely related to adiposity [201].

Hypothesis: Cardiovascular risk (measured by arterial stiffness, common carotid intima-media thickness and myocardial dysfunction) is increased in young women with PCOS independently of obesity compared to healthy volunteers.

Aims

1. Determine whether cardiovascular risk is increased in young women with PCOS independently of obesity.
2. Explore the relationship between regional body composition and markers of subclinical cardiovascular disease in young women with PCOS.
3. Explore the relationship between insulin sensitivity and markers of subclinical cardiovascular disease in young women with PCOS.

CHAPTER 2: METHODS

2.1 Outline of Study

2.1.1 Study Approval

The study was approved by the Research and Development department at the University Hospital of Wales (UHW) (Ref 08/RPM/4276) and the South East Wales Research Ethics Committee (Ref 08/WSE04/53), before it commenced. The study was sponsored by Cardiff University (CU) (Ref SPON CU 523-08).

2.1.2 Recruitment

Patients aged between 16 and 45 years of age were recruited from local endocrinology, dermatology and gynaecology clinics. They were provided with an information sheet giving details about the study (Appendix 1). The initial contact was followed up with a telephone call to determine interest and confirm eligibility. Healthy volunteers (HVs) aged between 16 and 45 years, were recruited from UHW and CU using poster advertisements and intranet postings (Appendix 2). Further recruitment of HVs was achieved by advertising in the local paper (Appendix 3). All interested, eligible women were provided with an information sheet (Appendix 4) and were telephoned a week later to determine if they wished to participate or not.

2.1.3 Inclusion and Exclusion Criteria of Study Participants

Patients who met the Rotterdam criteria for diagnosis of PCOS were included [6]. Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumours, hyperprolactinaemia and thyroid dysfunction were excluded by biochemical testing. Women were excluded from participation if they were pregnant, breastfeeding or if they had a history of diabetes, hypertension or hyperlipidaemia. Potential participants who were taking, or had taken in the previous 3 months, anti-

hypertensive agents, lipid-lowering agents, glucose lowering agents, weight reducing agents or glucocorticoids were also excluded.

The healthy state of the volunteers was established by history, physical examination and biochemical testing. They were included if they had regular menstrual cycles (every 27-32 days) and excluded if there was a family history of PCOS, evidence of hyperandrogenism or if they had a history of hypertension, hyperlipidaemia or impaired glucose metabolism.

All women were investigated during the follicular phase of their menstrual cycle if they had mild oligomenorrhoea or eumenorrhoea. No restrictions were applied to women with severe oligomenorrhoea or amenorrhoea.

2.1.4 Consent

All participants gave written informed consent prior to entering the study (Appendices 5 and 6).

2.1.5 Protocol

Participants attended the Clinical Research Facility (CRF), UHW at 8am following an overnight fast. All subjects had a pregnancy test to confirm that they were not pregnant before a clinical assessment, venepuncture and oral glucose tolerance test were undertaken. Computed tomography (CT) and dual energy x ray absorptiometry (DEXA) scans were performed at the Medical Physics Department in UHW, carotid ultrasound and echocardiography at The Wales Heart Institute, UHW and applanation tonometry at the CRF. The information acquired was recorded on case report forms (Appendices 7 and 8).

2.2 Clinical Assessment

The clinical assessment was undertaken by me.

2.2.1 History and Examination

Details of past medical history, medication taken currently and in the previous 3 months, contraceptive use, smoking history, family history and menstrual history were recorded. A routine physical examination was performed.

2.2.2 Blood Pressure Measurement

After five minutes of rest in a seated position, three blood pressure recordings were taken from the right brachial artery using a validated semi-automated oscillometric device (Omron 7051T; Omron Corporation, Tokyo, Japan). The mean of these recordings was used in this study.

2.3 Biochemical and Metabolic Measurements

The blood sampling was undertaken by me.

2.3.1 Sample Collection and Storage

With the subject at rest, an intravenous cannula or butterfly needle was inserted into a suitable vein in the antecubital fossa or forearm and secured. Blood was obtained directly into blood bottles via a vaccutainer or via a syringe and then decanted into blood bottles. The cannula or butterfly needle was then flushed with 5 millilitres (mls) of normal saline. The bottles were marked with the subject number and 0 minutes. The subject then received a drink containing 113 mls of Polycal® with 187 mls of water. Thirty, 60, 90 and 120 minutes later further blood samples were collected and marked with the subject number and time. The first 5 mls of blood at each collection were discarded and the cannula was flushed with 5 mls of normal saline after the sampling was completed. The cannula or butterfly needle was

removed after the 120 minutes sample was taken. Samples were centrifuged at 4000 rpm for 8 minutes and stored at -30°C prior to analysis.

2.3.2 Assays

Serum total cholesterol, high density lipoprotein cholesterol (HDL) and triglycerides were assayed using an Aeroset automated analyser (Abbott Diagnostics, Berkshire, UK); LDL cholesterol (LDL) was calculated using Friedewald's formula. Insulin was measured using an immunometric assay specific for human insulin (Invitron, Monmouth, UK) and glucose was measured using the Aeroset chemistry system (Abbott Diagnostics, Berkshire, UK). High sensitivity C-reactive protein (hsCRP) was assayed by nephelometry (BN™ II system, Dade Behring, Milton Keynes, UK) and total testosterone was measured by liquid chromatography-tandem mass spectrometry (Quattro™ Premier XE triple quadruple tandem mass spectrometer, Waters Ltd, Watford, UK). High molecular weight (HMW) adiponectin was measured by ELISA (EMD Millipore, Billerica, MA). The intra- and inter-assay coefficients of variation were all less than 9%.

2.3.3 Estimations of Insulin Sensitivity and Insulin Resistance

The hyperinsulinaemic-euglycaemic clamp is the gold standard method to measure insulin sensitivity [286]. Insulin is infused intravenously at a constant rate in subjects who have fasted overnight. Blood glucose levels are measured every 5 to 10 minutes and 20% dextrose is given intravenously at a variable rate to maintain euglycaemia. It is assumed that the hyperinsulinaemic state suppresses hepatic gluconeogenesis. After several hours of constant insulin infusion, steady state conditions are achieved for plasma insulin, blood glucose and glucose infusion. As there is no net change in glucose, the glucose infusion rate equals glucose disposal rate (GDR) in the subject. To generate an estimate of insulin sensitivity, GDR is

normalised to body weight or fat free mass (FFM). Alternatively, an insulin sensitivity index is calculated using the formula:

$SI_{Clamp} = M/(G \times \Delta I)$

where M = glucose infusion rate, G = steady state blood glucose concentration and ΔI = difference between fasting and steady-state insulin concentrations. Tam *et al* have determined that an individual with an M or GDR value of <5.6mg/kgFFM+17.7·min has an 80% chance of being insulin resistant [287]. The main advantage of this method is that whole body glucose disposal is measured directly at a defined level of hyperinsulinaemia. However, the method is labour-intensive, time consuming and expensive and for these reasons was not used as there were large numbers of subjects recruited to the study.

Alternative methods have been established to overcome the limitations of the hyperinsulinaemic-euglycaemic clamp. Two well described and widely published methods are the homeostatic model assessment (HOMA) [288] and the quantitative insulin sensitivity check index (QUICKI) [289]. HOMA-IR is a paradigm model derived from a mathematical assessment of the interaction between β-cell function and IR and is calculated using the formula:

HOMA-IR = (FPI x FPG)/22.5

where FPI = fasting plasma insulin (mU/l) and FPG = fasting plasma glucose (mmol/l). QUICKI uses a log transform of the insulin-glucose product and therefore correlates to HOMA-IR. These methods require a single fasting insulin and fasting glucose measurement and are therefore ideal for studies involving large numbers of subjects. HOMA-IR was used in this study for these reasons and because it correlates highly (R_s=0.88, P<0.0001) with the hyperinsulinaemic-euglycaemic clamp [288].

The above methods do not measure insulin sensitivity in a dynamic state. An OGTT enables assessment of glucose tolerance and insulin secretion in response to a glucose challenge. Insulin measurements at 0, 30, 60, 90 and 120 minutes after a 75 grams glucose challenge allow calculation of area under curve (AUC) insulin. The AUC insulin will be greater in an insulin resistant subject than a normal subject, as more insulin will be secreted in response to the glucose load. This is clinically important and has been associated with cardiovascular mortality [290]. Post-challenge insulin levels have also been shown to improve the performance of visceral fat adiposity in identifying subjects with metabolic disease [291]. Glucose levels 2 hours after an oral glucose challenge have also been associated with cardiovascular mortality [292, 293].

2.4 Body Composition Measurements

2.4.1 Anthropometric Measurements

The anthropometric measurements were taken by me.

Height was measured to the nearest 0.1 centimetre (cm) using a stadiometer and recorded in metres (m). Measurements were taken with the subject's footwear removed. Their head, back, buttocks and heels were placed against the wall and they were asked to look straight ahead. The headboard was then moved until firmly pushing on the vertex.

Weight was measured to the nearest 0.5 kilogram using digital weighing scales (Omron Monitor BF500, Omron Corporation, Japan). Subjects wore light clothing and had footwear removed.

Body Mass Index (BMI) was calculated as weight (kg) divided by height (m) squared.

Waist circumference was measured at minimal respiration to the nearest 0.5 cm by positioning a tape parallel to the floor and immediately above the superior iliac crests. Hip circumference was measured to the nearest 0.5 cm by positioning a tape parallel to the floor and at the greatest protrusion of the buttocks.

2.4.2 Dual Energy X Ray Absorptiometry

A whole body image was obtained (Hologic Discovery A absorptiometer) with the subject in the supine position. Body composition was determined using Auto Whole Body Fan Beam software version 13.3.0.1:3 by a single operator, Dr Sarah Darlington who was blind to the subject status. An example of the image recorded is shown in Figure 2.1. The image was subdivided into different regions by adjusting the white lines on the image to ensure all tissues were enclosed. Fat mass, lean mass and percentage fat were determined for regional areas and in total. An example of the output generated is shown in Figure 2.2.

Figure 2.1 An Example of the DEXA Image Acquired

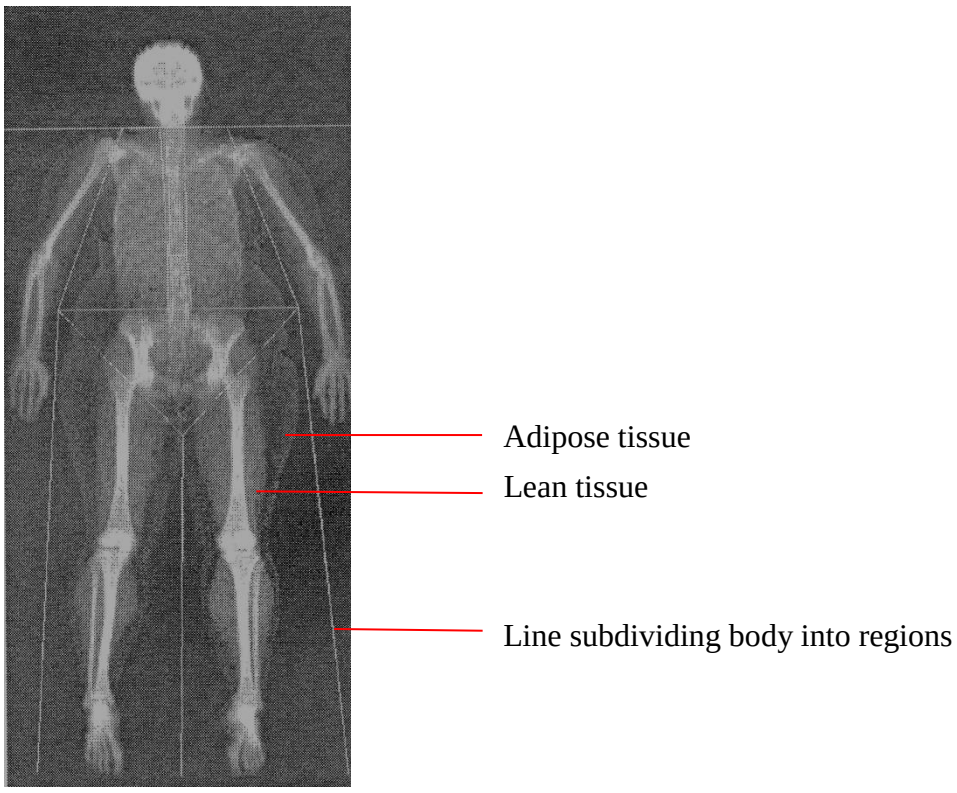


Figure 2.2 An Example of the Body Composition Results

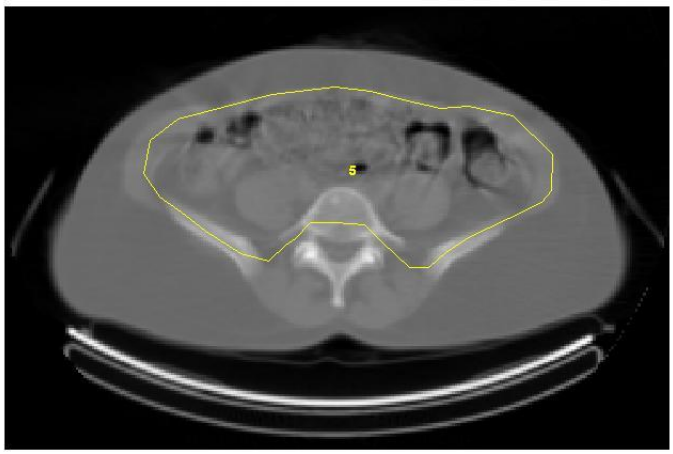
Body Composition Results

Region	Fat Mass (g)	Lean+ BMC (g)	Total Mass (g)	% Fat	%Fat Percentile YN	%Fat Percentile AM
L Arm	2006	2353	4359	46.0		
R Arm	2003	2174	4176	48.0		
Trunk	16343	22859	39201	41.7		
L Leg	7872	8048	15920	49.4		
R Leg	8153	8363	16516	49.4		
Subtotal	36376	43796	80172	45.4		
Head	1021	3093	4114	24.8		
Total	**37397**	**46889**	**84286**	**44.4**		**89**
Android (A)	2551	3777	6327	40.3		
Gynoid (G)	6574	8066	14640	44.9		

2.4.3 Computed Tomography

With the subject in the supine position, one cross-sectional scan was obtained by CT (Hawkeye, GE Medical Systems) using standard acquisition parameters (140kV, 2.5mA, 10mm slice width, 13.6 s rotation time, 256^2 pixel matrix) at the level of the fourth and fifth lumbar spines. The image was imported into MATLAB (Maths Works) and analysed by a single operator, Dr Helen Blundell who was blind to subject status. The image was segmented into areas of non-adipose tissue and adipose tissue using a fixed range of CT numbers (-120 to -80 to represent fat) derived from a previously published study [294]. The visceral adipose tissue area and total adipose tissue areas were calculated by segmenting an intra-peritoneal region and the whole image respectively as shown in Figure 2.3. Subcutaneous adipose tissue area was calculated by subtracting the visceral fat area from the total fat area.

Figure 2.3 Cross-sectional CT Scan Image



Intraperitoneal region enclosed within the yellow line.

2.5 Vascular Measurements

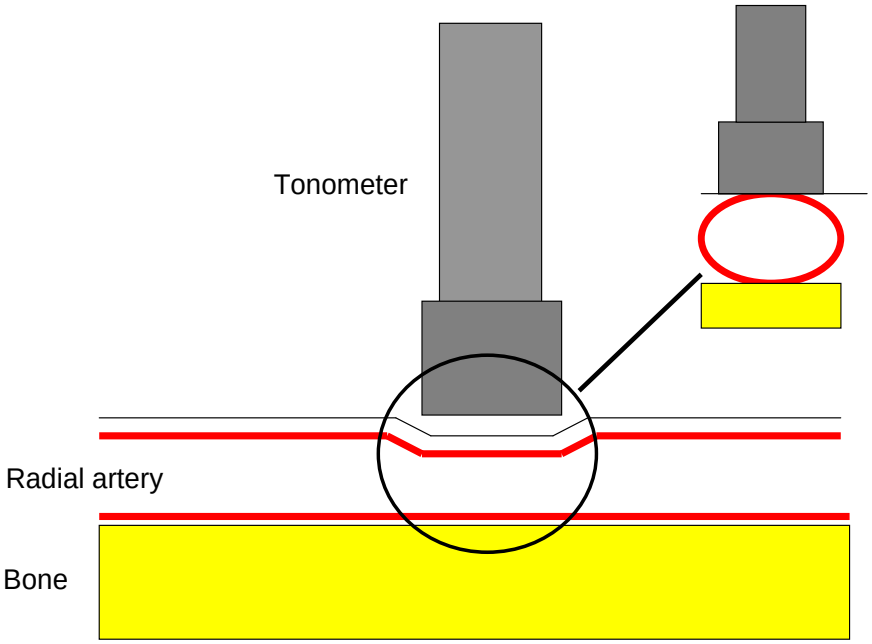
Arterial stiffness and carotid intima thickness were measured to assess the vascular status of the subjects. The methods used were applanation tonometry and carotid ultrasound, respectively. These methods were chosen because they are non-invasive and highly reproducible and therefore ideal for studies involving large numbers of subjects.

