The Effects of Obesity and Pre-Diabetic Conditions on Ventricular-Arterial Coupling in Women

A Thesis submitted for the Degree of Doctor of Philosophy

by

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Declaration

This work has not been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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Dedication

In memory of my grandfather

Sidney Davies

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This work was initiated following a meeting with Professor Julian Halcox who listened patiently to my early ramblings about ventricular-arterial coupling and kindly helped to shape those ideas into a sensible project. I am grateful that he saw some potential in the work and in my ability to undertake it. Our discussions helped me to keep an open-mind and to remember the broader perspective. His light touch in providing supervision helped me to flourish as an independent researcher. I would also like to extend my heartfelt thanks for his compassion and support when personal challenges threatened to bring my studies to a halt.

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Abstract

Obesity is associated with an increased risk of developing type 2 diabetes and women with diabetes have a higher relative-risk of cardiovascular mortality than men. Polycystic ovary syndrome (PCOS) is a common condition that is associated with obesity and confers a particularly high risk of diabetes.

There is a need to identify cardiovascular dysfunction in the pre-diabetic stage because, once diabetes is evident, it is difficult to improve the prognosis. Left ventricular hypertrophy (LVH) and diastolic dysfunction are well-established consequences of obesity, but the mechanisms which underpin these findings are not well-defined.

Measurements of ventricular-arterial coupling characterise the load which the ventricle must overcome to eject blood. This thesis investigated whether quantitative measures of ventricular-arterial coupling could explain the development of LVH and diastolic dysfunction in young women at risk of diabetes. Two methods of quantifying ventricular-arterial coupling were used (i) a comparison of arterial and ventricular end-systolic elastance, and (ii) the amplitude and timing of wave reflections in the carotid artery using wave intensity.

Increases in elastance were associated with general and central obesity. In contrast, increased wave reflections were predominantly associated with fat around the organs and with worse metabolic health. Arterial elastance and wave reflections were independent contributors of left ventricular mass but did not independently contribute to diastolic function.

Women with PCOS had similar cardiovascular risk, elastance and diastolic function to matched controls. However, they had a lower odds-ratio of LVH which appeared to be explained by lower amplitude wave reflections. There may be an aspect of PCOS which mitigates the effects of obesity and pre-diabetic states on the pulsatile loading of the left ventricle.

The quantification of ventricular-arterial coupling provides new insights to the effects of obesity and pre-diabetic states on sub-clinical cardiovascular disease.

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Explanation of Study Acronyms

DECODE	Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe
DECODA	Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Asia
ACCORD	Action to Control Cardiovascular Risk in Diabetes
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation Trial
VADT	Veterans Affairs Diabetes Trial
ORIGIN	Outcome Reduction with Initial Glargine Intervention
ARIC	Atherosclerosis Risk in Communities
MESA	Multi-Ethnic Study of Atherosclerosis
CARDIA	Coronary Artery Risk Development in Young Adults

1. General Introduction

Obesity is driving an increase in the prevalence of diabetes

The modern lifestyle of nutritional excess and reduced physical activity is leading to epidemic levels of obesity with up to two thirds of the population being overweight or obese.[1] Obesity is driving an increase in the prevalence of type 2 diabetes,[2] a large-scale problem with significant healthcare costs. In 2013, the prevalence of diabetes in European adults was 8.5% (~ 56 million people) and 95% of these had type 2 diabetes. The prevalence is projected to rise to 10.3% by 2035.[3]

Type 2 diabetes lies at the end of a continuum of disorders of glucose metabolism that also includes insulin resistance, impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). With the exception of insulin resistance, all stages are defined by diagnostic thresholds in European guidelines (Table 1-1). It is noteworthy that the diagnostic threshold for type 2 diabetes is based on the level of glucose at which microvascular disease occurs, but there is evidence that the larger blood vessels may be affected in the pre-diabetic stage.[4]

Table 1-1. Diagnostic criteria for pre-diabetic and diabetic conditions.[4]

Diagnosis	**Criteria**
Impaired fasting glucose	Fasting plasma glucose 6.1- 6.9mmol/L AND 2-hr post-load plasma glucose <7.8mmol/L
Impaired glucose tolerance	Fasting plasma glucose <7.0mmol/L AND 2-hr post-load plasma glucose ≥7.8<11.1mmol/L
Diabetes	Fasting plasma glucose ≥7.0mmol/L OR 2-hr post-load glucose ≥11.1mmol/L

In the earliest stages of abnormal glucose metabolism there is insulin resistance. Cells become less sensitive to circulating insulin and higher levels are required for glucose uptake so pancreatic beta cells produce more insulin to maintain normal blood glucose (euglycaemia). Over time, the higher levels of insulin (hyperinsulinaemia) may not be sufficient to regulate fluctuations in blood glucose after eating and there is IGT. Finally, beta cells will fail, resulting in IFG or type 2 diabetes.

Many individuals with type 2 diabetes remain asymptomatic until complications occur several years after the development of high blood glucose. A lack of symptoms in early stages of the disease results in around half of all cases being undiagnosed at any one time [5] and this precludes early intervention which may delay the onset of complications. Screening for diabetes at a population level is not currently indicated because there is insufficient evidence that early diagnosis and intervention can affect the prognosis of cardiovascular disease. In addition, there is no simple biomarker that can identify the pre-cursors to diabetes. Accurate diagnosis is based on fasting plasma glucose and an oral glucose tolerance test; these are relatively costly and time-consuming at a population level.

An increase in the prevalence of diabetes is also partly driven by a rising proportion of elderly individuals. The DECODE and DECODA studies demonstrated that the prevalence of diagnosed type 2 diabetes increased with age in European and Asian cohorts. [5,6] The combined prevalence in European cohorts was less than 10% in those under 60 years, rising to 10-20% in those between 60 and 80 years.

The prevalence of *undiagnosed* diabetes and pre-diabetic conditions may depend on the diagnostic criteria used in screening. One study found that when the diagnosis was defined by the presence of hyperglycaemia at two hours after an oral glucose load, the prevalence of undiagnosed diabetes and IGT was higher in women. In contrast, a diagnosis based on an isolated fasting glucose revealed higher prevalences of diabetes and IFG in men.[5] Furthermore, European women were more likely to have IGT than men in all age groups up to 70 years. These data support the use of a diagnostic strategy that includes both fasting plasma glucose and oral glucose

tolerance testing in order to avoid under-diagnosis of abnormalities in glucose metabolism.

An increase in the prevalence of diabetes does not necessarily reflect an accumulation of new cases since prevalence is also affected by differences in survival. Data on the incidence of diabetes provide a better insight to how the patterns of the condition have changed over time. Results for the Framingham Offspring Study confirmed that the age-adjusted 8-year incidence rate of diabetes increased between 1970 and 1990. Most of the increase occurred in obese individuals and the increase in incidence of diabetes was lower in women than in men.[2] These data confirm that obesity is contributing to higher levels of type 2 diabetes in modern society.