2.5.1 Applanation Tonometry

The Sphygmocor® apparatus (AtCor Medical, Sydney, Australia) was used to measure peripheral arterial wave forms from which measures of arterial stiffness were derived. A highly sensitive pressure transducer (tonometer) was placed over the point of maximal palpable pulsation of the radial, carotid or femoral artery. Gentle downward pressure was applied to the sensor to partially compress the artery beneath underlying structures, as shown in Figure 2.4. The sensor detected dynamic

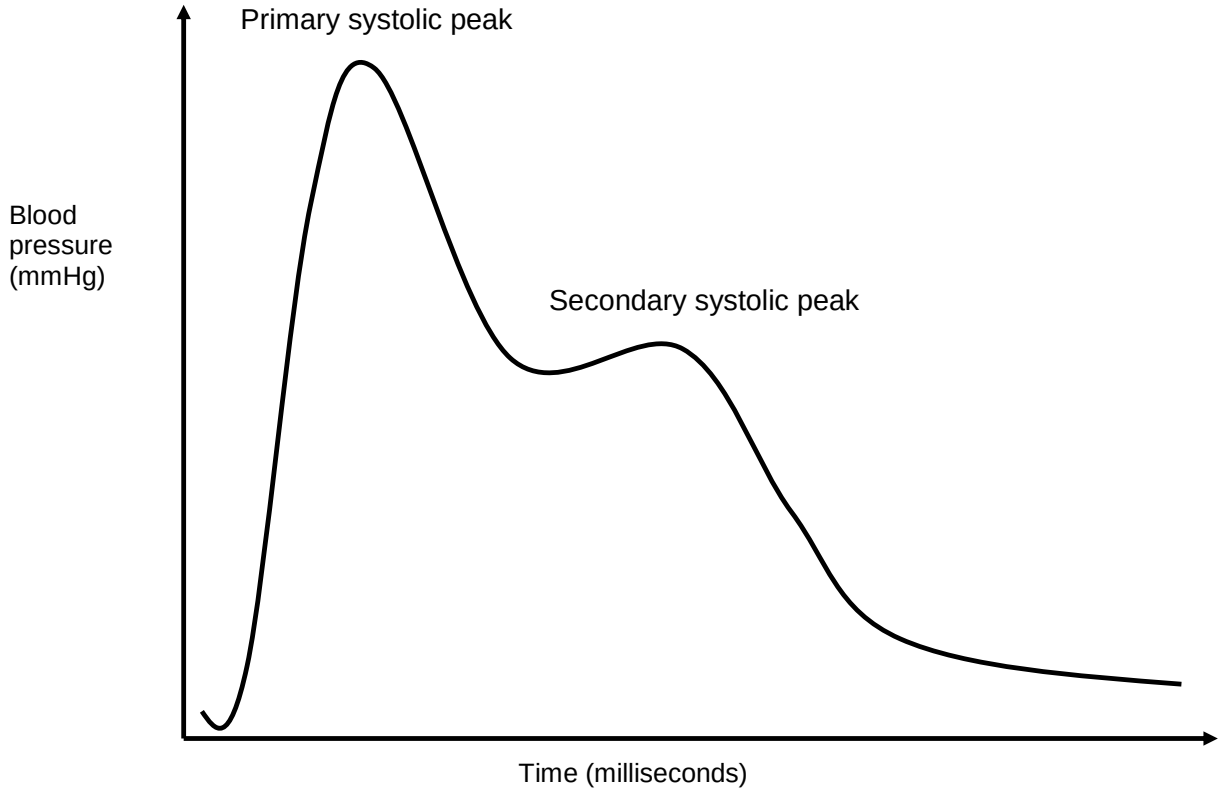
pressure and volume changes in the artery and generated a peripheral arterial waveform.

Figure 2.4 Applanation Tonometry Schematic



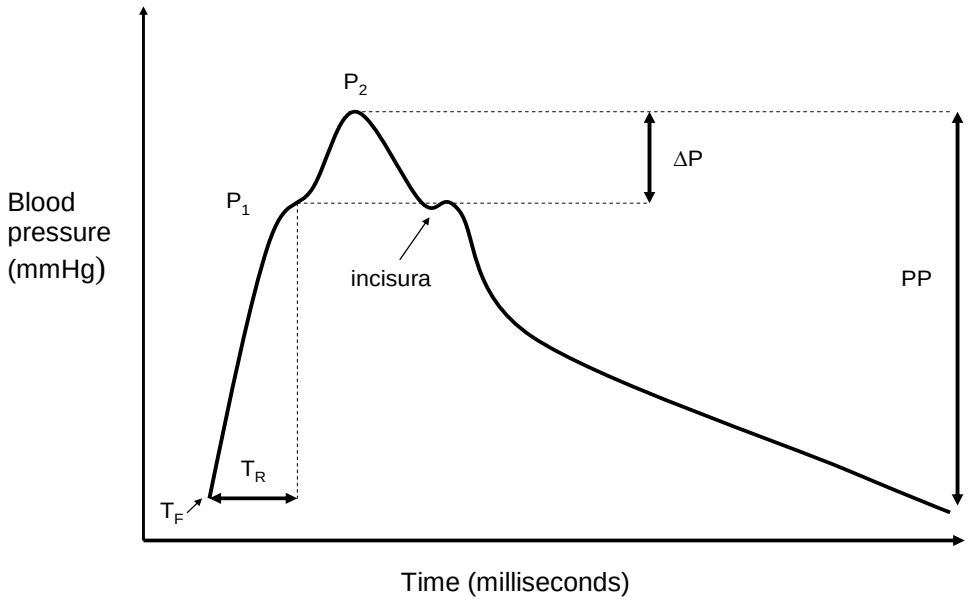
The tonometer interfaces with the software to record the peripheral pressure waveforms. These waveforms are a composite of the forward pressure wave created by ventricular contraction, the first peak, and the reflection back of this wave from peripheral resistance vessels, the second peak as shown in Figure 2.5.

Fig. 2.5 Radial Peripheral Waveform Recorded by Applanation Tonometry
(adapted from Oliver *et al* 2003 [295])



The Sphygmocor® software derives a central pressure waveform, as shown in Figure 2.6, from 20 recorded and averaged peripheral waveforms using a validated, generalised transfer function [296]. Augmentation index (Ai) (the difference between the first and second systolic peaks (ΔP) expressed as a percentage of the pulse pressure (PP)), is a measure of arterial stiffness and can be derived from the central pressure waveform.

Figure 2.6 Central Aortic Waveform Derived From Peripheral Wave Form using a Generalised Transfer Factor (adapted from Oliver *et al* 2003 [295])



P_1 First systolic peak
P_2 Second systolic peak
ΔP Difference between first and second systolic peaks
PP Pulse pressure
T_F Foot of wave
T_R Time between T_F and inflection

Before clinical cardiovascular events occur, arterial walls becomes less elastic or distensible resulting in a reduction of the vessel capacity to accommodate volume changes throughout the cardiac cycle. Higher systolic pressures are generated as the aorta distends less well, maintenance of the pulse pressure is impaired due to reduced elastic recoil and pressure waves and their reflections from peripheral resistance vessels are transmitted at higher velocities. Therefore, in stiffer arteries the reflected wave is seen earlier and if it appears before aortic valve closure will add to or augment the pressure produced by the initial wave resulting in higher central arterial pressures and a higher Ai.

Ai and pulse wave velocity (PWV), measures of arterial stiffness, independently predict cardiovascular events and/or mortality in the general population [189, 190],

the elderly [191, 192] and in disease states including end-stage renal disease [193, 194], hypertension [195, 196] and impaired glucose tolerance/diabetes [197]. Arterial stiffness has also been associated with cardiovascular risk factors including hypercholesterolaemia [198] and smoking [199].

Measurements were taken by me in accordance with international recommendations [297].

Formal training was undertaken for pulse wave analysis using the sphygmocor at the 'Arterial Stiffness Theory and Practice' course at the Vascular Research Unit in Addenbrooke's Hospital. Prior to commencement of the study, intra-operator variability was established by performing pulse wave analysis on 15 volunteers at two time points, ten minutes apart. The mean difference and the standard deviation of the difference between the readings were calculated for augmentation index and aortic pulse wave velocity and were 2.93% and 1.98% and 0.35m/s and 0.29m/s respectively, as shown in Table 2.1. This is comparable with previously published data which found the intra-operator standard deviation of the difference between readings to be 5.37% for augmentation index and 1.09 m/s for aortic pulse wave velocity [298].

Table 2.1 Intra-operator Variability for Pulse Wave Analysis

Age	AIx 1	AIx 2	Difference AIx 1 & 2	aPWV 1	aPWV 2	Difference aPWV 1 &2
22	3.0	2.0	1	5.9	5.6	0.3
39	0.0	2.0	2	7.6	6.8	0.8
36	16	11	5	5.7	5.8	0.1
34	10	10	0	7.7	7.5	0.2
34	23	25	2	6.5	5.8	0.7
26	-6.0	-8.0	2	6.5	6.5	0.0
45	30	30	0	6.1	7.0	0.9
40	35	30	5	7.4	6.7	0.7
42	16	13	3	8.6	8.7	0.1
38	5.0	10	5	8.3	8.4	0.1
27	22	27	5	5.2	5.3	0.1
40	7.0	13	6	6.8	7.2	0.4
21	0.0	-3.0	3	5.7	5.4	0.3
25	-4.0	-5.0	1	5.7	5.9	0.2
36	14	10	4	6.9	6.6	0.3
Mean			2.93			0.35
SD			1.98			0.29

AIx, Augmentation Index; aPWV, aortic pulse wave velocity

2.5.1.1 Pulse Wave Analysis

Peripheral pressure wave forms were recorded from the right radial artery with the subject at rest for 10 minutes, as described above. Ai and central aortic pressure were derived from analysis of the central waveform. Ai was adjusted to a heart rate of 75 beats per minute.

2.5.1.2 Pulse Wave Velocity

During pressure wave recordings for PWV, the subject had continuous 3 lead ECG recording allowing gating of the pressure wave to the peak of the R wave on the ECG. This allowed the time for the pressure wave to move from the ascending aorta to the peripheral pulse to be calculated. Measurements were taken from the carotid notch to the carotid, radial and femoral pulses to determine distance. From these

measurements, brachial (determined from recordings at the carotid and radial pulses) and aortic (determined from recordings at the carotid and femoral) pulse wave velocities were calculated as the distance/time in metre per second.

2.5.2 Carotid Ultrasound

Ultrasound imaging (Aloka Prosound SSD-5500, 7.5MHz linear array transducer) was undertaken by a single investigator, Mrs Emma Rees, who was blind to the subject status. An image was recorded of the right and left common carotid artery with the subject lying supine after 5 minutes of rest.

2.5.2.1 Carotid Intima Media Thickness

Measurements of carotid intima-media thickness (CIMT) were taken off-line, from a one centimetre segment of the common carotid artery proximal to the bifurcation, using automated-tracking and analysis software (Carotid Analyzer, Medical Imaging Applications). A mean of left and right sided values was used to calculate CIMT. The coefficient of variance for the right and left common carotid arteries were 5% and 6% respectively. These were calculated on a sample of 20 subjects selected with a random number generator, from repeated analysis of the raw Digital Imaging and Communications in Medicine (DICOM) files for a single session.

2.6 Cardiac Measurements

Echocardiography (Vivid 7, GE Medical Systems, Horten, Norway, with a 2.5MHz transducer and harmonic imaging) was undertaken by Mrs Emma Rees who was blind to subject status. Images were acquired at passive end-expiration where possible. Standard echocardiographic measurements and calculations were performed during off-line analysis using EchoPAC PC software version 110.0.0 (GE

Healthcare) according to the recommendations of the European Association of Echocardiography [299].

2.6.1 Myocardial Function

Longitudinal systolic function (s') and diastolic function (e', a' and e':a' ratio) were assessed by pulsed Doppler myocardial velocity imaging. The e' and a' are measures of left ventricular myocardial velocity related to passive filling and the component of filling related to atrial contraction, respectively. Reproducibility for resting myocardial velocities are well defined in the Wales Heart Research Institute (WHRI) echo laboratory and are all <10%, this was not repeated for the PCOS data. All echocardiograms in this study were undertaken in the WHRI echo laboratory.

2.7 Statistical Analyses

Statistical analyses were undertaken with the help of Professor Frank Dunstan, statistician, Institute of Primary Care and Public Health, Cardiff University.

2.7.1 Sample Size Calculation

The planned sample size was 80 in each group. This would give 80% power for detecting a difference of 0.45m/sec in aortic PWV or one of 0.25 in e':a'. I regarded these differences in PWV and e':a' as being aetiologically significant. The likely power for the regression analyses was harder to estimate without detailed knowledge of the relationships but estimated to be 80% for detecting partial correlation coefficients of around 0.25.

2.7.2 Regression Analyses

Comparisons between patients with PCOS and controls, both unadjusted and adjusted, were performed by using linear models when distributions of variables were approximately normally distributed and using bootstrapping when this was not

the case. Relationships with age and BMI were checked for linearity using graphical methods and examining residuals.

Relationships between fat measures and arterial stiffness parameters were also estimated using linear modelling. Fat measures were first converted into z-scores to facilitate comparison between models; a regression coefficient associated with a fat measure could then be interpreted as the effect on the outcome of a change of one standard deviation of the fat measure. Models were adjusted for age, central pulse pressure and, where there was evidence that body size was a confounder, for height. Interaction terms between PCOS status and the explanatory factors were included in the models to test if the relationships varied between PCOS subjects and controls.

CHAPTER 3: Demographic, Metabolic and Anthropometric Results

3.1. Introduction

Risk factors for cardiovascular disease include impaired glucose tolerance (IGT), Type 2 diabetes, an adverse lipid profile, elevated high sensitivity C reactive protein (hsCRP), obesity, visceral adiposity, hypertension and smoking. Factors which may explain the mechanisms of cardiovascular disease include the distribution of adipose tissue, adipocytokines, insulin resistance, hyperglycaemia, oxidative stress and androgens. This chapter will explore many of these risk factors and potential mediators of cardiovascular disease in our study population. As discussed in the introduction, the best method for screening PCOS women for dysglycaemia is debated and in a subgroup of the PCOS cohort we measured fasting glucose, glycated haemoglobin (HbA1C) and performed an oral glucose tolerance test to determine the best method in our local population.

The aims of this chapter are to:

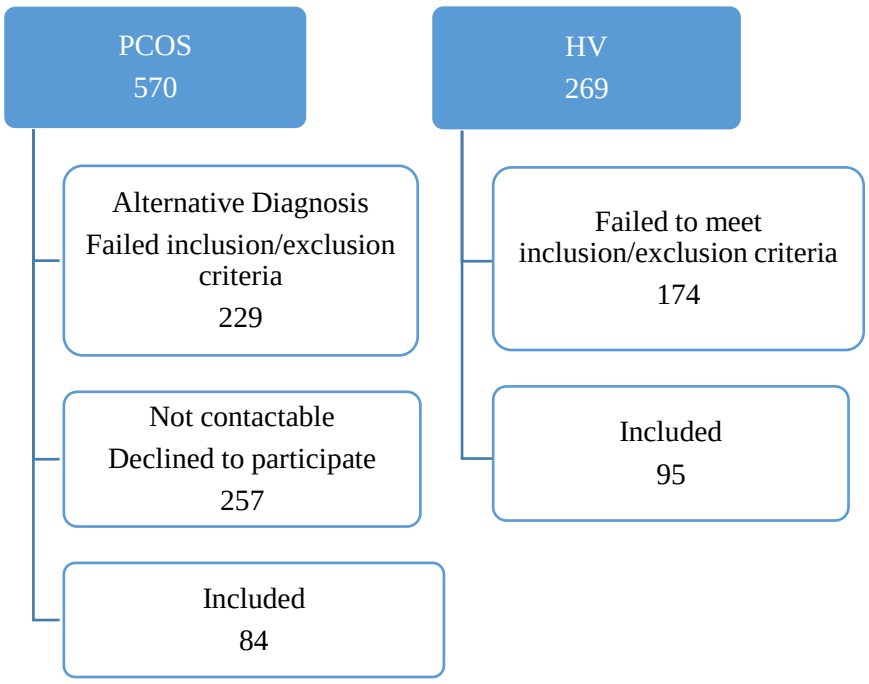
1. Present the data on study recruitment
2. Present the age and BMI distribution of the population and explain what adjustments were needed
3. Present the ethnicity, smoking status and medications taken by the subjects
4. Explore any differences between the PCOS group and HV groups in terms of metabolic factors; glucose tolerance, insulin area under the curve (AUC), glucose AUC, lipid profile, hsCRP, adiponectin and testosterone
5. Examine the different screening methods for identifying dysglycaemia in PCOS

6. Explore any differences between the PCOS group and HV group in terms of anthropometric data; waist and hip circumferences, waist to hip ratio, total body fat, total lean mass and trunk fat measured by dual energy x ray absorptiometry (DEXA); and total fat, visceral fat and subcutaneous fat as measured by Computed Tomography (CT)

3.2 Study Recruitment

From the departmental database and outpatient clinics at the University Hospital of Wales, women of the correct age with a presentation of hirsutism, oligo/amenorrhoea, infertility, biochemical hyperandrogenaemia or obesity were identified. Of these, 229 had an alternative diagnosis to PCOS or failed to meet the inclusion/exclusion criteria. A further 257 were not contactable or declined to participate. From local advertisement, 269 expressed an interest in being a healthy volunteer. One hundred and seventy four were excluded as they did not meet the inclusion and exclusion criteria. Please see Figure 3.1.

Figure 3.1 Study Recruitment Flow Diagram



3.3 Demographic Data

The numbers of subjects in each group, their age, BMI, smoking status, ethnicity and medications taken are presented below. The PCOS group was analysed with respect to the three different diagnostic criteria for PCOS.

3.3.1 Number of Subjects

Eighty-four women with PCOS and 95 healthy volunteers participated in the study.

3.3.2 PCOS Subjects and Different Diagnostic Criteria

The Rotterdam criteria were used in the recruitment of PCOS subjects. The NIH criteria are the least inclusive requiring a diagnosis of hyperandrogenism and chronic anovulation. The AE-PCOS criteria are broader including hyperandrogenism and ovulatory dysfunction (olig- or an-ovulation or polycystic ovaries) and the

Rotterdam are the most inclusive as a diagnosis of hyperandrogenism is not needed. This is reflected in the numbers of women in this study meeting the different diagnostic criteria as shown in Table 3.1.

Table 3.1 Polycystic Ovary Syndrome Subjects in Different Diagnostic Criteria Groups

Diagnostic Criteria	NIH	Rotterdam	AE-PCOS
Number of subjects	66	84	75

3.3.3 Age and BMI Distribution

During recruitment it was noticed that the HV group had a lower mean BMI than the PCOS group. To address this, an advert was placed in the local paper for healthy volunteers with a dress size of 16 or more (Appendix 3). Dress size was used to reflect a larger BMI, as it was thought most women would know their dress size but may not know their BMI. The mean age and standard deviation (SD) of the PCOS and HV groups were 29.8 years ± 6.7 and 32.6 years ± 7.9 respectively, which was significantly higher in the HV group (p=0.01). The mean BMI and SD of the PCOS and HV groups were 33.3 kg/m^2 ± 7.8 and 27.6 kg/m^2 ± 6.3 respectively, which was a significant difference (p<0.001). Results are displayed in Table 3.2.