The proportion of cardiovascular disease cases attributable to diabetes is increasing

The cardiovascular mortality rate in the European general population has fallen by more than 30% in recent years.[7] This improvement may be attributed, at least in part, to better control of cardiovascular risk factors by medications such as aspirin, anti-hypertensive drugs and statins. The absolute risk of cardiovascular disease in those with diabetes has fallen in a similar manner, with large longitudinal studies such as the Framingham Heart Study reporting a 49% reduction between the 1950s and 1990s.[8] However, in the same study, the *relative* risk of developing cardiovascular disease in those with diabetes did not fall, remaining at double the risk of those without diabetes. As a result, the proportion of all cardiovascular disease cases attributable to diabetes has increased.[9]

The risk of cardiovascular mortality increases with the duration of diabetes and those who develop diabetes under 55 years have a greater risk than those who are older.[10] The Hoorn study compared all-cause mortality in subjects with diabetes identified by (i) screening, (ii) known diabetes of short duration and (iii) known diabetes of long duration.[11] The age- and sex-adjusted relative risks of mortality increased from 2.06 in the screening group to 3.19 in the known diabetes groups. In those with short duration diabetes, the elevated mortality risk was predominantly

explained by risk factors other than diabetes, while in those with long-duration diabetes, the elevated mortality risk was independent of such risk factors.

The actual duration of diabetes in these subjects is likely to be underestimated because individuals are frequently asymptomatic at the true onset of hyperglycaemia. In addition, those with known diabetes may have presented with symptoms because of a more 'aggressive' form of diabetes that could affect the mortality risk beyond the duration of the condition. However, if long-duration diabetes is an independent risk factor for mortality, this might suggest that chronic hyperglycaemia is associated with irreversible alteration to cardiovascular structure and function. This is further supported by a review of the largest randomised controlled trials on tight glycaemic control in diabetes (ACCORD, ADVANCE, VADT, ORIGIN) which failed to demonstrate clear evidence of reduced medium-term adverse outcomes.[12] Furthermore, there is some evidence that sub-clinical cardiovascular dysfunction progresses despite good glycaemic control.[13] The reasons for this are unclear. It may be that macrovascular and cardiac changes occur at a pre-clinical stage in the development of type 2 diabetes and are irreversible by the time of diagnosis. In addition, the macrovascular complications may be more strongly related to other co-morbidities such as dyslipidaemia, pro-thrombotic state and vascular dysfunction than to glucose levels.

Women with diabetes have a higher relative risk of cardiovascular mortality than men

Men have a higher absolute risk of cardiovascular mortality but the relative risk associated with diabetes is higher in women.[10] The reasons for this are unclear. However, compared with men, it appears that women have to gain more weight to develop diabetes.[14] The greater storage capacity of subcutaneous fat in women means that greater adiposity is needed to result in ectopic fat deposits that contribute to insulin resistance. In addition, data from the British Regional Heart Study/ British Women's Heart and Health Study suggested that diabetes may have a more adverse influence on adiposity, inflammation and vascular function in women.[15] It appears that compared with men, women with diabetes have undergone more significant

alterations in a cluster of risk factors, and this might have a greater impact on their cardiovascular morbidity and mortality.

Fat is an endocrine organ

Adipose tissue comprises adipocytes, connective tissue matrix, nerve tissue, stromovascular cells, and immune cells. The primary role of adipocytes is to store energy. In situations of calorie excess, free fatty acids (FFAs) in the form of triglycerides are stored in adipose tissue which increases in mass. This fuel can be released back into the circulation at times of calorie deficit and contributes to survival. Obesity is therefore characterised by increased storage of FFAs in a significantly expanded tissue mass.

In 1953, Kennedy suggested that a circulating metabolite may be acting upon the hypothalamus to reduce food intake in the event of reduced energy expenditure and increased fat mass.[16] His hypothesis of 'lipostasis' in the rat centred around adipose tissue producing a metabolite that initiated a negative feedback loop to prevent the rat from becoming obese. This is believed to be the first description of adipose tissue as an endocrine organ. The circulating metabolite was not identified until 1994 when Zhang identified and sequenced the mouse obese (*ob*) gene and its product, leptin.[17]

In animal models leptin causes decreased food intake, reduced body fat and restores euglycaemia.[18] However, obese humans appear to develop leptin resistance which limits its role in regulating metabolism. One study in obese men demonstrated that while recombinant leptin injection decreased appetite, it did not affect basal metabolic rate, body mass or composition.[19] This suggests that while the central effects of leptin may be preserved in obesity, there is likely to be peripheral leptin resistance in metabolically active peripheral tissues such as skeletal muscle.

Following the discovery of leptin a series of other secretory proteins were identified in fat, including adiponectin, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). The modern understanding of adipose tissue is that together, its components work as an integrated unit to express, secrete and react to a range of proteins that have autocrine, paracrine (local) and endocrine (systemic) effects.[20]

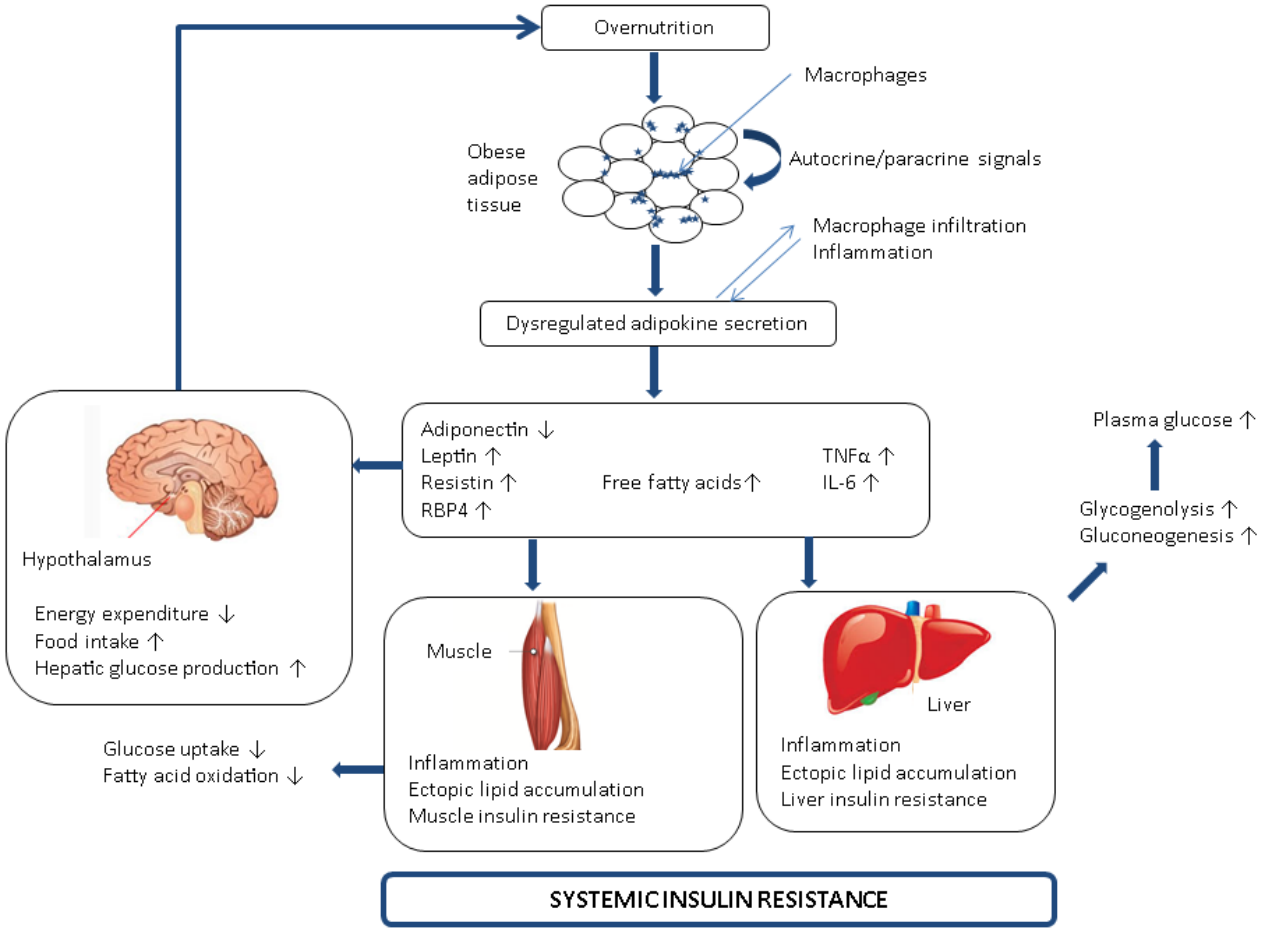

Figure 1-1. Fat as an endocrine organ. Adapted from Galic, Oakhill and Steinberg. [18]

Several detailed reviews of adipose tissue as an endocrine organ can be found in the published literature.[18, 20] Figure 1-1 summarises the key concepts. Greater detail about the effects of adiponectin is given below since this is relevant to subsequent discussion chapters.

Adiponectin

Adiponectin is a protein secreted by fat cells which increases hepatic insulin sensitivity and decreases hepatic glucose production, as well as being involved in the metabolism of lipids. Therefore, this may be one of the mechanisms linking obesity with atherosclerosis and with insulin resistance. A detailed review of the basic and clinical studies of the anti-atherosclerotic and insulin-sensitizing effects of adiponectin was published by Han *et al.* in 2009.[21]

The protein was first described in mice by Scherer in 1995 [22] who found that it was exclusively secreted by adipose tissue and noted to circulate at high concentrations in plasma. Several single nucleotide polymorphisms in the adiponectin gene have been identified and these have been associated with obesity, type 2 diabetes and coronary artery disease.[23] Adiponectin can assemble into a form with three macromolecules (trimeric complex), and can further combine into hexameric or high-molecular weight forms; the latter having 18-36 molecules. The most metabolically-active form of adiponectin is believed to be the high-molecular weight (HMW) form.[24]

Unlike leptin, adiponectin is *decreased* in obesity and increases with weight loss. Levels are maintained in a narrow physiological range and it has a long half-life so it does not alter significantly with dietary challenges. Changes in concentration occur over a prolonged period of time as a result in changes to body weight, hormone levels and insulin sensitivity.[24]

Females tend to have higher levels of HMW adiponectin than males [24] and this is thought to be because of higher levels of subcutaneous fat [25] and because in men, the hormone testosterone inhibits secretion of adiponectin from adipocytes.[26, 27] Thus adiponectin may contribute to the finding that males have a higher degree of insulin resistance than females.

There is evidence that adiponectin has a protective effect against the development of type 2 diabetes regardless of ethnicity and gender.[28-32] The largest of these studies was conducted in the ARIC population (Atherosclerosis Risk in Communities) comprising 1153 white and African-American subjects (581 incident diabetes cases).[29] Those with the highest quartile of adiponectin had 40% lower risk of developing diabetes compared with those in the lowest quartile. However, this difference was absent in smokers and in those with high markers of inflammation, suggesting that there are conditions within which adiponectin is less effective at counteracting metabolic abnormalities.

In addition to its insulin sensitizing effects, adiponectin has anti-inflammatory and anti-atherogenic properties [21] which should mean that it protects against cardiovascular events and mortality. However, a recent review reports a paradoxical positive association between adiponectin and cardiovascular mortality.[33] Most of this evidence is drawn from studies of older people often with established cardiovascular disease but data from the Dallas Heart study extends these findings to a younger population (43±10 years) who were free from overt cardiovascular disease.[34] These authors found that increasing adiponectin quartiles were associated with a better cardiovascular risk profile but in multivariate models adjusted for other cardiovascular risk factors, increasing total adiponectin quartiles were associated with higher rates of all-cause mortality, cardiovascular mortality and cardiovascular events.

The mechanisms underpinning this apparent paradoxical positive association are yet to be defined and it may be that there is a subset of the population with pathologically elevated adiponectin which is a biomarker of an as yet unappreciated risk factor. However, Moreno *et al*. [35] have suggested a cause-effect relationship between adiponectin and cardiovascular mortality in subjects with type 2 diabetes and coronary artery disease enrolled in the Gargano Heart Study (n=356). Their findings are based on the A allele of single nucleotide polymorphism rs822354 (located in the ADIPOQ locus, the gene that encodes for adiponectin) which was associated with total and HMW adiponectin levels as well as a high cardiovascular mortality rate. Further studies with larger sample sizes are needed to confirm this hypothesis.

Not all fat depots are equal

Several large-scale studies have demonstrated that central obesity is more strongly associated with diabetes and cardiovascular disease than is body mass index (BMI).[1, 36, 37] Waist circumference is a simple and inexpensive measure of central obesity which makes it suitable for epidemiological studies across wide geographic areas. The IDEA study [1] investigated the relationship of waist circumference with diabetes and cardiovascular disease in 168,000 people across 63 countries. Waist circumference was consistently associated with higher odds-ratios for diabetes and cardiovascular disease than was BMI. Women with waist circumference greater than 88cm had an odds ratio of 1.97 for cardiovascular disease and 3.94 for diabetes. The relationship between waist circumference and diabetes or cardiovascular disease extended to those who were classified as 'lean' by BMI. These data add to earlier questionnaire-based studies showing the relative risk of diabetes associated with waist circumference in US males [38] and females.[39]

The endocrine function of adipose tissue varies according to the location of the fat. Hormones derived from fat around the organs (visceral fat) are secreted into the portal vein which supplies ~80% of blood flow to the liver. Therefore, visceral fat may have a greater effect on hepatic metabolism than subcutaneous fat which releases hormones into the systemic circulation. In addition, visceral fat expresses and secretes proportionally greater levels of cytokines such as interleukin-6 (IL-6) which correlates with arterial C-reactive protein (CRP), a measure of systemic inflammation.[40] Systemic inflammation is known to induce insulin resistance. Finally, each depot may have specific receptor patterns that determine their ability to respond to other circulating factors such as androgens and angiotensinogen.[41]

It is possible to differentiate visceral from subcutaneous fat using non-invasive imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI) and dual-energy x-ray absorptiometry (DEXA). Many research studies have used these techniques to ascertain whether visceral fat is more strongly associated with cardiovascular risk factors and dysfunction than general obesity.

In the population-based Framingham Heart Study [42] both visceral and subcutaneous fat were associated with the cardio-metabolic risk factors of blood

pressure, fasting glucose and triglycerides. However, the associations were stronger with visceral fat, and in a multivariate analysis visceral fat contributed to risk factor variation beyond the proportion explained by BMI and waist circumference. Similar results were found in African-American subjects in The Jackson Heart Study.[43] In both studies there was a gender difference in relationships; visceral fat was associated with higher odds-ratios of cardiovascular risk and diabetes in women than in men.