Table 3.2 Age and Body Mass Index of Study Population

	PCOS Mean (SD)	HV Mean (SD)	Difference (PCOS-HV)	95% CI	p-value
Age (years)	29.8 (6.7)	32.6 (7.9)	-2.9	-5.0, -0.7	0.01
BMI (kg/m^2)	33.3 (7.8)	27.6 (6.3)	5.7	3.6, 7.8	<0.001

SD, standard deviation

3.3.3.1 Adjustments for Age and BMI

Matching for BMI and age within the study group resulted in a reduction in the number of subjects, under-powering the study. As these were possible important confounding factors for cardiovascular disease, further analyses were undertaken taking age and BMI into account. Linear regression models were used to assess measurements. Subject status, age and BMI were put into the model as explanatory/independent variables and the measurement of interest was the outcome/dependent variable. This made the assumption that relationships were linear and this was checked using scatter plots.

3.3.4 Ethnicity

The majority of the study population were Caucasian; 89% of the total study population, 82% of the PCOS group and 94.7% of the healthy volunteers. There was a significant difference in the number of Caucasians in each group (p=0.009). Further details of ethnicity are provided in Table 3.3.

Table 3.3 Ethnicity of Study Population

Ethnicity	PCOS Number (%)	Healthy Volunteers Number (%)
Asian	7 (8.3)	3 (3.2)
Arab	2 (2.4)	0 (0.0)
Afro-Caribbean	4 (4.8)	1 (1.1)
Caucasian	69 (82)	90 (94.7)
Mixed Race	2 (2.4)	1 (1.1)

3.3.5 Smoking Status

A third of the PCOS group (32%) and a third of the healthy volunteer (31%) were current or ex-smokers. There was no significant difference in the number of ex- and

current smokers compared to non-smokers in each group (p=1.0). Results are displayed in Table 3.4.

Table 3.4 Smoking Status of Study Population

Smoking Status	PCOS Number (%)	Healthy Volunteers Number (%)
Non-smoker	57 (68)	65 (68)
Ex-smoker	12 (14)	19 (20)
Smoker	15 (18)	11 (12)

3.3.6 Medication

As this was a study of young women, ladies taking contraceptive pills were included. Recruitment would have been more difficult if this was an exclusion criterion as they are widely used in this age group. Twenty-eight (29.5%) of the HVs and 19 (22.6%) of the PCOS group were taking a contraceptive pill; there was no significant difference between the two groups, p=0.3. Twelve of the PCOS group and 8 of the HVs were taking antidepressants. Two PCOS women were taking a proton-pump inhibitor and one of the HVs. One of the HVs was taking acetazolamide and three others were taking antihistamines as required.

3.4 Metabolic Characteristics of Study Population

The glucose tolerance status, measures of insulin resistance, glucose AUC, lipid profile, hsCRP, testosterone and high molecular weight (HMW) adiponectin data are presented in this section. Data on screening for dysglycaemia in PCOS are also presented.

3.4.1 Glucose Tolerance Status

All women had a 75 gram oral glucose tolerance test (OGTT); samples from two healthy volunteers were lost in the lab and one PCOS subject did not complete the OGTT. None of the subjects had any known pre-existing diabetes or pre-diabetes states. There was an abnormality of the OGTT in 16% of the PCOS subjects compared to 4% of the HVs, which was significantly higher (p=0.02). Details are shown in Table 3.5.

Table 3.5 Glucose Tolerance Status of Study Population

	Normal Number (%)	Diabetes Number (%)	IGT Number (%)	IFG Number (%)
PCOS	70 (84)	5 (6)	8 (10)	0 (0)
HV	89 (96)	0 (0)	4 (4)	0 (0)

IGT- Impaired glucose tolerance, IFG – Impaired fasting glycaemia.

3.4.1.1 Screening for Glucose Intolerance in PCOS

In a subgroup of PCOS subjects (n=34), HbA1c was measured along with the OGTT. Seven women were identified as having abnormal glucose tolerance on one or more of the three screening tests and the results are displayed in table 3.6. If an OGTT had not been performed, 5 diagnoses of IGT or diabetes would have been missed (subject numbers 1-5). The HbA1c identified subject number 6 as having diabetes who had a normal fasting glucose and OGTT by World Health Organisation (WHO) diagnostic criteria, although if the American Diabetes Association (ADA) criteria were applied she would have been defined as having impaired fasting glycaemia. Subject number 7 was identified as having diabetes by all three screening tests. All subjects except subject number 6, who was identified by HbA1c alone, had a BMI>37 kg/m^2.

Table 3.6 Outcome of Screening tests for Diabetes and Dysglycaemia in Polycystic Ovary Syndrome

Subject Number	Diagnosis	HbA1c (mmol/mol)	FPG (mmol/l)	2 hour glucose (mmol/l)	BMI (kg/m^2)	Age (years)	Ethnicity
1	IGT	29	4.4	8.2	43	30	Caucasian
2	Diabetes	36	4.8	11.6	37.5	37	Caucasian
3	Diabetes	37	5.6	11.3	37	40	Caucasian
4	IGT	39	5.6	7.9	45.7	22	Caucasian
5	IGT	40	5.6	8.0	42.7	35	Caucasian
6	Diabetes	48	5.6	4.9	27.8	24	Arab
7	Diabetes	54	8.7	14.1	42.7	29	Caucasian

FPG, fasting plasma glucose; BMI, body mass index; IGT, impaired glucose tolerance

Diabetes: WHO/ADA HbA1c ≥ 48 mmol/mol or FPG ≥7.0mmol/l or 2 hour glucose OGTT ≥11.1mmol/l

Impaired glucose tolerance: WHO/ADA FPG <7.0mmol/l and 2 hour glucose OGTT ≥ 7.8mmol/l

Impaired fasting glycaemia: WHO FPG ≥ 6.1 mmol/l and <7.0 mmol/l, ADA FPG ≥5.6 mmol/l and < 7.0 mmol/l

High risk of diabetes: WHO HbA1c 42-47mmol/mol, ADA 39mmol/mol-47mmol/mol

Results displayed in red are abnormal as per WHO criteria

Results displayed in blue are abnormal according to ADA criteria; this is also the cut off for an oral glucose tolerance test according to NICE guidelines

3.4.2 Insulin Resistance and Glucose Area Under the Curve

Insulin resistance was assessed by measuring insulin AUC and homeostatic model assessment for insulin resistance (HOMA-IR). The mean insulin AUC in the PCOS and HV groups was 101000 pmol min/l and 50900 pmol min/l respectively which was significant before ($p<0.001$) and after adjustment ($p<0.001$) for age and BMI, as shown in Table 3.7. The mean HOMA-IR in the PCOS and HV groups was 4.94 and 2.33 respectively, which was also significant before ($p<0.001$) and after adjustments ($p=0.003$) for age and BMI, as shown in Table 3.7. The mean glucose AUC was higher in the PCOS group than the HV group, 812 mmol min/l and 699 mmol min/l respectively, which was significant before ($p<0.001$) and after ($p=0.005$) adjustments for age and BMI, as shown in Table 3.7. Adjusting for age only, resulted in little change in the mean difference between the two groups for IAUC, HOMA-IR and glucose AUC but the p value remained significant. Adjusting for BMI only, resulted in a greater change in the mean difference between the two groups for IAUC, HOMA-IR and glucose AUC and this was also significant. BMI was having a greater influence on the difference between the two groups, although after adjustment for age and BMI there remained a significant difference between the two groups.

3.4.3 Lipid Profile

The mean total cholesterol was 4.80 mmol/l and 4.63 mmol/l in the PCOS and HV groups, respectively. In the PCOS group the mean HDL-cholesterol was 1.32 mmol/l and in the HV group 1.39 mmol/l. The mean LDL-cholesterol was 2.97 mmol/l in the PCOS group and 2.79 mmol/l in the HV group. There were no significant differences in total cholesterol, HDL-cholesterol or LDL-cholesterol

between the two groups before or after adjustment for age and BMI. The mean triglycerides in the PCOS group was 1.11 mmol/l compared to 0.96 mmol/l in the HV group. This was significant before adjustment for age and BMI (p=0.031) but not after the adjustment was made (p=0.841). These results are presented in Table 3.7. BMI accounted for the difference in triglycerides between the two groups.

3.4.4 High Sensitivity C Reactive Protein

The mean hsCRP was 4.67 mg/l in the PCOS group and 2.49 mg/l in the HV group. This was significantly higher in the PCOS group in the unadjusted analysis (p=0.002) but not after adjustments for age and BMI (p=0.553), please see Table 3.7. Adjustments for age only and BMI only were done. Adjusting for BMI only, the difference became non-significant suggesting that BMI was contributing to the difference rather than age.

3.4.5 Testosterone

The mean testosterone was 1.39 nmol/l in the PCOS group and 0.77 nmol/l in the HV group. Testosterone was significantly higher in the PCOS group in unadjusted (p<0.001) and adjusted (p<0.001) analyses. Please see Table 3.7.

3.4.6 High Molecular Weight Adiponectin

In the PCOS group the mean HMW adiponectin was 10.5 µg/ml and in the HV group 10.1 µg/ml. There was no significant difference in unadjusted analyses (p=0.723) but mean HMW adiponectin became higher in the PCOS group after adjustment (p=0.02). Please see Table 3.7. When the adjustment for BMI only was done, the adiponectin was significantly higher in the PCOS group suggesting that BMI was contributing to the difference.

Table 3.7 Metabolic Characteristics of the Study Population Unadjusted and Adjusted for Age and Body Mass Index

			Unadjusted			Adjusted		
	PCOS Mean (SD)	**HV Mean (SD)**	**Difference (PCOS-HV)**	**95% CI**	**P-value**	**Difference (PCOS-HV)**	**95% CI**	**P-value**
Insulin AUC (pmol min/l)	101000 (66000)	50900 (34700)	50000	32800, 68400	<0.001	35900	19900, 52600	<0.001
HOMA-IR	4.94 (4.15)	2.33 (1.45)	2.61	1.71, 3.52	<0.001	1.33	0.45, 2.21	0.003
Glucose AUC (mmol min/l)	812 (250)	699 (134)	113	54, 169	<0.001	78.4	26.9, 131.6	0.005
TC (mmol/l)	4.80 (0.75)	4.63 (0.83)	0.17	-0.06, 0.41	0.152	0.19	-0.06, 0.45	0.139
HDL-C (mmol/l)	1.32 (0.35)	1.39 (0.33)	-0.07	-0.17, 0.03	0.166	0.07	-0.04, 0.17	0.196
LDL-C (mmol/l)	2.97 (0.68)	2.79 (0.78)	0.18	-0.04, 0.40	0.115	0.11	-0.13, 0.35	0.346
TG (mmol/l)	1.11 (0.47)	0.96 (0.45)	0.15	0.01, 0.29	0.031	0.01	-0.13, 0.16	0.841
hsCRP (mg/l)	4.67 (5.92)	2.49 (3.04)	2.18	0.81, 3.55	0.002	0.41	-0.95, 1.78	0.553
Testosterone (nmol/l)	1.39 (0.81)	0.77 (0.38)	0.61	0.43, 0.80	<0.001	0.57	0.36, 0.78	<0.001
Adiponectin (µg/ml)	10.5 (8.9)	10.1 (7.0)	0.4	-1.98, 2.84	0.723	3.01	0.49, 5.53	0.020

AUC, area under the curve; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TG, triglycerides; hsCRP, high sensitivity C reactive protein

3.5 Anthropometric Characteristics of Study Population

The waist and hip circumferences, waist to hip ratio measurements and body composition as assessed by DEXA and CT are presented below.

3.5.1 Waist and Hip Circumference

The mean waist circumferences were 98.1cm and 85.4 cm in the PCOS and HV groups, respectively. The mean hip circumferences were 117.5 cm and 105.5 cm in the PCOS and HV groups, respectively. The mean waist to hip ratio was 0.83 and 0.81 in the PCOS and HV groups. All parameters were significantly higher in the PCOS group in the unadjusted analyses (waist circumference, $p<0.001$; hip circumference $p<0.001$ and waist hip ratio, $p<0.03$) but differences were not significant after adjustments (waist circumference, $p=0.101$; hip circumference, $p=0.91$ and waist to hip ratio, $p=0.09$). Please see Table 3.8. Adjusting for age and BMI separately, suggested that BMI was contributing to the difference between the two groups.

3.5.2 Total Body Fat, Regional Body Fat and Lean Mass Assessed by DEXA

The Hologic Discovery A absorptiometer was not always available due to servicing or awaiting repairs, therefore only 146 women had a DEXA scan. The mean total lean mass, mean total body fat and mean trunk fat in the PCOS group were 46477 grams, 41281 grams and 20574 grams respectively. In the HV group, the mean total lean mass, mean total body fat and mean trunk fat were 40937 grams, 29058 grams and 13130 grams respectively. PCOS women had a higher lean mass, total body fat and trunk fat mass compared to HVs ($p<0.001$ for all parameters) but after adjustments for age and BMI there were no significant differences ($p=0.513$, $p=0.401$

and p=0.515 respectively). Please see Table 3.8. Adjusting for age and BMI separately, suggested that BMI was contributing to the difference.

3.5.3 Subcutaneous and Visceral Fat Measurement Assessed by CT

In the PCOS group, the mean total fat, subcutaneous fat and visceral fat areas were 361 cm^2, 322 cm^2 and 39.1 cm^2 respectively. In the HV group, the mean total fat, subcutaneous fat and visceral fat areas were 286 cm^2, 257 cm^2 and 28.5 cm^2 respectively. Before adjustments for age and BMI, the mean of all parameters were higher in the PCOS group compared to the HV group (p=0.001 for total fat and subcutaneous fat and p=0.007 for visceral fat). After adjustment there were no differences between the two groups for total fat (p=0.904), subcutaneous fat (p=0.783) or visceral fat (p=0.529). Please see Table 3.8. Adjusting for age and BMI separately, suggested that BMI was contributing to the difference.

Table 3.8 Anthropometric Characteristics of the Study Population Unadjusted and Adjusted for Age and Body Mass Index

			Unadjusted			Adjusted		
	PCOS Mean (SD)	**HV Mean (SD)**	**Difference (PCOS-HV)**	**95% CI**	**P-value**	**Difference (PCOS-HV)**	**95% CI**	**P-value**
WC (cm)	98.1 (17.9)	85.4 (13.8)	12.7	8, 17.4	<0.001	1.65	-0.33, 3.62	0.101
HC (cm)	117.5 (18.0)	105.5 (14.4)	12.1	7.3, 16.9	<0.001	-0.09	-1.66, 1.48	0.91
Waist: Hip ratio	0.83 (0.05)	0.81 (0.05)	0.02	0.01, 0.04	<0.003	0.02	-0.002, 0.03	0.09
DEXA total lean mass (g)	46477 (9700)	40937 (5551)	5540	2839, 8082	<0.001	-630	-2371, 1130	0.513
DEXA total body fat (g)	41281 (15811)	29058 (11934)	12222	7747, 17041	<0.001	-620	-2250, 738	0.401
DEXA trunk fat (g)	20574 (9232)	13130 (6766)	7444	4861, 10204	<0.001	273	-643, 1182	0.515
CT Total fat (cm^2)	361 (126)	286 (130)	75	30.6,110.8	0.001	-1.6	-27.3, 24.1	0.904
CT SC fat (cm^2)	322 (110)	257 (115)	65	25.2, 96.1	0.001	-3.4	-27.7, 20.9	0.783
CT Visceral fat (cm^2)	39.1 (28.2)	28.5 (19.5)	10.6	2.7, 17.4	0.007	1.8	-3.9, 7.5	0.529

WC, waist circumference; HC, hip circumference; SC, subcutaneous

3.6 Summary of Demographic, Metabolic and Anthropometric Results

All PCOS women met the Rotterdam criteria, 66 met the NIH criteria and 75 met the AE-PCOS criteria. Women with PCOS were significantly heavier and younger than the HVs, so adjustments were made for these in further analyses as they were potential confounding factors for cardiovascular disease. The majority of women were Caucasian, however there were significantly more Caucasians in the HV group compared to the PCOS group. There were no significant differences between the two groups with respect to numbers of smokers/exsmokers and the number of women taking the contraceptive pill.

In the PCOS group, 5 women were found to have diabetes and 8 IGT compared to 4 HVs having IGT. There was a significant difference between the two groups in the numbers of women having an abnormal OGTT. In a subgroup of PCOS women, HbA1c was not able to identify all women with an abnormal OGTT result. Markers of insulin resistance (HOMA-IR and insulin AUC), glucose AUC and testosterone were significantly higher in the PCOS group before and after adjustment. There were no significant differences in cholesterol measurements. Triglycerides and hsCRP were significantly higher in the PCOS group before adjustment but there were no differences after adjustment. High molecular weight (HMW) adiponectin did not differ between the groups in the unadjusted analyses but became significantly higher in the PCOS group after adjustment.

All anthropometric measurements were significantly higher in the PCOS group in unadjusted analyses, but none were significant after adjustments for age and BMI. Not surprisingly, adjusting for BMI alone, resulted in no significant difference between the two groups.

3.7 Discussion

All women with PCOS met the Rotterdam criteria for diagnosis of PCOS as this was part of the inclusion criteria for the study. The AE-PCOS criteria were met by more women than the NIH criteria, the latter has the strictest criteria for the diagnosis of PCOS. This was not surprising and reflects the prevalence of PCOS by the different criteria in the general population; the prevalence of PCOS diagnosed by the Rotterdam criteria being the highest and the prevalence of PCOS diagnosed by the NIH criteria being the lowest [50, 51]. This study was not designed to determine if there were any differences in cardiovascular risk between the different diagnostic groups. It has been suggested that non-NIH PCOS may present with a less adverse cardiometabolic profile than women with PCOS diagnosed by NIH criteria [52, 301]. A study examining vascular function in the different categories of PCOS found elevated asymmetric dimethylarginine (ADMA), an inhibitor of nitric oxide production, in both NIH-PCOS and non-NIH PCOS patients which were significantly higher than controls; however, there were no differences between NIH PCOS and non-NIH PCOS and controls for other measures of vascular health including central aortic pulse wave velocity and endothelial function [302].