Lower levels of insulin resistance and higher subcutaneous fat levels in women [25] have led to speculation that subcutaneous fat may be protective. The theory centres on an 'overspill' hypothesis that ectopic fat stores develop when subcutaneous fat becomes saturated with FFAs because of chronic energy excess. Therefore, those with greater capacity for subcutaneous fat are less likely to develop significant levels of visceral fat. One study has demonstrated that for each standard deviation increase in visceral fat, the odds of insulin resistance increased by 80%, while for each standard deviation increase in subcutaneous fat the odds decreased by 48%.[44] Furthermore, when grouped by visceral fat tertile, those who were insulin sensitive had significantly more subcutaneous fat.

Obesity and insulin resistance affect left ventricular mass

Left ventricular (LV) remodelling or hypertrophy [45-47] and diastolic dysfunction [48] are well-established consequences of obesity.

Data from the Framingham study has shown that left ventricular hypertrophy is an important marker of cardiovascular risk.[49] Historically, uncomplicated obesity was thought to result in an eccentric pattern of hypertrophy while the addition of hypertension would result in a more concentric pattern. More recent evidence has shown that this is not necessarily accurate and that even those who are obese without hypertension may have a concentric pattern. Indeed, a recent systematic review concluded that both eccentric and concentric patterns of hypertrophy were evident in those who are obese [45] and this mixed pattern appears to be more evident in women than in men. The precise mechanisms underlying the *pattern* of remodelling are yet to be clearly defined and this may have therapeutic implications. Whatever the

mechanisms involved, both patterns are associated with adverse cardiovascular outcomes in those with heart failure and preserved ejection fraction (HFpEF).[50]

A detailed description of all potential mechanisms linking obesity and ventricular hypertrophy is beyond the scope of this thesis. A review of such mechanisms was published by de Simone *et al.* in 2013 [51] and the key components identified in that review are highlighted in Figure 1-2.

In general, mechanisms may be considered as having a haemodynamic or non-haemodynamic effect but some mechanisms appear to affect haemodynamic load and have a direct effect on the structure and composition of the myocardium. Particular attention is given below to the major haemodynamic effects of general obesity, central obesity and insulin resistance, since these have direct relevance to the results chapters which follow.

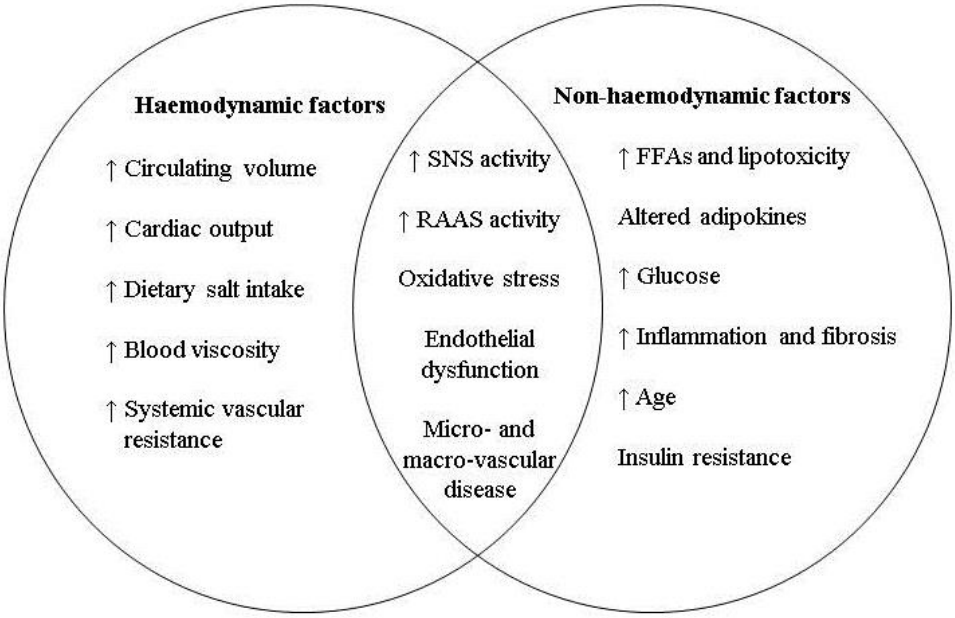

Figure 1-2. Haemodynamic and non-haemodynamic factors influencing the development of increased ventricular mass in obesity. Adapted from de Simone *et al.* [51] SNS: sympathetic nervous system, RAAS: renin-angiotensin-aldosterone system, FFA: free fatty acids.

General obesity is associated with an increased fat and lean mass (predominantly comprising skeletal muscle) and both are positively associated with increased LV mass.[52] It is well established that general obesity is associated with a greater circulating blood volume and that the additional tissues require an increased cardiac output to meet the demands of metabolic function. This increased volume-loading of the LV results in increased wall stress and hypertrophy develops as a compensatory mechanism. The increased cross-sectional area of the vascular bed in obesity might be expected to result in a decreased systemic vascular resistance so that blood pressure remains normal even in the presence of increased cardiac output. In fact, systemic vascular resistance is variable in obese subjects and has been found to be low, pseudo-normal or elevated, depending on the population studied.[53] A pseudo-normal systemic vascular resistance coupled with increased circulating volume would contribute to a higher mean arterial pressure, even in the absence of overt hypertension.

There is also more recent evidence that obesity is associated with a relative deficiency of lean mass likely due to lower muscle mass than is expected for body size. The term sarcopenia is sometimes applied to this finding although it is more traditionally used to describe a loss of muscle mass associated with ageing and chronic disease. In the Strong Heart Study, obese subjects who had a relative reduction in lean mass also had *increased* left ventricular mass,[46] an effect that was particularly evident in women. This may be explained by the fact that individuals with reduced lean mass tend to have even higher levels of adipose tissue with reduced perfusion,[54] demanding greater cardiac output for metabolism.

Central obesity appears to contribute independently to the development of abnormal ventricular geometry, across a wide range of BMI and obese subjects. In the Multi-Ethnic Study of Atherosclerosis (MESA), central obesity and insulin resistance were independently associated with concentric left ventricular remodelling after adjustment for BMI and other risk factors such as hypertension.[47] Central obesity suggests increased visceral fat, which secretes a range of pro-inflammatory molecules that contribute to insulin resistance, cardiac remodelling and heart failure. In addition, visceral fat is particularly metabolically active and requires a more substantial blood supply to support its metabolic activity than subcutaneous fat.[51]

Arterial stiffness is a predictor of mortality [55] and it is likely that the haemodynamic effects of increased stiffness explain some of the increased mortality through the development of ventricular hypertrophy. Studies have reported that obesity is associated with increased arterial stiffness even in children [56] and before the development of hypertension [57]. In addition, this is reversible with weight loss in a young population [58] which suggests that uncomplicated obesity may be associated with increased arterial stiffness before there are any irreversible structural changes.

The most commonly used method to assess arterial stiffness is carotid-femoral pulse wave velocity (PWV) which gives a measure of regional aortic stiffness. This is considered a gold-standard technique which is reproducible, inexpensive [59] and predicts cardiovascular events as well as all-cause mortality.[55] However, PWV is an integrated measure affected by functional haemodynamic factors such as heart rate and blood pressure (which is influenced by cardiac output), as well as by structural stiffness.[60] This probably contributes to its usefulness as a powerful predictor of mortality but also means that, in isolation, it does not help unpick the effects of obesity on these component parts. In addition, accurate measurement of PWV depends on assessing the central aortic path length from measurements made on the body surface which can be significantly overestimated in those who are centrally obese.