The BMI of the PCOS group was significantly higher than the HV group in our study. This reflects the high levels of obesity in women with PCOS [56]. As obesity is associated with hypertension and dysgylcaemia, this may have contributed to difficulty in recruiting 'healthy' volunteers who are obese and well. It may be that obese women are less likely to be volunteers in research studies than women of a normal weight. The HV group were older than the PCOS group and this could have occurred as older women may be more concerned about their health than younger women and more likely to volunteer in a study investigating cardiovascular risk.

The majority of women in the study were Caucasian but there were more ethnic minorities represented in the PCOS group. Asians and Arabs are at higher risk of developing glucose intolerance than Caucasians and this could have affected the results but the numbers involved are small and are unlikely to have made a big difference.

Smoking is a risk factor for cardiovascular disease but there were no significant differences in history of smoking between the PCOS and HV groups in our study and so unlikely to have affected our results. However, the total smoking pack year history was not ascertained and the effect of this on cardiovascular risk was not determined. The prevalence of smokers or ex-smokers in this study was 32% which is similar to that of work done by own group looking at a large community-database when the prevalence was 39% [67].

There is some evidence to suggest that oral contraceptive pills containing higher levels of ethinyl estradiol, are associated with impaired glucose tolerance and insulin resistance in the general population [303], increasing cardiovascular risk. However, a review suggests that the risk of cardiovascular events is minimal particularly in women who do not smoke and are not hypertensive [304]. A systematic review and meta-analysis of oral contraceptive use in women with PCOS also failed to find any clinically significant adverse metabolic profiles [305]. In our study there were no differences in oral contraceptive use between the PCOS group and HV group and therefore any increased cardiometabolic risk from the oral contraceptive pill, if present, is unlikely to have affected our results.

More IGT and diabetes were found in the PCOS group than in the healthy volunteers. This is not surprising as women with PCOS are at increased risk of developing glucose intolerance and diabetes [67, 100]. The prevalence rates of diabetes and IGT in PCOS are 1.6-10% [75-82] and 9.4-35% [75-82] respectively. The prevalence rates I found in my study were similar, 6% had diabetes and 10% had IGT. As known IGT and diabetes were exclusion

criteria in this study, the actual prevalence of IGT and diabetes in our local PCOS population may be higher. The mean BMI was higher in the PCOS group and this may be a factor in explaining the higher prevalence of dysglycaemia in the PCOS group; however, the measures of insulin resistance and glucose AUC were higher in the PCOS group than the HV group even after BMI- and age-matching. Therefore differences in BMI are unlikely to contribute solely to the differences in the prevalence of diabetes/IGT between the two groups.

The OGTT is the gold standard for diagnosing abnormalities of glucose metabolism. Alternatives are the fasting plasma glucose and the HbA1c which are more convenient, less costly and less labour intensive than the OGTT. In our population, HbA1c was not able to identify all women with dysglycaemia and if this had been used alone, five out of six (83%) ladies with an abnormal OGTT would have been missed. Therefore, in patients with PCOS HbA1c may not be an adequate screening test for diagnosing glucose intolerance and diabetes in PCOS. This is in agreement with other studies [109-110]. Following the NICE guidance [107] would have identified all women in this subgroup with IGT/diabetes as all had a fasting plasma glucose ≥5.6 mmol or a BMI more than 30. However, as the mean BMI in this population was 33.3 there may not be many women who would be excluded from having an OGTT.

Markers of insulin resistance (HOMA-IR and Insulin AUC) were significantly higher in the PCOS group both before and after adjustments for age and BMI. This is consistent with other studies. Previous authors have also shown basal- and glucose- stimulated hyperinsulinaemia in women with PCOS compared to weight-matched controls [19] and IR is present in PCOS independently of obesity [58].

This study found no differences in total or LDL-cholesterol between the two groups. Trigylcerides were higher in the PCOS group compared to the HV group in the unadjusted

analyses, but no differences were seen between the two groups after adjustment for age and BMI. After adjustment for age alone, the difference remained significant; whereas adjusting for BMI alone negated the differences, implying that BMI was driving the difference between the two groups. The most common adverse lipid profile in PCOS is elevated triglycerides and decreased HDL-cholesterol and has been reported in up to 70% of American women with PCOS [134]. Our findings are not consistent with a meta-analysis which found adverse lipid profiles remained in PCOS women after BMI matching [60]. This study did not match for age and they found that women with PCOS diagnosed by NIH criteria had worse lipid profiles than women diagnosed by Rotterdam criteria; this may partially explain the differences with my results.

A meta-analysis found that high sensitivity CRP was elevated in women with PCOS compared to controls, an observation which remained after studies with mismatches for BMI or prevalence of obesity were excluded [130]. Our study is not in agreement with this finding as there was no difference in hsCRP after matching for age and BMI. The meta-analysis included studies using NIH criteria and Rotterdam criteria to diagnose PCOS and this may partly explain the differences between our study and the meta-analysis.

Testosterone was significantly higher in the PCOS group before and after matching for age and BMI. This was expected as hyperandrogenism is common in women with PCOS, although the diagnosis of PCOS using the Rotterdam criteria does not necessarily require biochemical hyperandrogenism to be present.

A systematic review and meta-analysis found that adiponectin levels were significantly lower in PCOS women than BMI-matched controls [224]. However, some studies have not found any difference in adiponectin levels between PCOS and controls [306,307]. This study found no difference in HMW adiponectin between the two groups in unadjusted analyses;

after adjustment for age and BMI, HMW adiponectin was higher in the PCOS group (p=0.02). In the meta-analysis, the majority of studies had measured total adiponectin and used NIH criteria for diagnosis of PCOS, whereas I measured HMW adiponectin and used Rotterdam criteria for diagnosis of PCOS which could be an explanation for the discrepancy in findings. The PCOS women were more insulin resistant than the HVs in our study, even after adjustments for age and BMI, and therefore, it would be expected that the HMW adiponectin levels would be lower in the PCOS group as increasing insulin resistance has been associated with lower HMW adiponectin levels. However, there is some evidence to suggest that the morphology of adipocytes in subcutaneous and visceral adipose tissue may be important in regulating the secretion of adiponectin. One study has shown that hypertrophied adipocytes in subcutaneous adipose tissue secrete less adiponectin than smaller adipocytes and that the percentage of small fat cells in subcutaneous adipose tissue correlates with serum HMW adiponectin [308]. It could be that in our population, PCOS women had more small fat cells than the HVs explaining my finding of increased HMW adiponectin in the PCOS group.

All anthropometric measures and assessments of body composition were significantly higher in the PCOS group in the unadjusted analyses but no differences were seen between the two groups after adjustment for age and BMI. A meta-analysis found that women with PCOS had greater central adiposity than BMI-matched controls [56] and a further study found that non-obese PCOS women had greater accumulation of central fat mass than BMI-matched controls [118]. Neither of these studies matched for age and the former used waist circumference >80cm and waist:hip ratio >0.85 to define central obesity whilst the latter used ultrasound. We adjusted for age and used CT to assess fat distribution which is a more accurate method. A study using MRI (the gold standard along with CT) to assess body fat distribution did not find any differences in body fat distribution between PCOS patients and

controls [119] which is in agreement with our findings. As higher BMI can reflect a greater lean mass, DEXA was used to assess lean and fat mass in our population. A higher proportion of lean mass may protect against cardiovascular disease; however, there was no difference in lean mass after adjustment for age and BMI between the two groups.

CHAPTER 4: Cardiovascular Results

4.1 Introduction

Higher resting heart rate and hypertension are risk factors for cardiovascular disease and therefore resting heart rate and blood pressure were measured in our study population. As we were investigating a young population who had no known cardiovascular disease, surrogate markers of cardiovascular disease were measured to identify any subclinical cardiovascular disease. These included augmentation index (Ai), pulse wave velocity (PWV), common carotid intima-media thickness (ccIMT) and diastolic heart function. Aortic PWV, ccIMT and diastolic heart function are all independent predictors of cardiovascular disease [179, 189, 190, 206, 207].

The aims of this chapter are to:

1. Present the data on resting heart rate and blood pressure
2. Present the data on Ai, brachial PWV and aortic PWV
3. Present the data on ccIMT measurements
4. Present the data on echocardiogram measurements
5. Compare the findings with other studies

As significant differences between the age and body mass index (BMI) of the polycystic ovary syndrome (PCOS) group and healthy volunteer (HV) group were found (see Chapter 3), results are presented unadjusted and adjusted for age and BMI.

4.2 Resting Heart Rate

The mean resting heart rate was higher in the PCOS group 74.6 beats per minute (bpm) compared to the HV group 70.1 bpm, p=0.009. However, after adjustments the difference was no longer significant, p=0.250. Please see Table 4.1. After adjusting for age alone, there remained a significant difference in the results suggesting BMI was mediating the difference in heart rate in the unadjusted analyses.

4.3 Blood Pressure

The peripheral blood pressure and central blood pressure measurements are presented below. The central blood pressure was derived from data collected during applanation tonometry.

4.3.1 Peripheral Blood Pressure

None of the subjects included in the study had a systolic blood pressure greater than or equal to 140 mmHg. Four of the PCOS group (4.8%) and no-one in the HV group had a diastolic blood pressure greater than or equal to 90 mmHg (p=0.047). The mean systolic pressure in the PCOS group was 114.4 mmHg and in the HV group 112.1 mmHg, p=0.135. After adjustments for age and BMI, there was no significant difference in mean systolic blood pressure between the two groups, p=0.313. The mean diastolic blood pressure in the PCOS group was 67.2 mmHg and in the HV group 64.4 mmHg, which was significantly higher, p=0.047. After adjustments for age and BMI this was no longer significant, p=0.480. Please see Table 4.1. Adjusting for age alone, diastolic pressure remained higher in the PCOS group but no significant differences were found when adjusting for BMI alone; suggesting that

BMI exerted the main influence on the difference in diastolic blood pressure between the two groups.

4.3.2 Central Blood Pressure

Central systolic blood pressure was no different between the two groups before (p=0.381) and after adjustment for age and BMI (p=0.543). Mean central diastolic blood pressure was higher in the PCOS group before adjustments, PCOS mean 68.6 mmHg and HV mean 65.2 mmHg, p=0.02. After adjustment there was no difference, p=0.269. Please see Table 4.1. Adjusting for age only, the significant difference in central diastolic blood pressure remained but not after adjusting for BMI alone; also suggesting that BMI was mediating the difference between the two groups in the unadjusted analyses.

4.4 Augmentation Index

The mean Ai, adjusted to a heart rate of 75 bpm, in the PCOS group was 3.72% and in the HV group 5.6%. There were no differences between the two groups in the unadjusted (p=0.343) or age and BMI adjusted analyses (p=0.935). Please see Table 4.1. Adjusting for BMI alone resulted in a lower mean Ai in the PCOS group whereas adjusting for age alone had no significant effect.

4.5 Pulse Wave Velocity

The mean aortic PWV in the PCOS group was 6.40 metres per second (m/s) and in the HV group 6.41 m/s and was no different before (p=0.921) or after full adjustment (p=0.334). The mean brachial PWV in the PCOS group was 7.17 m/s and in the HV

group 7.16 m/s, which was also not different in unadjusted (p=0.976) or adjusted analyses (p=0.977). Please see Table 4.1. Adjusting for age only, all parameters remained non-significant. However, adjusting for BMI alone resulted in the aortic PWV just becoming significantly lower in the PCOS group compared to the controls (p=0.016).

4.6 Carotid Intima Media Thickness

The mean common carotid artery intima media thickness was 0.50 mm in the PCOS group and 0.51mm in the HV group. There was no difference before (p=0.100) or after adjustment (p=0.133). Please see Table 4.1. Adjusting for BMI alone, the ccIMT was significantly lower in the PCOS group than the HV group. There were no differences when adjusting for age alone.

Table 4.1 Cardiovascular Characteristics of the Study Population Unadjusted and Adjusted for Age and Body Mass Index

			Unadjusted			Adjusted		
	PCOS Mean (SD)	**HV Mean (SD)**	**Difference (PCOS-HV)**	**95% CI**	**P-value**	**Difference (PCOS-HV)**	**95% CI**	**P-value**
HR (bpm)	74.6 (11.0)	70.1 (10.9)	3.5	1.11, 7.80	0.009	2.10	-1.49,5.69	0.250
Peripheral SBP (mmHg)	114.4 (9.7)	112.1 (10.3)	2.3	0.71, 5.26	0.135	-1.50	-4.43, 1.43	0.313
Peripheral DBP (mmHg)	67.2 (9.5)	64.4 (9.1)	2.8	0.04, 5.60	0.047	1.02	-1.82, 3.85	0.480
Central SBP (mmHg)	97.3 (9.5)	95.9 (10.9)	1.4	-1.71, 4.45	0.381	-0.88	-3.74, 1.97	0.543
Central DBP (mmHg)	68.6 (9.6)	65.2 (9.4)	3.4	0.53, 6.21	0.02	1.63	-1.27, 4.52	0.269
AI (75bpm)	3.72 (11.31)	5.69 (15.41)	-1.97	-6.06, 2.12	0.343	0.15	-3.40, 3.69	0.935
aPWV (m/s)	6.40 (1.03)	6.41 (0.97)	-0.02	-0.32, 0.29	0.921	-0.13	-0.40, 0.14	0.334
bPWV (m/s)	7.17 (1.05)	7.16 (1.31)	0.01	-0.35, 0.36	0.976	0.01	-0.37, 0.38	0.977
ccIMT (mm)	0.50 (0.06)	0.51 (0.06)	-0.01	-0.04, 0.00	0.100	-0.01	-0.03, 0.00	0.133

HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; AI, augmentation index; aPWV, aortic pulse wave velocity; bPWV, brachial pulse wave velocity; ccIMT, common carotid intima media thickness

4.7 Echocardiographic Measures

The mean of the following echocardiographic variables were no different between the PCOS group and HV group before or after adjustments for age and BMI; left ventricular (LV) posterior wall thickness, LV end-diastolic septal thickness, LV end-diastolic dimension, LV end-diastolic volume, LV end-systolic volume, stroke volume and ejection fraction. Fractional shortening was higher in the PCOS group prior to adjustment for age and BMI but not after. The echocardiographic measures that were particularly of interest, as they are independent predictors for cardiovascular mortality, were the longitudinal systolic myocardial velocity (s'), the early diastolic myocardial velocity and the early to late myocardial velocity ratio. The mean early diastolic myocardial velocity was 14.71 cm/s in the PCOS group and 14.57 cm/s in the HV group. The mean systolic myocardial velocity was 9.84 cm/s in the PCOS group and 9.83 cm/s in the HV group. The mean early:late diastolic myocardial velocity ratio was 1.82 in the PCOS group and 1.76 in the HV group. There was no difference between the two groups for early diastolic myocardial velocity, systolic myocardial velocity or the early:late diastolic myocardial velocity ratio before adjustments ($p=0.756$, $p=0.974$ and $p=0.586$ respectively) or after adjustments ($p=0.345$, $p=0.511$ and $p=0.860$ respectively). Please see Table 4.2.

Table 4.2 Echocardiographic Measures Unadjusted and Adjusted for Age and Body Mass Index

			Unadjusted			Adjusted		
	PCOS Mean (SD)	**HV Mean (SD)**	**Difference (PCOS-HV)**	**95% CI**	**P-value**	**Difference (PCOS-HV)**	**95% CI**	**P-value**
LV posterior wall thickness (cm)	0.86 (0.14)	0.85 (0.13)	0.01	-0.03, 0.05	0.662	-0.04	-0.88 ,0.01	0.059
LV end-diastolic septal thickness (cm)	0.85 (0.14)	0.81 (0.13)	0.04	-0.01, 0.08	0.084	-0.01	-0.05, 0.03	0.578
LV end-diastolic dimension (cm)	4.65 (0.38)	4.63 (0.38)	0.03	-0.09, 0.15	0.634	-0.04	-0.17, 0.08	0.492
Fractional shortening (%)	35.4 (5.0)	33.6 (5.5)	1.8	0.17, 3.42	0.031	1.28	-0.47, 3.03	0.151
LV end-diastolic volume (ml)	85.8 (13.6)	84.7 (16.4)	1.0	-4.76, 6.79	0.729	-3.72	-9.14, 1.71	0.177
LV end-systolic volume (ml)	35.5 (6.9)	33.8 (6.8)	1.7	-0.95, 4.26	0.210	-0.63	3.16, 1.90	0.623
Stroke volume (ml)	65.6 (12.9)	64.0 (11.1)	1.6	-2.03, 5.18	0.389	-0.15	-3.91, 3.61	0.936
Ejection fraction (%)	58.7 (4.0)	59.9 (4.5)	-1.3	-2.92, -0.37	0.125	-0.78	-2.55, 1.00	0.387
Early diastolic myocardial velocity (cm/s)	14.71 (2.72)	14.57 (2.68)	0.14	-0.76, 1.05	0.756	0.42	-0.45, 1.29	0.345
Systolic myocardial velocity (cm/s)	9.84 (1.64)	9.83 (1.78)	0.01	-0.57, 0.59	0.974	-0.22	-0.88, 0.44	0.511
Early:late diastolic myocardial velocity	1.82 (0.57)	1.76 (0.58)	-0.05	-0.25, 0.14	0.586	-0.01	-0.18, 0.15	0.860

4.8 Summary of Cardiovascular Results

The resting heart rate, peripheral and central diastolic blood pressures were significantly higher in the PCOS group in the unadjusted analyses, but were not significant after adjustment for age and BMI. Adjusting for BMI alone removed the differences between the two groups, suggesting that the difference in the unadjusted analyses was mediated by BMI. There were no differences in the central and peripheral systolic blood pressures, Ai, aortic PWV, brachial PWV or ccIMT between the two groups before or after adjustments for age and BMI. The measures of cardiac function were not different between the two groups before or after adjustments for age and BMI except for fractional shortening; this was significantly higher in the PCOS group in the unadjusted analyses but not after the adjustment for age and BMI was made.

4.9 Discussion

The resting heart rate was significantly higher in the PCOS group compared to the HV group before adjustments were made. This is in agreement with another study which found significantly higher heart rate in women with PCOS compared to age-matched controls [309]. However, this study had significant differences in BMI between the PCOS group and controls. After adjustments for BMI there were no differences between the two groups in resting heart rate which is in agreement with other studies who have age- and BMI-matched their subjects [166, 204]. The difference between the unadjusted and adjusted analyses in my study is likely to be due to obesity.

Defining hypertension as a peripheral blood pressure greater than or equal to 140/90 mmHg, the prevalence of hypertension was 4.8% in the PCOS group and 0% in the HV group. This is much lower than the prevalence reported by a Chinese study, 19.2% [131] and the Dallas heart study, 29.2% [174]. In the Dallas heart study, the PCOS group had a significantly higher BMI than the controls. As women with hypertension and those on antihypertensive medications were excluded from participating in this study, it is not surprising that the prevalence rates were lower. The mean diastolic blood pressure was significantly higher in the PCOS group in the unadjusted analyses but not after adjustment. Blood pressure is a continuous variable and increases with age and BMI. The difference seen in the unadjusted analyses may be related to the higher BMI in the PCOS group which was a greater significant difference than the older age in the HV group. The potential mechanisms causing hypertension in obesity include hyperinsulinaemia, activation of the renin-angiotensin-aldosterone system, sympathetic nervous system stimulation and abnormal levels of leptin [310].

Atcor medical, the company manufacturing the sphygmocor apparatus which was used in this study, have published mean values and reference ranges for Ai in a healthy population; for ages 20-, 30- and 40-years the mean Ai is -4.67%, 3.03% and 10.73% respectively. In the PCOS group the mean age was 29.8 years and the mean Ai was 3.72%. In the HV group the mean age was 32.6 years and the mean Ai was 5.69%. Both the PCOS and HV groups had comparable results with the published age-specific mean Ai values. In a healthy population the mean aortic PWV is 6.1 m/s in the under 30 year age group and 6.6 m/s in the 30-39 year age group [311]. In this study the mean aortic PWV was 6.4 m/s in the PCOS group and 6.41 m/s in the HV

group. These both fall within the published normal range of 4.6-7.5 m/s for the under 30 year olds and 4.4-8.1 m/s in the 30-39 year olds [311].

There was no evidence of increased arterial stiffness (measured by aortic and brachial PWV) in this population of PCOS women compared to healthy volunteers. This is in agreement with Muneyyirci-Deale *et al* [202], Ketel *et al* [201] and Moran *et al* [302] who found no relationship between arterial stiffness and PCOS after adjustment for BMI and age. Ketel *et al* also used Rotterdam criteria to diagnose PCOS but had fewer subjects in their study than mine [201]. The mean aortic PWV was 6.31 m/s in the lean PCOS group and 6.82 m/s in the obese PCOS group [201] comparable with the mean 6.4 m/s in this study. Moran *et al* included women diagnosed by NIH criteria and non-NIH criteria but found no differences between these groups [302]. Cussons *et al* [203] also found no difference between non-obese PCOS and controls in arterial stiffness, as measured by PWV and Ai. However, other studies have found evidence of increased arterial stiffness in women with PCOS [164, 166, 200]. Meyer *et al* also measured arterial stiffness by PWV but the mean age and BMI of their population were higher than this study which may partially explain the differences in findings [164]. Soares *et al* used carotid ultrasound to assess artery distensibilty and stiffness index, a different method to that used in this study [166].

There was no difference in ccIMT between the PCOS and HV groups. This is in contrast with a meta-analysis which found that women with PCOS had ccIMT around 0.08mm greater than matched controls [188]. The mean ccIMT of the PCOS patients included in the meta-analysis varied from 0.41 mm to 0.7 mm [188] and the mean ccIMT of PCOS patients in my study was 0.5 mm. The meta-analysis considered the potential confounders of age and obesity but included women who were diagnosed with PCOS using the three different criteria. This study included women with PCOS

diagnosed by the Rotterdam criteria and this may explain some of the difference in outcomes of the studies. Talbott *et al* [187] also failed to find a difference in ccIMT in PCOS patients under the age of 45 years. As subjects were young in this study population, it may be that the metabolic alterations were not yet sufficient to result in measurable ccIMT changes; repeating the study in 15-20 years might conceivably yield different results.

There were no differences in echocardiographic measures between the two groups before or after adjustment for age and BMI except for fractional shortening which was significant in the unadjusted but not the adjusted analysis. One study has reported increased left ventricular posterior wall thickness and ejection fraction in women with PCOS compared to controls [180] whilst others have not [209, 312]. Some studies have reported impaired diastolic function in women with PCOS compared to age- and BMI- matched controls [180, 208] while others have not [209, 312]. The differences in results may be related, in part, to the lack of sensitivity of traditional left ventricular echocardiographic measures.

CHAPTER 5 – Associations of Metabolic Parameters and Body Composition with Cardiovascular Measures

5.1 Introduction

The previous two results chapters have compared the metabolic, anthropometric and cardiovascular data between the polycystic ovary syndrome (PCOS) group and healthy volunteer (HV) group, unadjusted and adjusted for age and body mass index (BMI). Insulin resistance (a risk factor for cardiovascular disease) and testosterone (a possible protector against the development of cardiovascular disease) were significantly different between the two groups after adjustment for age and BMI. Adiponectin, which has anti-atherogenic properties, was significantly higher in the PCOS group in the adjusted analyses. These metabolic variables were explored further to identify any influence on cardiovascular function.

Obesity is common in PCOS and is itself a risk factor for cardiovascular disease. Studies to date have reported conflicting results on the risk of cardiovascular disease and cardiovascular events in women with PCOS. Obesity could partly explain these differing results. Body composition variables were explored further to determine any influence on cardiovascular measures in our population.

As metabolic and body composition could be jointly contributing to cardiovascular outcomes these were explored in multivariable analyses. The models were then adjusted to explore whether adiposity and insulin resistance affected cardiovascular function independently of each other.

Three cardiovascular measures were selected for analyses: aortic pulse wave velocity (aPWV), common carotid intima-media thickness (ccIMT) and the ratio of early to

late diastolic mitral velocity (e':a'). These were chosen as aPWV and ccIMT have the greatest validity as predictors of cardiovascular events in the general population [189, 190, 179]. The measure of diastolic function chosen was e':a' as this is less sensitive to change in pre-load [299], an important consideration because of the potential influence of obesity in this population, and because it is an independent predictor of death [206].

The aims of the chapter are to:

1. Determine the effect of insulin resistance, testosterone and adiponectin on cardiovascular measures in women with PCOS.
2. Determine the effect of BMI on cardiovascular measurements and metabolic parameters in women with PCOS.
3. Explore any association between body composition and insulin resistance with cardiovascular function
4. Explore any association between insulin resistance and cardiovascular function adjusted for visceral fat

5.2 Metabolic Parameters and Cardiovascular Function in Women with PCOS

The PCOS population were initially divided into four groups, according to quartiles of HOMA-IR, testosterone and adiponectin. The mean and standard deviation for the chosen cardiovascular measures were calculated for each group. Comparisons were then made between the quartiles.

5.2.1 Insulin Resistance

With increasing quartiles of HOMA-IR, there was an increase in the mean aortic PWV, 6.00 m/s, 6.10 m/s, 6.50 m/s and 7.10 m/s. However, the mean ccIMT was similar across the quartiles, 0.49 mm, 0.50 mm, 0.51 mm and 0.50 mm for quartiles 1 to 4 respectively. The mean e':a' ratio was lower in quartiles 3 and 4 (1.43 and 1.56 respectively), than 1 and 2 (1.67 and 1.71 respectively). Quartiles 1 and 2 had a significantly lower aortic PWV when compared to quartile 4, p=0.007 and p=0.014 respectively. There were no significant differences across the quartiles with respect to ccIMT and diastolic function. Please see Table 5.1.

Table 5.1 Effect of Insulin Resistance on Cardiovascular Measures in Women with PCOS

Quartile of HOMA-IR	Aortic PWV (m/s)	ccIMT (mm)	Early:late diastolic myocardial velocity (e':a')
1	6.00 (0.6)	0.49 (0.06)	1.67 (0.4)
2	6.10 (0.7)	0.50 (0.08)	1.71 (0.4)
3	6.50 (0.9)	0.51(0.07)	1.43 (0.4)
4	7.10 (1.4)	0.50 (0.05)	1.56 (0.4)
1 versus 4	-1.1 (-1.4 – -0.74); p=0.007	-0.01 (-0.008 – -0.01); p=0.594	0.11 (0.082 – 0.093); p=0.412
2 versus 4	-1.0 (-1.3 – -0.7); p=0.014	0.00 (-0.01 – 0.01); p=0.775	0.15 (0.13 – 0.17); p=0.308
3 versus 4	-0.6 (-0.9 – -0.2); p=0.151	0.01 (0.016 – 0.02); p=0.375	-0.13 (-0.17 – - 0.19); p=0.363

Summary measures by quartile are expressed as mean (Standard Deviation). Comparisons between each quartile and the most insulin resistant quartile are expressed as mean and confidence intervals for the difference. PWV, pulse wave velocity; ccIMT common carotid intima media thickness

5.2.2 Testosterone

The mean aortic PWV increased within the first three quartiles, 5.90 m/s, 6.41 m/s and 6.66 m/s respectively. However, there appeared to be no relationship between

testosterone quartile and mean ccIMT or mean e':a'. There were no significant relationships between quartiles of testosterone and the cardiovascular markers. Please see Table 5.2.

Table 5.2 Effect of Testosterone on Cardiovascular Measures in Women with PCOS

Quartile of Testosterone	Aortic PWV (m/s)	ccIMT (mm)	Early:late diastolic myocardial velocity (e':a')
1	5.90 (0.6)	0.51 (0.07)	1.71 (0.4)
2	6.41 (1.4)	0.46 (0.11)	1.60 (0.5)
3	6.66 (1.1)	0.51 (0.07)	1.45 (0.4)
4	6.62 (1.4)	0.50 (0.06)	1.61 (0.4)
1 versus 4	-0.72 (-1.1 – -0.4); p=0.08	0.01 (0.01 – 0.02); p=0.327	0.1 (0.09 – 0.12); p=0.490
2 versus 4	-0.21 (-0.22 – -0.20); p=0.585	-0.04 (-0.01 – -0.06); p=0.061	-0.01 (-0.04 – 0.04); p=0.493
3 versus 4	0.04 (-0.9 – 0.17); p=0.927	0.01 (0.007 – 0.02); p=0.536	-0.16 (-0.17 – -0.15); p=0.226

Summary measures by quartile are expressed as mean (standard deviation). Comparisons between each quartile and the highest testosterone quartile are expressed as mean and confidence intervals for the difference. PWV, pulse wave velocity; ccIMT common carotid intima media thickness.

5.2.3 Adiponectin

With increasing quartiles of adiponectin, mean aortic PWV decreased; quartile one, 6.74 m/s, quartile two, 6.49 m/s, quartile 3, 6.34 m/s and quartile 4, 6.06 m/s. This reached statistical significance for quartile 1 versus quartile 4. The mean ccIMT decreased across the four quartiles; quartile one, 0.51mm, quartile two 0.51 mm, quartile 3, 0.50 mm and quartile 4, 0.47mm but none of these reached significance. There appeared to be no relationship with quartile of adiponectin and e':a' with the exception of quartile 3 which was significantly lower than quartile 4. Please see Table 5.3.

Table 5.3 Effect of Adiponectin on Cardiovascular Measures in Women with PCOS

Quartile of Adiponectin	Aortic PWV (m/s)	ccIMT (mm)	Early:late diastolic myocardial velocity (e':a')
1	6.74 (1.1)	0.51 (0.07)	1.56
2	6.49 (1.1)	0.51(0.06)	1.56
3	6.34 (1.0)	0.50 (0.07)	1.46
4	6.06 (0.8)	0.47 (0.07)	1.77
1 versus 4	0.68 (0.56 – 0.81); p=0.053	0.04 (0.036 – 0.042); p=0.067	-0.21 (-0.23 – -0.19); p=0.163
2 versus 4	0.43 (0.29 – 0.57); p=0.198	0.04 (0.03 – 0.04); p=0.087	-0.21 (-0.25 – -0.17); p=0.433
3 versus 4	0.28 (0.21 – 0.36); p=0.534	0.03 (0.019 – 0.025); p=0.285	-0.31 (-0.37 – -0.25); p=0.028

Summary measures by quartile are expressed as mean (SD). Comparisons between each quartile and the highest adiponectin quartile are expressed as mean and confidence intervals for the difference

5.3 Body Mass Index and Metabolic and Cardiovascular Measures in Women with PCOS

Women with PCOS were divided into four groups according to quartile of BMI. Mean and standard deviation for each metabolic parameter (HOMA-IR, testosterone and adiponectin) and cardiovascular measure (aortic PWV, ccIMT and e':a') were calculated for each group. Comparisons were made between the lower BMI quartiles and the most obese BMI quartile.

5.3.1 Body Mass Index and Metabolic Parameters

The mean HOMA-IR increased with increasing quartile of BMI; 3.13, 3.21, 5.68 and 7.87 for quartiles one to four respectively. There appeared to be no relationship with testosterone and quartile of BMI and this was confirmed when comparing the mean difference between quartiles. Mean adiponectin decreased with increasing quartiles of BMI; 17.2 µg/ml, 10.9 µg/ml, 9.1 µg/ml and 4.6 µg/ml. The lower two quartiles

compared to quartile 4 for HOMA-IR and adiponectin revealed significant relationships. The lowest two BMI quartiles were less insulin resistant than the highest quartile (p=0.001 for both) and had higher levels of adiponectin (p<0.001 and p=0.003). A comparison of quartiles 3 and 4 for adiponection also reached statistical significance. Please see Table 5.4.

Table 5.4 Effect of Body Mass Index on Metabolic Parameters in Women with PCOS

Quartile of BMI	HOMA-IR	Testosterone (nmol/l)	Adiponectin (µg/ml)
1	3.13 (3.3)	1.58 (0.7)	17.2 (11.3)
2	3.21 (3.2)	1.62 (0.7)	10.9 (8.2)
3	5.68 (4.2)	2.37 (1.2)	9.1 (6.2)
4	7.87 (5.1)	1.99 (0.9)	4.6 (2.5)
1 versus 4	-4.74 (-5.50 – -4.70); p=0.001	-0.41 (-50 – -0.33); p=0.166	12.6 (8.8 – 16.5); p<0.001
2 versus 4	-4.66 (-6.37 – -2.92); p=0.001	-0.37 (-0.40 – -0.30), p=0.219	6.3 (3.9 – 8.9); p=0.003
3 versus 4	-2.19 (-2.58 – -1.80); p=0.150	0.38 (0.25 – 0.50); p=0.291	4.5 (2.9 – 6.2); p=0.006

Summary measures by quartile are expressed as mean (SD). Comparisons between each quartile and the most obese are expressed as mean and confidence intervals for the difference

5.3.2 Body Mass Index and Cardiovascular Function

With increasing quartiles of BMI there was an increase in mean aPWV; quartile one, 5.86 m/s; quartile two, 6.09 m/s; quartile three, 6.41 m/s and quartile four, 7.30 m/s. Mean ccIMT measurements also increased with increasing quartile; first quartile, 0.47mm; second quartile, 0.49 mm; third quartile, 0.51 mm and fourth quartile, 0.52 mm. Mean e':a' ratios decreased with increasing quartile; quartile 1, 2.16;quartile 2, 1.78; quartile 3, 1.68 and quartile 4, 1.35.

Comparisons were made between each quartile and the most obese quartile to see if the relationships were significant. Adjustments were made for age and central pulse pressure as these were potential confounding factors. The lowest three quartiles had a significantly lower aPWV than the most obese quartile; quartile 1 versus 4 mean difference -1.27 ($p<0.001$), quartile 2 versus 4 mean difference -1.14 ($p<0.001$) and quartile 3 versus 4 mean difference -0.79 ($p=0.009$). There were no significant differences between the quartiles when ccIMT was investigated; quartile 1 versus 4 mean difference -0.25 ($p=0.167$), quartile 2 versus 4 mean difference -0.11 ($p=0.560$) and quartile 3 versus 4 mean difference -0.01 ($p=0.947$). The lower two quartiles had a significantly higher e':a' than the most obese quartile and there was no difference between quartiles 3 and 4; quartile 1 versus 4 mean difference 0.72 ($p<0.001$), quartile 2 versus 4 mean difference 0.36 ($p=0.035$) and quartile 3 versus 4 mean difference 0.30 ($p=0.112$). Please see Table 5.5.

Table 5.5 Effect of BMI on Cardiovascular Measures in Women with PCOS

Quartile of BMI	Aortic PWV (m/s)	ccIMT (mm)	Early:late diastolic myocardial velocity (e':a')
1	5.86 (0.72)	0.47 (0.06)	2.16 (0.54)
2	6.09 (0.64)	0.49 (0.04)	1.78 (0.61)
3	6.41 (1.04)	0.51 (0.07)	1.68 (0.43)
4	7.30 (1.10)	0.52 (0.07)	1.35 (0.34)
1 versus 4	-1.27 (-1.85, -0.70); p<0.001	-0.25 (-0.62, 0.11); p=0.167	0.72 (0.36, 1.08); p<0.001
2 versus 4	-1.14 (-1.71, -0.57); p<0.001	-0.11 (-0.46, 0.25); p=0.560	0.36 (0.03, 0.69); p=0.035
3 versus 4	-0.79 (-1.38, -0.21); p=0.009	-0.01 (-0.37, 0.35); p=0.947	0.30 (-0.07, 0.67); p=0.112

Summary measures by quartile are expressed as mean (SD). Comparisons between each quartile and the most obese are expressed as mean and confidence intervals for the difference

5.4 Association of Body Composition and Insulin Resistance with Cardiovascular Measures

Multivariable analyses were undertaken to examine the relationships between obesity and insulin resistance with cardiovascular function. Potential confounders of age, height, central pulse pressure and LDL cholesterol were controlled for. Interaction terms between PCOS status and the explanatory factors were included in the models to see if the relationships varied between PCOS subjects and controls. The standardised regression coefficient, 95% confidence interval, p value and the percentage variation explained in the models were calculated.

Body composition markers (log visceral fat area and BMI) were strongly associated with diastolic function (e':a') and aortic PWV ($p<0.0001$ for all relationships). There was no association between log visceral fat area and BMI with ccIMT ($p=0.382$ and $p=0.101$ respectively). The markers of insulin resistance (HOMA-IR and Insulin AUC) were strongly associated with diastolic function (e':a') and aortic PWV ($p<0.0001$ for all relationships). The interaction terms were not significant suggesting that the relationships between the cardiovascular risk factors and the explanatory variables were similar in both the PCOS and HV groups. There was no significant overall association with HOMA-IR and ccIMT ($p=0.750$). However, the association between insulin AUC and ccIMT varied in the two groups with no association seen in the PCOS group ($p=0.650$) but a strong association in the HV group ($p=0.009$). Please see Table 5.6.