Obesity and insulin resistance affect diastolic function

Impaired diastolic function is evident in the earliest stages of uncomplicated obesity in children and young adults.[48, 61] The typical finding is a pattern of reduced early diastolic function and filling with an increased active atrial component. This pattern is not simply a consequence of increased circulating volume since increased venous return would typically cause an increase in early diastolic filling. Obese individuals also tend to have increased left atrial volume, although in the earliest stages of disease this may be related to haemodynamic load and increased filling rather than as a consequence of increased left ventricular end-diastolic pressure. Later in life and with increasing co-morbidities such as hypertension and diabetes, there may be overt diastolic dysfunction which is the underlying pathophysiology in patients with symptoms of HFpEF.[62] Despite this evidence, the precise mechanisms underpinning diastolic dysfunction in obesity are not clearly defined.

Diastolic function appears to be more strongly associated with central obesity and insulin resistance than with general obesity. A study of cardiovascular function in overweight Australian youths (10-19 years) reported that those who were overweight and insulin resistant had worse diastolic function than those who were overweight and metabolically healthy, but only the difference between the insulin resistant group and controls was statistically significant.[48] In multiple regression analysis insulin resistance (HOMA-IR), inflammation (CRP) and adiponectin were significant independent predictors of the early diastolic tissue velocity after adjusting for BMI.

A detailed review of the potential mechanisms linking obesity and myocardial dysfunction was published by Wong and Marwick in 2007.[63] There is considerable overlap in the mechanisms linking obesity with hypertrophy and with diastolic dysfunction. Briefly, the key mechanisms are thought to include insulin resistance, increased free fatty acids (FFA), altered adipokines, activation of the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS), and small vessel disease (as well as myocardial remodelling).

Few studies consider the effect of epicardial fat on diastolic function. Epicardial fat is the visceral fat around the heart. Obese subjects with high levels of abdominal visceral fat also tend to have increased epicardial fat which may affect cardiac function through non-haemodynamic pathways such as by paracrine secretion of inflammatory cytokines. A detailed description of the effects of epicardial fat on myocardial metabolism has been published by Iacobellis, Malavazos and Corsi.[64] Epicardial fat volume measured by CT scanning appears to independently explain some of the variation in diastolic function in a healthy population of older subjects (55±13 years) after accounting for comorbid factors such as hypertension and metabolic syndrome. In addition, it was a stronger correlate of the early diastolic tissue velocity than BMI.[65] Similar results have been found using the echocardiographic method validated by Iacobellis [66] in older subjects with metabolic syndrome.[67]

Obesity and interventional studies

While many of the studies demonstrating diastolic dysfunction and increased left ventricular mass in obesity are cross-sectional in nature, there is evidence from interventional studies that weight loss improves ventricular geometry and diastolic function. A review of these studies showed that dietary interventions resulting in successful weight loss were associated with improved diastolic function with and without accompanying changes in left ventricular mass.[68] Similar findings are described in patients who achieved weight loss following bariatric surgery. There is also some evidence that the combination of exercise and dietary weight loss appears to have a greater impact on the improvement in diastolic function than weight loss alone.

Together, these studies imply that alterations in cardiac structure and function are affected by haemodynamic factors (loading) related to increased body size and non-haemodynamic factors such as visceral fat and insulin resistance. While many of the key epidemiological studies include obese subjects with confounding factors such as hypertension or diabetes, there is emerging evidence that similar results are found in obese children who have yet to develop co-existing pathologies. Kozakova *et al.* recently reported that obese children (age ~13years) had ventricular remodelling that was most strongly associated with body size implying that loading was a significant factor, while diastolic function was most strongly associated with abdominal obesity and fasting insulin.[61]

Therefore, it appears that general obesity, central obesity and insulin resistance impact different aspects of cardiovascular structure and function. It is unclear whether quantification of the ventricular arterial interaction, particularly pulsatile loading, might help to explain the mechanisms linking obesity and increased LV mass or diastolic dysfunction.

Polycystic ovary syndrome and insulin resistance

It is difficult to assess the independent and additive effects of obesity and insulin resistance in the general population since they tend to exist together in many individuals. However, exploration of their individual effects on cardiovascular function may provide insights that are relevant to therapeutic interventions.

Polycystic ovary syndrome (PCOS) is a common condition in women. Although diagnosis is based upon hyperandrogenism and dysfunctional or polycystic ovaries, insulin resistance is present in the majority of women with the disorder, including those who are lean. Therefore, PCOS may provide a model through which to study the individual and combined effects of insulin resistance and obesity on the cardiovasculature. In addition, PCOS is considered to be a risk factor for diabetes by the American Diabetes Association.[69] For these reasons women with PCOS were included as a comparative group in this thesis.

Interdisciplinary interest, uncertainty about the aetiology and significant variation in presentation has led to three different diagnostic definitions for PCOS which identify several different reproductive phenotypes (Table 1-2).

Table 1-2 Diagnostic criteria for PCOS (adapted from Dunaif and Fauser).[70]

	Criteria
National Institute of Health	BOTH hyperandrogenism and chronic anovulation
Rotterdam [71]	Two of the following: hyperandrogenism, chronic anovulation, polycystic ovaries
Androgen Excess Society [72]	Hyperandrogenism plus ovarian dysfunction (oligo/amenorrhea) and/or polycystic ovaries

The National Institute of Health (NIH) definition focuses on the endocrine abnormalities of chronic anovulation *and* hyperandrogenism; this is considered the 'classic' PCOS phenotype. The Rotterdam criteria do not require both these to be present but permit ovarian morphology to be considered, leading to a greater number

of potentially mild phenotypes.[71] The Androgen Excess Society has attempted to bring these definitions together but their diagnostic criteria are used infrequently.[70] The current position of the European Society of Endocrinology is to support the use of the more inclusive Rotterdam criteria in diagnosis, but clinicians and researchers are urged to consider how the specific phenotype will affect risk factors and treatment.[73]

Many studies have examined insulin sensitivity in women with PCOS but there has been debate about whether insulin resistance is extrinsic i.e. solely attributable to visceral adiposity, or intrinsic to the condition. Reports of the prevalence of insulin resistance have been heterogeneous due to differing methods of measuring insulin resistance, thresholds for diagnosing resistance and PCOS diagnostic criteria.

Stepto *et al*. recently reported their results of euglycaemic-hyperinsulinaemic clamp (considered to be a gold-standard technique) in women with PCOS according to the Rotterdam criteria.[74] Insulin resistance was defined by clamp-derived glucose infusion rate (GIR) below the 25th centile of lean, matched controls. This comprehensive study found that insulin resistance was present in 75% of lean women with PCOS and 95% of overweight women with PCOS compared with 62% of overweight controls. Furthermore, the negative relationship between GIR and BMI was more marked in women with PCOS than in controls ($p<.01$). These data support a theory of intrinsic insulin resistance in PCOS, even in those who are lean or from milder phenotypical categories. There appears to be an additional extrinsic level of insulin resistance in the presence of obesity with a greater impact of BMI in PCOS than in controls.