Table 5.6 Associations of Measures of Body Composition and Insulin Resistance with Diastolic Function, Aortic Pulse Wave Velocity and Common Carotid Intima-Media Thickness

	Standardised regression coefficient	95% CI	P-value	Percentage variation explained
Log visceral fat area				
E':a'	-0.386	-0.536, -0.236	<0.0001	48.4
Aortic PWV	0.291	0.153, 0.434	<0.0001	32.4
Common carotid IMT	0.067	-0.084, 0.217	0.382	31.1
BMI				
E':a'	-0.449	-0.584, -0.315	<0.0001	48.8
Aortic PWV	0.383	0.253, 0.513	<0.0001	38.9
Common carotid IMT	0.114	-0.022, 0.251	0.101	30.3
HOMA-IR				
E':a'	-0.311	-0.452, -0.171	<0.0001	41.6
Aortic PWV	0.298	0.164, 0.432	<0.0001	34.1
Common carotid IMT	-0.022	-0.159, 0.115	0.750	30.2
Insulin AUC				
E':a'	-0.466	-0.609, -0.323	<0.0001	47.7
Aortic PWV	0.304	0.159, 0.449	<0.0001	33.9
Common carotid IMT PCOS	-0.047	-0.252, 0.158	0.650	37.3
Common carotid IMT HV	0.323	0.084, 0.562	0.009	31.6

Percentage variation explained includes age, height, central pulse pressure and LDL cholesterol**.** E':a', early:late mitral velocity ratio; PWV, pulse wave velocity; IMT, intima media thickness

5.5 Association of Insulin Resistance with Cardiovascular Measures Adjusted for Log Visceral Fat

Further analyses were undertaken to see if adiposity and insulin resistance affected cardiovascular function independently of each other. The results were adjusted on insulin sensitivity for log visceral fat. This adjustment reduced the strength of the associations between HOMA-IR and aortic PWV, and insulin AUC and aortic PWV which nevertheless remained significant (p=0.015, and p=0.015 respectively). There was no longer a significant association between HOMA-IR and diastolic function after adjustment for log visceral fat area (p=0.063) but the association between insulin AUC and e':a' remained highly significant (P<0.0001). The associations between HOMA-IR and ccIMT, and between insulin AUC and ccIMT in the PCOS group remained non-significant (p=0.270 and p=0.548). The association between insulin AUC and ccIMT in the HV group remained, but was less significant (p=0.028). Please see Table 5.7.

Table 5.7 Associations of Markers of Insulin Resistance with Cardiovascular Measures Adjusted for Log Visceral Fat Area

	Standardised regression coefficient	**95% CI**	**p-value**
HOMA-IR			
E':a'	-0.099	-0.180, 0.005	0.063
Aortic PWV	0.214	0.043, 0.385	0.015
Common carotid IMT	-0.099	-0.274, 0.077	0.270
Insulin AUC			
E':a'	-0.173	-0.266, -0.080	<0.0001
Aortic PWV	0.214	0.042, 0.385	0.015
Common carotid IMT PCOS	-0.076	-0.329, 0.176	0.548
Common carotid IMT HV	0.314	0.035, 0.593	0.028

E':a', early:late mitral velocity ratio; PWV, pulse wave velocity; IMT, intima media thickness

5.6 Summary of Results

In the PCOS group, there was a significant relationship between HOMA-IR and aortic PWV, but no relationship between testosterone and adiponectin with cardiovascular measures. This would suggest that worsening insulin resistance was associated with arterial stiffness. With increasing quartiles of BMI, mean HOMA-IR increased and adiponectin decreased suggesting that, as expected, more obese women with PCOS are more insulin resistant and have lower levels of adiponectin. There appeared to be no clear relationship with testosterone and BMI in PCOS women.

Mean aortic PWV and ccIMT increased with increasing quartiles of BMI in the PCOS group suggesting that obesity was associated with worse cardiovascular function. Diastolic function decreased with increasing quartiles of BMI. There was a significant difference across the lower three quartiles with the most obese quartile for aortic PWV and diastolic function (except quartile 3 compared to quartile 4 for diastolic function) but no significant differences between the groups with respect to ccIMT. Increasing BMI in women with PCOS appeared to be associated with stiffer arteries and worse diastolic function but there was no effect on ccIMT.

In multivariable analyses, body composition and insulin resistance measures were all significantly associated with aortic PWV and diastolic function, after controlling for the potential confounding influences of age, height, central pulse pressure and LDL-cholesterol. There was no association between log visceral fat, BMI, HOMA-IR and ccIMT. Insulin AUC was associated with ccIMT in the healthy volunteers but not in the PCOS group.

Further adjustments for log visceral fat area revealed that the strengths of the associations between measures of insulin resistance and both aortic PWV and diastolic function were reduced but remained significant. This suggests that insulin resistance may be contributing to increased arterial stiffness and diastolic dysfunction independently of visceral fat.

5.7 Discussion

In this study, central arterial stiffness and diastolic dysfunction were associated with insulin resistance and abdominal obesity in young women. It is likely that insulin resistance and abdominal obesity exert effects on cardiovascular function that are at least in part mediated independently of each other. PCOS does not appear to confer any additional cardiovascular risk in young women. Adiponectin and testosterone do not appear to be conferring any protection against cardiovascular risk in young women with PCOS.

Some studies have demonstrated that women with PCOS have stiffer arteries [164, 166, 200] than controls whereas others, like mine, have found no difference in arterial stiffness between women with PCOS and controls [201, 202, 203]. These differing results may be due to small numbers of subjects, failure to adjust fully for obesity, or estimating stiffness in a single arterial territory. Previous studies have used simple anthropometric measures to assess adiposity whereas in this study CT was incorporated, a more accurate and precise measure of regional fat distribution. DEXA was also used to assess lean mass and fat mass, as a higher BMI can reflect a greater lean mass as well as increased fat. This study has demonstrated that central obesity is associated with arterial stiffness in women with PCOS but this is not

affected by the syndrome itself. This is in agreement with Ketel *et al* [201] who used waist circumference as an indicator of central obesity whereas this study used CT. Ketel *et al* did not make an assessment of insulin resistance in their study [201] whereas this study did and found that insulin resistance was having an independent effect on arterial stiffness. Cussons *et al*, in agreement with my study, found no difference in arterial stiffness (assessed by PWV and Ai) between PCOS women and controls but did find that flow-mediated dilatation (FMD) was reduced in the PCOS group [203]. This group did not demonstrate any difference in markers of insulin resistance between the PCOS women and controls but did find elevated androgens in the PCOS group and attributed the difference in FMD to the androgens [203]. Meyer *et al* found that women with PCOS had stiffer arteries and were more insulin resistant than controls; they demonstrated that in women with PCOS, insulin resistance was making an independent contribution to the variance in PWV [164]. In this study, the PCOS group were significantly more insulin resistant and had higher testosterone levels that the HV group. Insulin resistance was independently associated with arterial stiffness but testosterone had no significant association with arterial stiffness.

There was no significant difference in ccIMT between the PCOS group and HVs in adjusted or unadjusted analyses. These results are in agreement with those of Talbott *et al* [187] whose PCOS population had a mean testosterone of 1.6mmol/l similar to our own. Studies reporting a greater ccIMT in PCOS women had higher mean testosterone values [180-182] than this study, but had used less sensitive methods than the mass spectrometry method adopted here to measure testosterone. Vryonidou *et al* have suggested that hyperandrogenism partly attenuates the difference in ccIMT that they found between PCOS women and controls [184].

However, I did not find any association between ccIMT and testosterone. The mean testosterone level was higher in the Vryonidou *et al* study but they used less sensitive methods to measure testosterone than I did. No association was found between insulin resistance or body composition with ccIMT in my study, except for insulin AUC in the HV group. This is in agreement with Soares *et al* who also failed to demonstrate a difference in ccIMT between PCOS women and healthy controls and found no difference in markers of insulin resistance between the two groups [166]. Vryonidou et al found no association between insulin resistance and ccIMT but did with BMI and ccIMT [184].

There were no difference in echocardiographic measures between PCOS women and HVs in this study. However, diastolic function was associated with insulin resistance and central adiposity. Kosmala *et al* [312] also found that impaired systolic and diastolic function were associated with insulin resistance in young, obese women with PCOS. In this study, lean women with PCOS were also included and therefore adds knowledge to the Kosmala *et al* study. Yarali *et al* also found that fasting insulin level correlated negatively with e':a' ratio and have suggested that there is an association between insulin resistance and diastolic dysfunction in women with PCOS [208].

CHAPTER 6: Discussion

6.1 Background

Polycystic ovary syndrome (PCOS) is characterised by hyperandrogenism, ovarian dysfunction and polycystic ovaries. The pathogenesis is debated but insulin resistance is thought to play a role. It is a common condition, affecting 4-20% of pre-menopausal women depending on criteria used for diagnosis and ethnicity.

Many cardiovascular risk factors have been documented to occur in the PCOS population including hypertension [61], dyslipidaemia [59, 60, 134], insulin resistance [57, 83, 84], type 2 diabetes [67, 75-82, 100] and impaired glucose tolerance [75-82]. Due to the presence of these cardiovascular risk factors, it is expected that women with PCOS are at greater risk of cardiovascular disease than women without the syndrome.

Three large prospective cohort studies have found an increased risk of cardiovascular events in women reporting a history of clinical features of PCOS [70-72]. A retrospective cohort study found increased odds ratio for the prevalence of myocardial infarction (MI) and angina in women with PCOS compared to the local female population; this was highest in women over the age of 65 years [148]. A meta-analysis in 2011, found the relative risk for coronary heart disease or stroke was 2.02 in women with PCOS compared to female controls [73]; this was reduced to 1.55 following adjustments for BMI [73]. However, two long-term follow-up studies failed to find a difference in the number of cardiovascular events between women with PCOS and controls [41, 147]. Work by our own group looked at a large community-based database and found no increased risk of large vessel disease in

women with PCOS [67]. A meta-analysis in 2014, found an increased risk of non-fatal stroke in women over 45 years but this observation did not remain after adjustment for BMI [146]; there was no significant increased risk of MI [146]. There may be an excess of cardiovascular events in older women with PCOS but there is no evidence currently of increased cardiovascular events in young women with PCOS.

Subclinical cardiovascular disease can be assessed by measuring endothelial dysfunction, carotid intima-media thickness (cIMT), arterial stiffness and myocardial dysfunction. Some [164, 165, 168] but not all [166, 167, 169-171] studies have found evidence of endothelial dysfunction in women with PCOS. Evidence of increased cIMT in women with PCOS has been demonstrated in some studies [180-185, 188]. However, others have failed to find any increased cIMT in women with PCOS [166, 186]; one study only found evidence of increased cIMT in older women with PCOS [187]. Arterial stiffness in women with PCOS has been demonstrated in some studies [164, 166, 200] but not in others [201-203]. Some studies have reported impaired diastolic function in women with PCOS compared to controls [180, 208] but others have not [209, 312].

Obesity is common in women with PCOS [56] and is itself a risk factor for cardiovascular disease. It is difficult to determine whether any cardiovascular risk in young women with PCOS is due to the syndrome itself or to obesity. Some people with obesity appear to be metabolically healthy and not at increased risk for cardiovascular disease [212, 213] whilst others are metabolically unhealthy and are at increased risk for cardiovascular disease. The distribution of body fat is important as visceral adiposity is an independent risk factor for cardiovascular disease [112-114]. There is some evidence to suggest women with PCOS may have greater

central adiposity than controls [56, 118]; however, Barber *et al*, using magnetic resonance imaging (MRI), the gold standard for measuring fat distribution, failed to find any evidence of increased visceral fat in women with PCOS compared to matched controls [119].

Possible mechanisms by which obesity contributes to cardiovascular disease are unclear but secretion of adipocytokines may play a role. Some adipocytokines have been associated with endothelial dysfunction and oxidative stress which contribute to the development of atherosclerosis [220]. Hyperinsulinaemia and insulin resistance have also been associated with altered adipocytokine secretion [235, 220]. Insulin resistance itself increases the risk for coronary artery disease [250] and has been shown to alter large artery compliance [253] and affect endothelial function [254]. Although obesity is common in PCOS it is not universal [56]. Insulin resistance is also common in PCOS [57, 83, 84] and is present in non-obese and obese women with PCOS [84].

In light of these considerations, this study sought to determine whether there was increased cardiovascular risk in young women with PCOS which was independent of obesity. The relationships between insulin resistance and cardiovascular function, and body composition and cardiovascular function were also explored.

6.2 Discussion of Results

This study has shown that arterial stiffness, carotid intima-media thickness and diastolic function are not increased in young women with PCOS compared to healthy volunteers, after careful adjustment for age and BMI. In this population, there was a positive association between insulin resistance and visceral adipose

tissue with central arterial stiffness and an inverse association of these measures with diastolic function. The association was reduced but still remained significant for insulin resistance and both central arterial stiffness and diastolic dysfunction after adjusting for log visceral fat. This suggests that insulin resistance and visceral fat were mediating an effect on the cardiovascular measures, independently of each other.

Some studies have demonstrated that women with PCOS have stiffer arteries [164, 166, 200] than controls whereas others, like mine, have found no difference in arterial stiffness between women with PCOS and controls [201-203]. These differing results may be due to small numbers of subjects, failure to adjust fully for obesity, or estimating stiffness in a single arterial territory. Previous studies have used simple anthropometric measures to assess adiposity whereas this study incorporated CT, a more accurate and precise measure of regional fat distribution. This study has demonstrated that central obesity is associated with arterial stiffness in women with PCOS but arterial stiffness is not affected by the syndrome itself. This is in agreement with Ketel *et al* [201] who used waist circumference as an indicator of central obesity. Central adiposity is a risk factor for cardiovascular disease in other populations [112-114]. Central adiposity has also been shown to be associated with increasing arterial stiffness in the elderly [313] and in healthy children [314]. Possible mechanisms for central adiposity to cause arterial stiffness include an unfavourable adipocytokine profile and/or insulin resistance.

In this study population, insulin resistance was associated with arterial stiffness independently of visceral fat. Markers of insulin resistance, HOMA-IR and insulin area under the curve (IAUC), were significantly higher in the PCOS group after adjustments for age and BMI. This is consistent with other studies in the literature

[19, 58, 84]. Insulin resistance disrupts insulin signalling pathways [252], resulting in a reduction in nitric oxide and a relative increase in endothelin-1. Both of these alterations may contribute to the development of atherosclerosis, as demonstrated in mice [251]. Hyperinsulinaemia has been shown to promote smooth muscle cell growth, endothelial dysfunction, oxidative stress and activate the sympathetic nervous system [315] which could all contribute to increased arterial stiffness. The finding that insulin resistance persists in women with PCOS compared to HV after adjustment for BMI and age, is potentially an important finding as it could provide a link between PCOS and cardiovascular disease independently of BMI.

There was no significant difference in ccIMT between the PCOS group and HVs in adjusted or unadjusted analyses. These results are in agreement with those of Talbott *et al* [187] whose PCOS population had a mean testosterone of 1.6mmol/l similar to our own. It is unclear whether testosterone is protective or detrimental to cardiovascular health. When quartiles of testosterone were examined in the PCOS group, there were no significant relationship with cardiovascular measures and therefore in this group testosterone does not appear to be playing a role in cardiovascular risk in women with PCOS. There was no association between insulin resistance or body composition with ccIMT.

This study did not demonstrate any differences in echocardiographic measures between PCOS and HVs. Some studies have reported impaired diastolic function in women with PCOS compared to age- and BMI- matched controls [180, 208] while others have not [209]. Differing results may be in part due to the lack of sensitivity of traditional left ventricular echocardiographic measures used. There was an association with insulin resistance and visceral adiposity with diastolic heart function in my study. Kosmala *et al* [312] also found that impaired systolic and diastolic

function were associated with insulin resistance in young, obese women with PCOS. Hyperinsulinaemia could potentially cause cardiac hypertrophy through myocyte growth and interstitial fibrosis, resulting in cardiac dysfunction. Obesity results in increased blood volume and blood pressure which can lead to compensatory cardiac hypertrophy and in time to cardiac dysfunction.

6.3 Study Limitations

There are a number of limitations in this study. The study was cross-sectional in design; therefore the possibility that the association seen between fat distribution, hyperinsulinaemia and cardiovascular dysfunction was mediated by an unmeasured common underlying factor cannot be discounted. This study does not give insight into the mechanisms causing cardiovascular dysfunction; it could be postulated that insulin resistance and central adiposity were influencing cardiovascular dysfunction independently of each other and therefore there may be more than one mechanism involved.

Selection bias may have influenced our results. Recruitment of PCOS women who by chance were healthier than non-enrolled PCOS women (bias towards the null) may have happened. This is because women with a history of diabetes, hypertension, dyslipidaemia or medication for these were excluded from participating. If women with these cardiovascular risk factors had been included, a difference in cardiovascular function between the two groups may have been found. The more inclusive Rotterdam criteria for diagnosis of PCOS were used in this study and may have resulted in the recruitment of women with milder phenotypes of the syndrome. A 'healthy worker' effect (bias away from the null) may have occurred

as some of the volunteers were recruited from medical staff and students at our institution. Due to the nature of their studies and work, they may have been more likely to have increased knowledge and awareness of cardiovascular disease than the general population. If this knowledge was put into practice then they may have been healthier than the general population.

The intention was to age- and BMI-match PCOS subjects with HVs but this was not achieved; however, these potential confounding factors were adjusted for carefully in statistical analyses to avoid any influence they may have had on cardiovascular outcome measures. Obesity itself is associated with hypertension, dyslipidaemia, diabetes and impaired glucose tolerance and therefore may have contributed to the difficulty of recruiting obese, 'healthy' volunteers. Obese women may be less likely to volunteer to participate in research studies than women with a normal BMI whereas obese women with PCOS may be more likely to participate as they have a medical diagnosis and hope to benefit their own health. Initially, healthy volunteers were recruited from our institution. Young students participated and there was no difficulty in age-matching at that time, although there was difficulty in BMI-matching. As recruitment was widened to the local community, the age of healthy volunteers increased. It may be that older women were more likely to take part as they had concerns about their health and were less likely to be responsible for the care of young children. As young women with PCOS were included in this study, the findings cannot be extrapolated to older women with PCOS.