There is little doubt that women with PCOS are at increased risk of developing impaired glucose tolerance (IGT) and type 2 diabetes compared with BMI-matched healthy women. A recent systematic review and meta-analysis calculated an odds-ratio of 2.54 (95% CI 1.44-4.47) for IGT and 4.00 (95% CI 1.97-8.10) for type 2 diabetes in studies where BMI was matched.[75] The magnitude of risk differs significantly between studies and this might be related to different phenotypes, race or ethnicity and levels of obesity.[76]

Impaired glucose tolerance and type 2 diabetes are associated with increased risk of cardiovascular disease in the general population [8] but it is unclear whether the insulin resistance related to PCOS necessarily progresses to dysglycaemia. Few longitudinal studies have examined the rates of progression from normal glucose tolerance to IGT and to type 2 diabetes.[76] In small studies the annual rate of conversion for women with PCOS is 2-3.6% [77, 78] but this is lower than the 7% annual conversion rate in the general population.[79] It may be that the rates in PCOS are an underestimate because of small sample sizes but it is also possible that the degree of dysglycaemia is particular to a PCOS phenotype with limited potential for conversion.

Polycystic ovary syndrome and obesity

Women with PCOS are frequently overweight or obese and this is known to have an adverse effect on the metabolic and reproductive features of the condition.[80]

It may be difficult to establish the precise prevalence of overweight status and of obesity in women with PCOS since this varies according to ethnicity, geographical location and recruitment source. Many studies recruit patients from clinics rather than from the general community and this may introduce selection bias with results tending towards exaggerated levels of adiposity.[81] Clinicians typically expect subjects with PCOS to be overweight or obese which may result in under-diagnosis of the condition in lean women who carry cardio-metabolic risk factors.

One recent systematic review and meta-analysis reported an increased prevalence of overweight status and obesity in women with PCOS [pooled risk ratio (95% CI) of 1.95 (1.52, 2.50) and 2.77 (1.88, 4.10) respectively].[82] In addition, Caucasian women with PCOS had a greater prevalence of obesity than Asian women with the condition [10.79 (5.36, 21.70) versus 2.31 (1.33, 4.00) $p<.001$ between-groups]. There were similar risks of obesity in patients with PCOS diagnosed by NIH and Rotterdam criteria but the studies included in the meta-analysis did not specifically compare mild and more severe phenotypes. A more recent study by Stepto *et al.* found greater adiposity in those with more severe phenotypes.[74]

There seems little doubt that women with 'classic phenotype' PCOS have increased cardiovascular risk factors (beyond insulin resistance) with reviews and meta-analyses confirming dyslipidaemia [83] and elevated CRP [84] amongst many other markers. However, there is debate about whether these risk factors are attributable to increased visceral adiposity in this group [81] and there are limited data on the cardio-metabolic risk in milder phenotypes defined by the Rotterdam criteria.

It is unclear whether any increased cardio-metabolic risk in PCOS translates to adverse cardiovascular outcomes. One systematic review and meta-analysis in 2011 concluded that women with PCOS had a two-fold risk of coronary heart disease or stroke compared with ovulatory controls.[85] The relative risk remained high when data were adjusted for BMI (1.55 95% CI 1.27, 1.89) suggesting that the risk is not entirely attributable to the effects of adiposity. However, the review was based on a very small number of eligible studies (five) and the authors noted significant heterogeneity related to PCOS definition, outcome data and study design. In contrast, a subsequent study in general practice comprising more than 21,000 women with PCOS compared with two groups matched for (1) geographic location and age, and (2) BMI, found an increased risk of diabetes but no increased risk for cancer, large vessel disease or all-cause mortality after adjustment for BMI.[86] This observational study included milder phenotypes of PCOS but the women may have been too young to have developed adverse cardiovascular outcomes. Large prospective longitudinal studies of women with clear definitions of PCOS phenotypes are needed to address this topic.

Even if the effect of PCOS on outcomes is unclear, there is emerging evidence from small studies that women with PCOS have subclinical arterial dysfunction with considerable debate about which aspects are important in the pathogenesis. Some authors suggest that arterial stiffness and/or endothelial dysfunction in those with PCOS are independent of obesity,[87-90] while others maintain that it is obesity and the accompanying insulin resistance which are important.[91, 92]

For example, Meyer *et al*. demonstrated that *overweight* women (BMI >27kg/m^2) with the classic PCOS phenotype had increased arterial stiffness (pulse wave velocity [PWV] 7.5±0.1 vs. 6.6±0.2m/s) and decreased brachial endothelial function (flow-mediated dilatation [FMD] 9.8±0.4 vs. 13.3±0.9%) compared with age- and

BMI-matched healthy controls.[87] The authors suggest that increased arterial stiffness is independent of obesity but lean women were not included in the study. One small case-control study (n=38) demonstrated that lean women with PCOS (BMI<30kg/m^2) had significantly worse endothelial function than controls but similar arterial stiffness (FMD 6.5±2.9% vs. 10.5±4.0% [p<.01], PWV 5.8±1.1 vs. 6.0±1.0 [p=.58]).[90] The authors conclude that a diagnosis of PCOS *per se* is associated with endothelial dysfunction (rather than the confounding factor of obesity). The women in this study were not necessarily typical of the PCOS population since they did not have the intrinsic insulin resistance or elevated testosterone confirmed in larger studies. This suggests that a significant proportion of the lean PCOS group were drawn from a 'mild' reproductive phenotype comprising anovulatory cycles and polycystic ovaries without hyperandrogenemia. It is possible that the finding of arterial stiffness differs according to reproductive phenotype.

A more recent, small, but comprehensive study found that artery stiffness in PCOS is solely attributable to the central obesity associated with the syndrome.[91] The authors used multiple methods of assessing arterial stiffness, considered different locations in the arterial tree, and included lean as well as obese women with PCOS. After careful adjustment for blood pressure and BMI a diagnosis of PCOS did not result in different carotid distensibility, compliance, elastic modulus or aortic augmentation index. Obesity itself, regardless of PCOS status, was associated with stiffer arteries when measures of distensibility, elastic modulus and compliance were considered. There were no differences in pulse wave velocity or aortic augmentation index. Regression analyses confirmed that obesity but not PCOS was associated with measures of arterial stiffness. Any associations between insulin resistance and measures of stiffness disappeared after adjustment for obesity.

Therefore, while obesity may have a more profound effect on arterial stiffness in women with PCOS than in controls, it is unclear whether lean women with insulin resistance and hyperandrogenemia have increased arterial stiffness.

Common carotid intima-media thickness (ccIMT) has been used as a marker of subclinical atherosclerosis in the general population with increased thickness associated with a higher number of cardiovascular events. There have been several

small studies of ccIMT in women with PCOS with disparate results. A systematic review and meta-analysis of these studies in 2012 pooled the results of 19 studies eligible for meta-analysis and found that women with PCOS had a ccIMT that was 0.072 – 0.084mm higher than controls.[93] The authors considered that this is clinically significant because a previous study has estimated that a thickening of just 0.10mm increases the risk of myocardial infarction by 15% and stroke by 18%. However, there was significant heterogeneity across studies both in terms of PCOS phenotypes and quality of ccIMT measurement methods. The review did not seek to establish which cardiovascular risk factors were associated with ccIMT.

Although increased ccIMT is used as a marker of subclinical atherosclerosis in the general population it does not necessarily follow that increased thickness equals atherosclerosis. In obesity, intima-media thickness may increase to compensate for increased circumferential wall stress because of a higher circulating volume which enlarges the lumen, and because of higher blood pressure. Therefore, in obese subjects an increased ccIMT might represent an adaptive vascular hypertrophy.[94] It is also unclear whether the altered ccIMT is present regardless of the metabolic phenotype and whether, given the lack of proven altered outcomes in PCOS, there is an aspect of the syndrome which mitigates this risk factor.

Given the proven trophic effects of insulin it seems logical to assume that the insulin resistance and obesity associated with PCOS may lead to left ventricular hypertrophy and diastolic dysfunction, but there are surprisingly few papers examining cardiac structure and function in this population. The studies tend to be small, using different PCOS diagnostic criteria, different echocardiographic methods, and have disparate results.

While some have found no differences in echocardiographic variables between those with PCOS and controls,[95-98] others have found a 10-40% increase in LV mass and an associated increase in left atrial diameter,[99, 100] either with [100] or without poorer diastolic function.[99]

One of the more frequently cited studies of left ventricular (LV) function is that of Orio *et al.* who studied women with the more severe NIH classification of PCOS.[100] This small prospective study examined LV mass (indexed by height$^{2.7}$),

systolic function (by ejection fraction) and diastolic function (by E/A) in 30 young women with PCOS compared with age- and BMI-matched controls. The women with PCOS had 40% increased LV mass index, poorer systolic function and poorer diastolic function. When subjects were grouped by BMI categories, even those PCOS subjects who were lean had higher LV mass index and worse diastolic function but the difference in systolic function was only evident in the obese category. In the final regression model only the HOMA index was related to LV mass index but it is unclear which other variables were used in the model as these are not reported. However, the authors do state that echocardiographic variables were not related to androgen levels. Since diastolic blood pressure was higher in those with PCOS the conclusion drawn was that insulin resistance in PCOS is associated with increased LV mass independent of obesity and that increased diastolic blood pressure may be the variable that connects these findings.

These data are supported by the results of a subset of the CARDIA women's study who reported that women with PCOS had an increase in LV mass/height$^{2.7}$ (10%) and left atrial diameter, after adjusting for age and race.[99] The magnitude of difference between-groups was significantly smaller than that reported by Orio and this may be the result of the different study designs (population-based vs. academic centre respectively).

In both these studies it appears that it is the insulin resistance which is important in the development of altered LV structure rather than obesity or other derangements associated with PCOS. It is unclear whether obese women with insulin resistance but without PCOS have comparable increases in LV mass index and reduced diastolic function.

The only study which appears to address this issue is that of Kosmala *et al*. examining 150 obese young women categorised by the presence or absence of PCOS (by Rotterdam criteria) and insulin resistance (defined by HOMA-IR threshold >2.5).[98] There were no differences in standard echo measures of LV mass/height$^{2.7}$, LA diameter, ejection fraction or E/A between groups, but obese subjects with insulin resistance had subclinical dysfunction by reduced systolic and diastolic strain whether they had PCOS or not. In multivariate analysis, fasting insulin and BMI emerged as independent predictors of both systolic and diastolic strain parameters.

Although women who were obese but 'metabolically healthy' had better strain and strain rate than those who were obese and had insulin resistance, it is not possible to ascertain whether strain and strain rate were comparable with lean subjects since these were not included in the study.

There are no studies demonstrating whether these potential subclinical alterations in LV structure and function progress to clinically relevant heart failure or worse outcomes in women with PCOS. An increase in LV mass may not be pathological and may be related to an increase in lean muscle mass in subjects with PCOS.[101]

There are no studies which comprehensively examine cardiac and arterial function in a cohort of well characterised women with PCOS across a wide range of metabolic phenotypes and in comparison with age- and BMI-matched controls. This is necessary to understand the confounding effects of obesity and insulin resistance and to explore the pathophysiology. For example, it is unclear whether any increase in LV mass is due to a direct trophic effect of insulin on the myocardium or secondary to increased arterial stiffness and altered wave reflections.

To better determine the most effective management strategy in PCOS a greater number of studies are needed to confirm (1) the causal links between PCOS and altered cardiovascular structure/function (2) the natural history of subclinical cardiovascular dysfunction (3) whether subclinical cardiovascular dysfunction results in altered morbidity and mortality. In the interim, it appears that a strategy of reducing obesity (particularly central obesity) in women with PCOS may at least ameliorate some of the cardiovascular effects of the syndrome.

The potential value of assessing the ventricular-arterial interaction

Cardiac structure and function are affected by arterial structure and function which determine the load that the LV has to pump against to eject blood. In addition, arterial structure and function are affected by systolic cardiac function since the arteries demonstrate increased stiffness at higher pressures and with more rapid ejection of blood by the LV.[102]

Despite this physiological systolic-coupling, many research studies typically assess LV function or arterial stiffness in isolation, providing a limited appraisal of

pathophysiology. In the case of uncomplicated obesity and pre-diabetic states, few studies have established whether any increase in arterial stiffness or pulsatile loading contributes to the development of left ventricular hypertrophy or diastolic dysfunction. This might have direct relevance to potential therapeutic treatments. To the best of my knowledge there are no case-control studies which comprehensively characterize body composition, metabolic health, cardiovascular structure and the ventricular-arterial interaction in obesity.

This thesis focuses on two non-invasive methods of quantifying the ventricular-arterial interaction. The first involves the calculation of arterial and ventricular end-systolic elastance from echocardiography and permits visualisation of the effects of a disease using simplified pressure-volume loops. The second involves deriving wave intensity (WI) signals from changes in pressure and velocity in the carotid artery.

Method 1: Ventricular-arterial coupling and the pressure-volume loop

The pressure-volume (PV) loop provides a detailed visual description of ventricular-arterial interaction across the cardiac cycle. Since its first description by Otto Frank in 1895, the PV loop has been used to understand the effects of pathology on the external stroke work that the ventricle does given a set of loading conditions. A review of the use of PV loops in clinical research was published by Burkhoff in 2013.[103]

Historically, PV loops have been derived from invasive studies that directly measure real-time changes in ventricular pressure while loading or contractility is altered in a step-wise and controlled manner. Ventricular end-diastolic volume (preload) is frequently reduced by transient balloon occlusion of the inferior vena cava (IVC), while the resistance that the ventricle is ejecting against (afterload) may be increased by aortic occlusion or decreased by administration of a vasodilator, and contractility may be altered by administration of a positive or negative inotrope.

Although PV loops provided an excellent assessment of the effects of arterial load on ventricular function, *quantification* of the ventricular-arterial interaction remained difficult because assessments of LV function were made in the time-

domain while those of arterial function were made in frequency-domain. This changed with the development of elastance theory which allowed both arterial and ventricular function to be assessed in the time-domain.

The concept of ventricular elastance

In the late 1960s and early 1970s, a series of papers from Hiroyuki Suga investigated the pumping properties of the LV using the time-course of the relationship between instantaneous pressure and volume. Using an experimental canine model, Suga described a new variable which characterised ventricular pumping properties using the equation

$$e(t) = \frac{p(t)}{v(t)}$$

where, e was a new concept of the reciprocal of compliance (elastance), p was LV systolic pressure, v was LV systolic volume and (t) was the time from the beginning of systole in a given cardiac cycle.[104]

Figure 1-3 demonstrates the typical time course of ventricular elastance in a cardiac cycle. The rising portion (during systole) tended to have two distinct slopes, a steeper early component and a more gradual component towards the peak elastance. The initial fall of elastance was very steep and then the slope became more gradual.