The majority of women enrolled in the study were Caucasian; however, significantly more ethnic minority groups were represented in the PCOS group. Asian and Middle Eastern populations are more at risk of developing diabetes than Caucasians.

This could have influenced the outcome of the study but the numbers were relatively small and therefore analyses were not undertaken excluding these women.

The amount of physical activity undertaken by the subjects was not assessed and this could have been a confounding factor. It is possible that women with PCOS were more physically active than the HVs thus offering them some cardiovascular protection.

A basic smoking history was taken to ascertain whether the subjects were current or ex-smokers as smoking is a risk factor for cardiovascular disease. Smoking pack year history was not assessed; this is a weakness in the study as some women may have had a greater lifetime exposure to cigarette smokers than others.

Women were identified to have diabetes or impaired glucose tolerance in both the PCOS group and HV group. This was unknown pathology and the women were not excluded from analyses. It could be that this affected the results, especially as there was a significant difference between the two groups. However, as there was no difference in cardiovascular function between the two groups it is unlikely to have had a significant effect. The models exploring body composition and insulin resistance markers with cardiovascular function did not find any differences between the groups except for insulin area under the curve and cIMT in the HVs.

It is not possible to completely exclude an association between PCOS and arterial stiffness or diastolic function because of the sample size. However, this is one of the largest studies on cardiovascular function in PCOS ever conducted, suggesting that any effects of the syndrome on these cardiovascular measures independent of obesity, if present, are likely to be small.

6.4 Implication of Results on Clinical Practice

This study has shown that a diagnosis of PCOS itself does not increase cardiovascular risk in young women with PCOS. Further studies are needed to confirm this, but it is reassuring information for the healthcare provider to give to the patient. However, this study has shown that central obesity and insulin resistance are associated with cardiovascular risk in young women, and patients should be counselled regarding this.

Obesity and central adiposity can be easily measured in clinic by measuring BMI and waist circumference. These are of low cost and do not expose the patient to radiation. They can be repeated to assess the effectiveness of any interventions. Insulin resistance is harder to assess in the clinical setting. A fasting glucose and fasting insulin sample are needed to calculate HOMA-IR and although this is feasible it does require patients to be fasted. Assessing patients for the development of diabetes and impaired glucose tolerance is most easily done by measuring glycated haemoglobin. However, this study and others [109, 110] have shown that this misses a significant number of cases and the clinician needs to be aware of the limitations of this test. An oral glucose tolerance test (OGTT) is the best method but is inconvenient and costly for the healthcare provider and patient. OGTTs should be performed at diagnosis and in high risk groups in the PCOS population as suggested in the NICE guidelines [107]. Women with PCOS should be strongly advised and encouraged to maintain a normal BMI (less than 25 in Caucasians and less than 23 in Asians) and waist circumference (less than 80 centimetres).

Interventions to assist weight loss, reduce central adiposity and improve insulin resistance in women with PCOS include lifestyle measures (diet and exercise) and

medication such as orlistat, glucagon-like peptide-1 (GLP-1) mimetics and metformin. Lifestyle intervention is recommended as the first line treatment for prevention of cardiovascular disease by the AE-PCOS society [316]. Calorie restriction can reduce BMI, abdominal obesity and insulin resistance in women with PCOS [317-319]. A 24 week structured exercise program consisting of three supervised 30 minute sessions on a bicycle ergometer with a target of 60-70% of maximal oxygen consumption resulted in reduction in weight, BMI, waist circumference and an improvement in insulin resistance indices in obese PCOS women [319]. However, Hutchinson *et al* found no change in weight or waist circumference with their exercise intervention program (12 weeks of three supervised, intensive one hour training sessions) but did find a significant improvement in insulin resistance, a reduction in visceral fat mass and triglycerides in obese PCOS women [320]. Two systematic reviews and meta-analyses have confirmed that lifestyle intervention in women with PCOS reduces weight and waist circumference and improves insulin resistance [321, 322]. Sprung *et al* found an improvement in endothelial function (measured by flow-mediated dilatation) in overweight and obese women with PCOS using a 16 week moderate-intensity aerobic exercise training program which was independent of improvements in body composition and insulin resistance [323]. They also found that compared to obese controls, women with PCOS had defective microvascular function which improved with a supervised 16 week moderate-intensity aerobic exercise program via the up regulation of nitric oxide [324]. In addition to the benefits to cardiovascular health outlined above, regular exercise also improves reproductive function [319] and cardiorespiratory fitness [325]. Women with PCOS should be strongly encouraged to undertake regular aerobic exercise even if weight loss is not achieved.

Orlistat and Liraglutide are the only licensed medications for weight loss in the UK. Orlistat is an established medication and has been shown to reduce weight in women with PCOS [326]. Liraglutide, a glucagon-like peptide-1 (GLP-1) mimetic, has very recently received a licence for weight loss in the UK. Several studies have demonstrated that liraglutide is effective in reducing weight in women with PCOS. One observational study of 84 obese and overweight PCOS women, who had failed to lose weight with metformin and lifestyle intervention, found a mean weight loss of 9 kilograms when treated with liraglutide [327]. A further observational study of 36 obese PCOS women treated with liraglutide for 12 weeks, found a mean weight loss of 3.8 kilograms and significant improvement in uncontrolled eating and emotional eating scores [328]. Liraglutide in combination with metformin resulted in greater weight loss and reduction in waist circumference than metformin or liraglutide alone in a randomised open-label study in obese PCOS women [329]. Six months treatment with liraglutide has also been shown to significantly reduce procollagen type III amino-terminal peptide (PIIINP), an early marker of liver fibrosis, in obese women with PCOS [330]. These women had also lost weight and therefore the reduction in PIIINP may have been related to the weight loss or the liraglutide treatment or a combination of the two. There is a potential risk of developing medullary thyroid cancer with liraglutide treatment, careful discussion with the patient should be undertaken before prescribing it for weight loss in women with PCOS. Exenatide, another GLP-1 has also been shown to reduce weight in women with PCOS [331] but does not have marketing authorisation for weight loss in the UK.

Metformin has been shown to reduce weight and/or waist circumference in women with PCOS in some [332, 333] but not all studies [334]. Work, by our own group

and Diamanti-Kandarakis *et al* found that metformin reduces arterial stiffness and/or improves endothelial function in women with PCOS [332, 335]. Metformin has also been shown to revert some women with PCOS and IGT to normal glucose tolerance [336] and reduce circulating CRP [337]. Metformin is safe in pregnancy and consideration should be given to prescribing this for PCOS women at higher risk of cardiovascular disease.

Attention should also be given to other modifiable cardiovascular risk factors; PCOS patients should be discouraged from smoking; blood pressure and lipid profiles should be assessed regularly and treated as needed.

6.5 Future Studies

This study looked at young women with PCOS and it could be that the population had yet to develop subclinical cardiovascular disease. Increasing age is associated with increased cardiovascular risk. In order to establish if cardiovascular events are affected in older women with PCOS, a sample of older women with PCOS and HVs should be investigated. Alternatively, this cohort of patients and healthy volunteers should be followed up in the future but results would not be available for many years. Larger and prospective longitudinal studies would also be useful to confirm if the findings of this study are reflected in an ongoing absence of an increase in cardiovascular risk.

As PCOS is a heterogeneous disorder, larger studies, sufficiently powered to look at subgroups within the PCOS population, are needed to identify who is most likely to develop cardiovascular risk factors and disease. This would help clinicians stratify who was at greatest risk and who would benefit most from intervention. This is

particularly important as PCOS is very common and potentially poses a large burden on the National Health Service.

Testosterone could be protective or detrimental in cardiovascular health and a study specifically designed to examine whether women with PCOS are offered some cardiovascular protection or not from testosterone should be undertaken.

6.6 Conclusion

This is the first study to comprehensively assess the relationships between carotid intima-media thickness, arterial stiffness, myocardial function, metabolic abnormalities and regional fat distribution in women with PCOS. It demonstrates that central arterial stiffness and diastolic dysfunction are not increased in young women with PCOS compared to healthy volunteers, after adjustment for age and BMI. Insulin resistance and visceral adipose tissue are associated with arterial stiffness and diastolic dysfunction in young women and are likely to exert their effects on cardiovascular risk independently of each other. This extends the understanding of cardiovascular risk in young women with PCOS. Further studies are needed to establish whether young women with PCOS are protected from cardiovascular dysfunction by an unknown factor related to the syndrome. Central obesity and insulin resistance are modifiable risk factors for cardiovascular disease in young women with PCOS, and lifestyle measures which target weight loss remain crucially important in disease management.

Appendix 1

VISCERAL FAT AND VASCULAR RISK IN POLYCYSTIC OVARY SYNDROME

PATIENT INFORMATION SHEET
(Version 2 September 2008)

1. Title of study

Stratification of cardiometabolic risk in Polycystic Ovary Syndrome: is visceral fat a major player?

2. Introduction

You are being invited to take part in a clinical research study. This study will also form part of a higher degree (MD) educational qualification. Before you decide whether you wish to become involved it is important that you understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask us if there is anything that is not clear or if you would like more information. Take time to consider whether or not you would wish to take part.

Thank you for reading this.

3. What is the purpose of this study?

Polycystic ovary syndrome (PCOS) is the commonest hormone condition in women of reproductive age, affecting up to 10% of the premenopausal population. In addition to its well-recognised effects on weight gain, excessive hair growth and infertility, it is becoming increasingly clear that PCOS is associated with long-term health risks including diabetes and arterial (blood vessel)/heart muscle disease, both of which are related to impaired action of insulin throughout the body, also known as insulin resistance.

There is increasing evidence that a major underlying cause of insulin resistance is a build up of a particular type of fat around the bowel called visceral fat. There have not been any studies on the relationship between visceral fat and arterial disease (or 'stiffness') or heart muscle function in young women with PCOS, but this may be an important area to study as research in middle-aged women with PCOS has shown that visceral fat is associated with increased 'furring' of the arteries in the neck, which is a marker for early heart disease.

We aim to measure visceral fat area in women with PCOS and

relate this to arterial stiffness and heart muscle function.

This study will involve two visits to the Clinical Research Facility and the Welsh Heart Research Institute at the Heath hospital, each lasting 2-3 hours or one visit lasting 6 hours.

4. Why have I been chosen?

You have been chosen for this study as your doctor has identified you as having PCOS. A total of 100 patients and 100 healthy volunteers will be studied.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

6. What will happen to me if I take part?

The study will take place at the Clinical Research Facility and the Welsh Heart Research Institute at the University Hospital of Wales. You will be asked to attend in the morning having only had water to drink (i.e. no breakfast, tea or coffee) on 2 separate days. Each visit will last approximately 2-3 hours. If you are a smoker we request that you avoid smoking on each morning.

We will firstly require you to have a pregnancy test before carrying out any measurements.

We will then measure your weight, height, blood pressure, waist circumference, hip circumference and take a brief clinical history. We will take blood samples (approximately four tablespoons) to check levels of 'fat' proteins (called adiponectin, PAI-1 and RBP-4), blood vessel proteins (called BNP, ICAM-1, VCAM-1 and TBARS) and to measure cholesterol, insulin, glucose and CRP (a marker of inflammation in the body). We will also measure a urine sample for a substance called 8-epi-PGF2α, which is a marker of cholesterol damage.

Next we will measure the amount of visceral fat in your body by three techniques: the BIA (bioimpedance) method, DEXA and CT scanning. The BIA technique is a simple, rapid and non-invasive technique which is used routinely in clinical practice to measure a patient's percentage body fat. It sends a very small electrical current through the body and measures the body's resistance to this current to determine the amount of fat, muscle and water present. The amount of electrical current is so small that you will be unable to feel it. The DEXA scan is also a simple, rapid and non-invasive technique which is used routinely in clinical practice. This scan does involve a very small dose of radiation, equivalent to what you would be exposed to simply by

living in Cardiff for 1 day. A CT scan will then be performed. Unlike CT scans used in medical practice this will be a single image (or 'slice') only, taken at the level of your lower spine. This will give us enough information to measure the amount of visceral fat present, while minimising your exposure to radiation. The amount of radiation you will receive is very small and similar to what you would be exposed to simply by living in Cardiff for 1 week.

We will then measure the stiffness of your blood vessels and your heart muscle function. The 'arterial stiffness' test will be performed using an item of equipment called a 'Sphygmocor' machine. This technique, which is completely painless and 'non-invasive', consists of a small pencil-like probe placed over the wrist, which measures the waveform of the pulse. Further measurements will be obtained over pulses in the neck (the carotid artery) and top of the leg (femoral artery). The 'heart muscle' test will be performed at the Welsh Heart Research Institute using a technique called echocardiography. This is a painless non-invasive ultrasound technique used routinely in clinical practice to give pictures of the heart muscle and chambers within the heart. It uses a very high frequency sound which cannot be heard, but can be emitted and detected by special machines.

Finally we will ask you to attend the Clinical Research Facility on a second morning for an oral glucose tolerance test (OGTT). This will assess how good your body is at handling sugar (termed 'insulin resistance'). We request that you attend in the morning having only had water to drink (i.e. no breakfast, tea or coffee). We will take a blood test from you (roughly one tablespoon) then we will ask you to take a drink containing a measured amount of glucose. Further blood samples (at 30, 60 and 90 minutes) will be taken during the test and a final test is taken at two hours. Each blood sample will be roughly one tablespoon.

If you wish we will be able to reimburse any travelling expenses / car parking fees incurred while attending for the study visits.

7. What do I have to do?

It is important that you take your regular medication in the normal way without altering the dose or timing of these. You should inform us of any dose adjustments.

There are no lifestyle or dietary restrictions and you can continue your daily activities normally. We request that you report any illnesses to us as they may influence the timing of your test visit.

You should inform us if there is any possibility of you being pregnant and this will be tested for in all participants. If you are pregnant you will not be able to participate in this study.

For the study visits, we ask that you attend the Clinical Research

Facility at 8 o'clock in the morning having fasted from midnight the previous night. You can drink water freely up to this point.

8. What are the possible disadvantages and risks of taking part?

Other than possible discomfort (temporary pain, swelling, bruising and rarely infection) caused by the collection of blood, no other side effects are anticipated from the study procedures, though as outlined above, a 'single slice' CT scan and DEXA scan do involve exposure to a small amount of radiation. In addition the CT scan could by chance pick up an unsuspected abnormality in your abdomen, in which case you will be given an opportunity to discuss these findings further with Dr Aled Rees. Similarly, the echocardiogram and blood tests may pick up unsuspected abnormalities in which case you will again be given an opportunity to discuss these findings further with Dr Rees.

9. What are the potential benefits of taking part?

There are unlikely to be any direct benefits for you but the study may provide us with important information in determining whether visceral fat could be important in the development of arterial disease and heart muscle dysfunction in women with PCOS. It may therefore be possible in future to use treatments which reduce visceral fat to prevent the development of arterial disease in patients with PCOS.

10. Will I be able to have the results of my tests?

We will contact you if there are any unsuspected abnormalities in your test results and you will be given an opportunity to discuss these findings with Dr Aled Rees. If you wish to have all your test results (including normal results), we will be able to provide these for you on your request.

11. What if something goes wrong?

This study is being indemnified by Cardiff University. If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

12. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. With

your permission your GP will be informed of your participation in this study. With your permission we may also look at sections of your medical notes which are relevant to the research study.

13. What will happen to the results of the research study?

The results of the research study will be prepared for publication in appropriate medical journals together with presentation at medical conferences. Patients participating in the study will be able to obtain a copy of the results after they have been published in the relevant journal(s). Patients will not be identified in any report/publication.

14. Who is organising and funding the research?

The study is being organised by Dr Aled Rees (the Principal Investigator) and Dr Rosie Hocking (Research Registrar) from the Centre for Endocrine and Diabetes Sciences at the University Hospital of Wales. Funding for the study is provided from funds within the Centre for Endocrine and Diabetes Sciences at the University Hospital of Wales. The doctors conducting the research are not being paid for including and looking after patients in the study.

15. Who has reviewed the study?

The study has been reviewed by the Cardiff and Vale NHS Trust Research and Development Office and by the South Wales Research Ethics Committee.

16. Contact for further information

Should you have any further queries regarding this research study, then please do not hesitate to contact me on 02920 742341. You can also contact me via e-mail on reesda@cf.ac.uk. Or you can contact Dr Rosie Hocking by email hockingrk1@cardiff.ac.uk or on 02920748251 or 07866771280.

Thank you for considering taking part in this study.

Dr Aled Rees
Senior Lecturer in Endocrinology

You will be given a copy of this Patient Information Sheet and a signed consent form to keep

Appendix 2: Healthy Volunteer Recruitment Advert

Are you interested in participating in research?

If you are female, aged 16-45 years old and in good health you could take part in our research.

You will have a test for diabetes, a cholesterol check, scans of your tummy to measure your fat, an assessment of your blood vessels and a heart scan.

The study is held at The University Hospital of Wales, Cardiff and will take 6 hours (over 1 or 2 visits).

You will be reimbursed for your time and for travelling expenses.

For more information please contact:

Dr Rosie Hocking hockingrk1@cardiff.ac.uk or 07866771280

Dr Aled Rees reesda@cardiff.ac.uk or 02920742341

Poster Recruitment Version 4 April 2010

Appendix 3: Healthy Volunteer with Higher BMI Recruitment Advert

Are you interested in participating in research?

If you are female, aged 16-45 years old and **wear clothes dress size 16 or above** you could take part in our research.

You will have a test for diabetes, a cholesterol check, scans of your tummy to measure your fat, an assessment of your blood vessels and a heart scan.

The study is held at The University Hospital of Wales, Cardiff and will take 6 hours (over 1 or 2 visits).

You will be reimbursed for your time and for travelling expenses.

For more information please contact:

Dr Rosie Hocking hockingrk1@cardiff.ac.uk or 07866771280

Dr Aled Rees reesda@cardiff.ac.uk or 02920742341

Poster Recruitment Version 5, October 2010

Appendix 4

VISCERAL FAT AND VASCULAR RISK IN POLYCYSTIC OVARY SYNDROME

HEALTHY VOLUNTEER INFORMATION SHEET
(Version 4 March 2010)

1. Title of study

Stratification of cardiometabolic risk in Polycystic Ovary Syndrome: is visceral fat a major player?

2. Introduction

You are being invited to take part in a clinical research study. This study will also form part of a higher degree (MD) educational qualification. Before you decide whether you wish to become involved it is important that you understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please do not hesitate to ask us if there is anything that is not clear or if you would like more information. Take time to consider whether or not you would wish to take part.

Thank you for reading this.

3. What is the purpose of this study?

Polycystic ovary syndrome (PCOS) is the commonest hormone condition in women of reproductive age, affecting up to 10% of the premenopausal population. In addition to its well-recognised effects on weight gain, excessive hair growth and infertility, it is becoming increasingly clear that PCOS is associated with long-term health risks including diabetes and arterial (blood vessel) disease, both of which are related to impaired action of insulin throughout the body, also known as insulin resistance.

There is increasing evidence that a major underlying cause of insulin resistance is a build up of a particular type of fat around the bowel called visceral fat. There have not been any studies on the relationship between visceral fat and arterial disease (or 'stiffness') or heart muscle function in young women with PCOS, but this may be an important area to study as research in middle-aged women with PCOS has shown that visceral fat is associated with increased 'furring' of the arteries in the neck, which is a marker for early heart disease.

We aim to measure visceral fat area in women with PCOS and relate this to arterial stiffness and heart muscle function. In order for us to investigate this we need to see how normal healthy

individuals, like yourself, who don't have PCOS, respond to the same tests to see if there is a difference with our study patients.

This study will involve two visits to the Clinical Research Facility and the Welsh Heart Research Institute at the Heath hospital, each lasting 2-3 hours or one visit lasting 6 hours.

4. Why have I been chosen?

You have been chosen for this study as you are healthy and similar in terms of age to the patients with PCOS. A total of 100 patients and 100 healthy volunteers will be studied.

5. Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

6. What will happen to me if I take part?

The study will take place at the Clinical Research Facility and the Welsh Heart Research Institute at the University Hospital of Wales. You will be asked to attend in the morning having only had water to drink (i.e. no breakfast, tea or coffee) on 2 separate days. Each visit will last approximately 2-3 hours. If you are a smoker we request that you avoid smoking on each morning.

We will firstly require you to have a pregnancy test before carrying out any measurements.

We will then measure your weight, height, blood pressure, waist circumference, hip circumference and take a brief clinical history. We will take blood samples (approximately six tablespoons) to check levels of your hormones (testosterone, thyroid function, prolactin and 17-hydroxyprogesterone), 'fat' proteins (called adiponectin, PAI-1 and RBP-4), blood vessel proteins (called BNP, ICAM-1, VCAM-1 and TBARS) and to measure cholesterol, insulin, glucose and CRP (a marker of inflammation in the body). We will also measure a urine sample for a substance called 8-epi-PGF2α, which is a marker of cholesterol damage.

Next we will measure the amount of visceral fat in your body by three techniques: the BIA (bioimpedance) method, DEXA and CT scanning. The BIA technique is a simple, rapid and non-invasive technique which is used routinely in clinical practice to measure a patient's percentage body fat. It sends a very small electrical current through the body and measures the body's resistance to this current to determine the amount of fat, muscle and water present. The amount of electrical current is so small that you will be unable to feel it. The DEXA scan is also a simple, rapid and non-invasive technique which is used routinely in clinical practice.

This scan does involve a very small dose of radiation, equivalent to what you would be exposed to simply by living in Cardiff for 1 days. A CT scan will then be performed. Unlike CT scans used in medical practice this will be a single image (or 'slice') only, taken at the level of your lower spine. This will give us enough information to measure the amount of visceral fat present, while minimising your exposure to radiation. The amount of radiation you will receive is very small and similar to what you would be exposed to simply by living in Cardiff for 1 week.

We will then measure the stiffness of your blood vessels and your heart muscle function. The 'arterial stiffness' test will be performed using an item of equipment called a 'Sphygmocor' machine. This technique, which is completely painless and 'non-invasive', consists of a small pencil-like probe placed over the wrist, which measures the waveform of the pulse. Further measurements will be obtained over pulses in the neck (the carotid artery) and top of the leg (femoral artery). The 'heart muscle' test will be performed at the Welsh Heart Research Institute using a technique called echocardiography. This is a painless non-invasive ultrasound technique used routinely in clinical practice to give pictures of the heart muscle and chambers within the heart. It uses a very high frequency sound which cannot be heard, but can be emitted and detected by special machines. Also at the Welsh Heart Research Institute we will examine the main artery in the neck (the carotid artery) using a special ultrasound probe. This is also a painless, non-invasive technique.

Finally we will ask you to attend the Clinical Research Facility on a second morning for an oral glucose tolerance test (OGTT). This will assess how good your body is at handling sugar (termed 'insulin resistance'). We request that you attend in the morning having only had water to drink (i.e. no breakfast, tea or coffee). We will take a blood test from you (roughly one tablespoon) then we will ask you to take a drink containing a measured amount of glucose. Further blood samples (at 30, 60 and 90 minutes) will be taken during the test and a final test is taken at two hours. Each blood sample will be roughly one tablespoon.

If you wish we will be able to reimburse any travelling expenses / car parking fees incurred while attending for the study visits. We will also give you a single payment of £50 to reimburse you for your time spent on the study.

7. What do I have to do?

It is important that you take any regular medication in the normal way without altering the dose or timing of these. You should inform us of any dose adjustments.

There are no lifestyle or dietary restrictions and you can continue your daily activities normally. We request that you report any illnesses to us as they may influence the timing of your test visit.

You should inform us if there is any possibility of you being pregnant and this will be tested for in all participants. If you are pregnant you will not be able to participate in this study.

For the study visit, we ask that you attend the Clinical Research Facility at 8 o'clock in the morning having fasted from midnight the previous night. You can drink water freely up to this point.

8. What are the possible disadvantages and risks of taking part?

Other than possible discomfort (temporary pain, swelling, bruising and rarely infection) caused by the collection of blood, no other side effects are anticipated from the study procedures, though as outlined above, a 'single slice' CT scan and DEXA scan do involve exposure to a small amount of radiation. In addition the CT scan could by chance pick up an unsuspected abnormality in your abdomen, in which case you will be given an opportunity to discuss these findings further with Dr Aled Rees. Similarly, the echocardiogram and blood tests may pick up unsuspected abnormalities in which case you will be given an opportunity to discuss these findings with Dr Rees.

9. What are the potential benefits of taking part?

There are unlikely to be any direct benefits for you but the study may provide us with important information in determining whether visceral fat could be important in the development of arterial disease in women with PCOS. It may therefore be possible in future to use treatments which reduce visceral fat to prevent the development of arterial disease in patients with PCOS.

10. Will I be able to have the results of my tests?

We will contact you if there are any unsuspected abnormalities in your test results and you will be given an opportunity to discuss these findings with Dr Aled Rees. If you wish to have all your test results (including normal results), we will be able to provide these for you on your request.

11. What if something goes wrong?

This study is being indemnified by Cardiff University. If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

12. Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed so that you cannot be recognised from it. With your permission your GP will be informed of your participation in this study. With your permission we may also look at sections of your medical notes which are relevant to the research study.

13. What will happen to the results of the research study?

The results of the research study will be prepared for publication in appropriate medical journals together with presentation at medical conferences. Patients participating in the study will be able to obtain a copy of the results after they have been published in the relevant journal(s). Patients will not be identified in any report/publication.

14. Who is organising and funding the research?

The study is being organised by Dr Aled Rees (the Principal Investigator) and Dr Rosie Hocking (Research Registrar) from the Centre for Endocrine and Diabetes Sciences at the University Hospital of Wales. Funding for the study is provided from funds within the Centre for Endocrine and Diabetes Sciences at the University Hospital of Wales. The doctors conducting the research are not being paid for including and looking after patients in the study.

15. Who has reviewed the study?

The study has been reviewed by the Cardiff and Vale NHS Trust Research and Development Office and by the South Wales Research Ethics Committee.

16. Contact for further information

Should you have any further queries regarding this research study, then please do not hesitate to contact me on 02920 745002. You can also contact me via e-mail on reesda@cf.ac.uk or Dr Rosie Hocking by email hockingrk1@cardiff.ac.uk or 07866771280.

Thank you for considering taking part in this study.

Dr Aled Rees
Senior Lecturer in Endocrinology

You will be given a copy of this Information Sheet and a signed consent form to keep

Appendix 5

VISCERAL FAT AND VASCULAR RISK IN POLYCYSTIC OVARY SYNDROME

Patient Identification Number for this study:

PATIENT CONSENT FORM

(Version 2, September 2008)

Title of Study: Stratification of cardiometabolic risk in Polycystic Ovary Syndrome: is visceral fat a major player?

Name of Researchers: Dr Aled Rees, Dr Rosie Hocking

Please initial box

1. I confirm that I have read and understood the information sheet dated September 2008 (version 2) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I consent to my GP being informed of my participation in the Study. ☐
5. I consent to a pregnancy test. ☐
6. I agree to take part in the above study. ☐

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature

1 copy for patient; 1 for researcher; 1 to be kept with hospital notes

Appendix 6

VISCERAL FAT AND VASCULAR RISK IN POLYCYSTIC OVARY SYNDROME

Subject Identification Number for this study:

HEALTHY VOLUNTEER CONSENT FORM

(Version 4 March 2010)

Title of Study: Stratification of cardiometabolic risk in Polycystic Ovary Syndrome: is visceral fat a major player?

Name of Researchers: Dr Aled Rees, Dr Rosie Hocking

Please initial box

1. I confirm that I have read and understood the information sheet dated March 2010(version 4) for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that sections of my medical notes may be looked at by responsible individuals from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
4. I consent to my GP being informed of my participation in the Study. ☐
5. I consent to a pregnancy test. ☐
6. I agree to take part in the above study. ☐

_____	_____	_____
Name of volunteer	Date	Signature
_____	_____	_____
Researcher	Date	Signature
_____	_____	_____
Name of person taking consent (if different from researcher)	Date	Signature

1 copy for patient; 1 for researcher; 1 to be kept with hospital notes

Subject Number:

VISCERAL FAT AND VASCULAR RISK IN PCOS: CASE REPORT FORM FOR PATIENTS

Patient Details

Enrolment Number ……..

Age …….

Date of Visit ……..

Inclusion Criteria

Diagnosis of PCOS (2 out of 3)

[ ] Ovulatory dysfunction (<6 cycles year)
[ ] Androgen excess (clinical or biochemical)
[ ] Polycystic ovaries on ultrasound

[ ] Age 16-45

Exclusion Criteria

[ ] Pregnancy
[ ] Breastfeeding
[ ] Hypertension
[ ] Diabetes
[ ] Glucocorticoids (current/last 3 mnth)
[ ] Lipid lowering agents (current/last 3 mnth)
[ ] Antihypertensives (current/last 3 mnth)
[ ] Antidiabetic agents (current/last 3 mnth)
[ ] Antiobesity agents (current/last 3 mnth)
[ ] CAH
[ ] Cushings
[ ] Hyperprolactinaemia
[ ] Thyroid disease
[ ] Androgen secreting tumour
[ ] Glucocorticoid resistance

Consent

[ ] Discuss Information Sheet and obtain informed consent

Pregnancy Test

[ ] Negative [ ] Positive

Batch No…… Expiry Date……

Performed by……. Witnessed by…….

Medical History

……

……

……..

Smoking Status

Nonsmoker Y/N Exsmoker (details)…….

Smoker (details)…….

Drug History

Drug.....

Dose.....

Duration.....

Allergies…….

Contraception……...

Family History

……

……

……

Menstrual History

Age at Menarche……...

Eumenorrhoeic cycle length…… Day no…….

Oligomenorrhoeic LMP…….

Amenorrhoeic LMP…….

Clinical Examination

BMI Height (m)……..Weight (kg)……BMI…….

Body composition Fat mass (kg)……...Fat %.....

Waist:Hip Waist (cm)……..Hip (cm)……..ratio……..

Cardiovascular HS
Murmurs
BP 1…….. 2……. 3……. Av…….

Abdominal Respiratory

Oral Glucose Tolerance Test Date…….

Time (mins)	0	30	60	90	120
Glucose					
Insulin					

Other Laboratory Investigations

Blood	Result	Action if abnormal
Overnight Dexamethasone Test (<50nmol/L)		
17-hydroxyprogesterone (2-6nmol/L)		
TSH/FT4 (mU/L/pmol/L)		
Prolactin (50-560mU/L)		
Testosterone (<3noml/L)		
Androstenidone (4-10.2 nmol/L)		
hs CRP		
Total cholesterol (mmol/L)		
Triglycerides(<2mmol/L)		
LDL-cholesterol (mmol/L)		
HDL-cholesterol (mmol/L)		
adiponectin		
Plasminogen activator-1		
Retinol binding protein 4		
Brain natriuretic peptide		
Intercellular adhesion molecule 1		
Vascular cell adhesion molecule 1		
Thiobarbituric acid reactive substance	0min 120 min	
Urine		
8-epi-prostaglandin-F2α		

Medical Physics Department

CT Abdomen Date……..

Total fat	Subcutaneous fat	Visceral fat

DEXA scan Date……..

Absolute total body fat (kg)	
Relative total body fat (%)	
Absolute total lean mass (kg)	
Relative total lean mass (%)	
Absolute total abdominal fat (kg)	
Relative total abdominal fat (%)	

Welsh Heart Research Institute **Date……..**

Augmentation Index and Pulse Wave Velocity

Distance

SSN to carotid pulse……..mm

SSN to radial pulse……mm

SSN to femoral pulse……mm

Blood Pressure

A……..mmHg

B……..mmHg

C……..mmHg

Average…….mmHg

Augmentation Index

	A	B	Average
Augmentation Index (%)			
Central aortic pressure (mmHg)			
Time to return of reflected wave Tr (ms)			
Heart Rate (bpm)			

Pulse Wave Velocity

	A	B	Average
Carotid-radial (m/s)			
Carotid-femoral (m/s)			

Carotid Intima

	mm	**mm**
Right carotid		
Left carotid		
Wave intensity		

Echocardiogram

Systolic fn – long axis fn	
Diastolic fn – e’	
Diastolic fn – a’	
Diastolic fn – e’:a’ ratio	

End of Study

Study completed: Yes [ ] Date……
No [ ] Date……..

If No, reason study not completed [ ] subject withdrawal of consent
[ ] Other……....

……
……

Adverse Events

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Subject Number:

VISCERAL FAT AND VASCULAR RISK IN PCOS: CASE REPORT FORM FOR HEALTHY VOLUNTEERS
Version 1 Oct 2008

Volunteer Details

Enrolment Number ……..

Age ……..

Date of Visit ……..

Inclusion Criteria

[ ] Age 16-45
[ ] Regular menstruation (27-32 days)

Exclusion Criteria

[ ] Pregnancy
[ ] Breastfeeding
[ ] Glucocorticoids (current/last 3 mnth)
[ ] Lipid lowering agents (current/last 3 mnth)
[ ] Antihypertensives (current/last 3 mnth)
[ ] Antidiabetic agents (current/last 3 mnth)
[ ] Antiobesity agents (current/last 3 mnth)
[ ] Family history of PCOS
[ ] Hirsutism
[ ] Hypertension
[ ] Diabetes

Consent

[ ] Discuss Information Sheet and obtain informed Consent

Pregnancy Test

[ ] Negative [ ] Positive

Batch No:…… Expiry Date……

Performed by…… Witnessed by……

Medical History

……

……

……..

Smoking Status

Nonsmoker Y/N Exsmoker (details)…….

Smoker (details)…….

Drug History

Drug.....

Dose.....

Duration.....

Allergies……..

Contraception……..……...

Family History

……

……

……

Menstrual History

Age at Menarche……...

Eumenorrhoeic cycle length…… Day no…….

Oligomenorrhoeic LMP…….

Amenorrhoeic LMP…….

Clinical Examination

BMI Height (m)……..Weight (kg)……BMI…….

Body composition Fat mass (kg)……..Fat %.....

Waist:Hip Waist (cm)……..Hip (cm)……..ratio……..

Cardiovascular HS
Murmurs
BP 1…….. 2……. 3……. Average…….

Abdominal Respiratory

Oral Glucose Tolerance Test Date……

Time (mins)	0	30	60	90	120
Glucose (mmol/L)					
Insulin					

Other Laboratory Investigations

Blood	Results	Action taken if abnormal
17-hydroxyprogesterone (2-6 nmol/L)		
TSH/FT4 (mU/L/pmol/L)		
Prolactin (50-560 mU/L)		
Testosterone (<3nmol/L)		
hs CRP		
Total cholesterol (mmol/L)		
Triglycerides (<2mmol/L)		
LDL-cholesterol (mmol/L)		
HDL-cholesterol (mmol/L)		
adiponectin		
Plasminogen activator-1		
Retinol binding protein 4		
Brain natriuretic peptide		
Intercellular adhesion molecule 1		
Vascular cell adhesion molecule 1		
Thiobarbituric acid reactive substance	0 min 120 min	
Urine		
8-epi-prostaglandin-F2α (pg/ml)		

Medical Physics Department, UHW

CT Abdomen Date……..

Total Fat	Subcutaneous fat	Visceral fat

DEXA scan Date……

Absolute total body fat (kg)	
Relative total body fat (%)	
Absolute total lean mass (kg)	
Relative total lean mass (%)	
Absolute total abdominal fat (kg)	
Relative total abdominal fat (%)	

Welsh Heart Research Institute Date…….

Augmentation Index and Pulse Wave Velocity

Distance

SSN to carotid pulse……..mm

SSN to radial pulse……mm

SSN to femoral pulse……mm

Blood Pressure

A……..mmHg

B……..mmHg

Average…….mmHg

Augmentation Index

	A	B	Average
Augmentation Index (%)			
Central aortic pressure (mmHg)			
Tr (ms)			
Heart Rate (bpm)			

Pulse Wave Velocity

	A	B	Average
Carotid-radial (m/s)			
Carotid-femoral (m/s)			

Carotid Intima

	mm	mm
Right Carotid		
Left Carotid		
Wave Intensity		

Echocardiogram

Systolic fn – long axis fn	
Diastolic fn – e’	
Diastolic fn – a’	
Diastolic fn – e’:a’ ratio	

End of Study

Study completed: Yes [ ] date……..
No [ ] date……..

If No, reason study not completed [ ] patient withdrawal of consent
[ ] other……
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……

Adverse Events

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