Suga established that the time-course of elastance retained this pattern regardless of alterations in preload [104] and afterload [105] within their physiologic ranges. However, during inotropic stimulation the maximum elastance increased significantly, and occurred sooner in the cardiac cycle.[106] This provided experimental evidence that the time-course of elastance was a robust and relatively load-independent measure of ventricular contractility. Suga also noted the relatively linear time-course of elastance during isovolumetric contraction, a feature that would be used to develop non-invasive methods many years later.

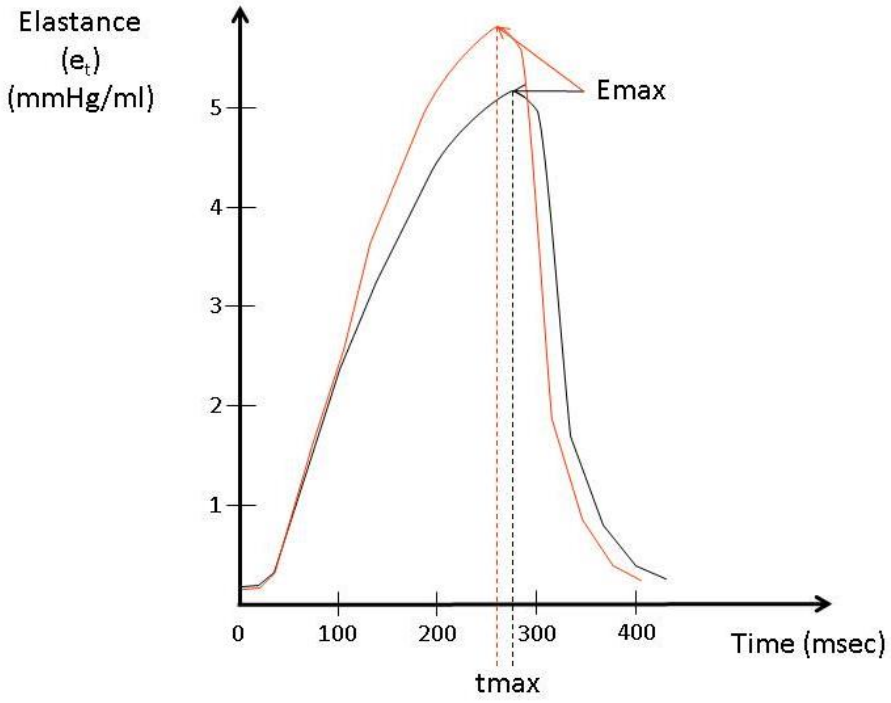

Figure 1-3. Drawing showing the typical time-course of LV elastance through a cardiac cycle. The red curve demonstrates the effects of inotropic stimulation; the maximum elastance increases and occurs sooner in the cardiac cycle. Adapted from Suga.[104]

These early experiments included indicator dilution techniques to estimate ventricular volume and electromagnetic measurement of aortic flow. Suga considered that these methods may have limited the accuracy of volume measurements and in a subsequent paper he, Kiichi Sagawa and Artin Shoukas, re-investigated the pressure-volume ratio with an improved method.[107] The 'cardiometer' involved closing the excised ventricle in an airtight chamber and using air pressure changes to measure changes in ventricular volume (plethysmography). The model was tested using a variety of loading conditions, heart rates and inotropic interventions, and confirmed that the pressure-volume relation was not significantly affected by preload or afterload. Despite an increase in elastance slope during inotropic stimulation, the extrapolated line intercepted the volume axis at a single point greater than zero. This was defined Vd, a correction for the 'dead volume'. The authors therefore assumed that changes in inotropic state would not alter the volume

intercept but would cause the elastance curve to pivot from this point. Therefore, the elastance at any point in time (t) could be calculated by

$$E(t) = \frac{P(t)}{V(t) - Vd}$$

While the elastance curves had similar shape during inotropic stimulation, they had greater amplitude and shorter duration. The authors found that all curves could be 'normalised' using the maximum elastance (E_{max}) and the time to this peak (T_{max}) so concluded that these variables should be considered the characteristic parameters of the elastance curve. E_{max} was defined by the upper leftmost corner of the pressure volume loop and T_{max} was the time taken from end-diastole to E_{max}. Neither variable was affected by changes in loading but both were specifically and sensitively affected by a change in contractile state (during epinephrine infusion). When heart rate was changed by pacing and contractile state was fixed, E_{max} did not alter significantly while T_{max} shortened. These results were confirmed by a subsequent similar study.[108]

In summary, ventricular elastance is a measurement of the stiffness of the LV. An increased elastance will result in a higher change in pressure for any given change in volume. The major determinant of ventricular end-systolic elastance is contractility. The maximum ventricular end-systolic elastance was initially called E_{max} but other terms have been used in the decades that followed the early experimental work. The most recent convention is to use the term E_{es} and this will be adopted in this thesis. The volume intercept will be called V_0 and not V_d as used in the original work.

The concept of arterial elastance

In 1983, Sunagawa, Maughan, Burkhoff and Sagawa presented a conceptual framework to determine whether the stroke volume ejected from the LV into the arterial system could be predicted by quantification of the ventricular-arterial interaction.[109] To achieve this they examined whether the arterial load could be characterised by the pressure-stroke volume relationship. They assumed that the arterial system could be treated as though it was an elastic chamber with a volume elastance (E_a) in the same way that Suga had treated the LV as an elastic chamber

with end-systolic volume elastance (E_{es}). Experimental results from a canine model revealed that the equation below was a very good approximation of the arterial loading system.

$$E_a = \frac{P_{es}}{SV}$$

where P_{es} was the end systolic pressure and SV was stroke volume.

Furthermore, the slope of the pressure-volume relation was highly dependent on arterial resistance (increased slope associated with increased resistance), and less influenced by arterial compliance. Thus, they concluded that it was a measure of 'effective' arterial resistance that included pulsatile load. Having established that the arterial system could be characterised in this way they assessed whether stroke volume could be predicted in any coupled system by simultaneously solving the equations for E_{es} and E_a so that,

$$SV = \frac{V_{ed} - V_0}{1 + {}^{E_a}/_{E_{es}}}$$

Predicted stroke volume was closely correlated with measured stroke volume (r=.985). This demonstrated that the stroke volume ejected into the arterial circulation was dependent on the ratio of elastances, and that the intersection of the slopes E_a and E_{es} could provide a visual means of understanding the effects of altered loading and contractility on stroke volume. The authors used diagrams as a means of communicating the effects of altered loading or contractility on stroke volume (drawings to show examples of these are shown in Figure 1-4). They suggested that in a normal heart an increase in preload would not affect the slope of the ventricular pressure-volume relation (E_{es}) but would cause it to shift to the right so that stroke volume is at a higher new equilibrium. If ventricular contractility was increased, then the slope E_{es} would increase causing an increase in stroke volume (assuming there were no changes in loading). If arterial resistance was increased the slope E_a would increase causing a decrease in stroke volume.

While this visual representation of the ventricular-arterial interaction is useful it does not fully represent intact physiology since a change in the condition of one factor will result in a change in another. For example, a decrease in stroke volume

would result in decrease in mean central blood pressure which, through the baroreflex, would trigger increased sympathetic drive to increase contractility and heart rate.

Using echocardiography, the same authors tested whether ultrasound could be used to quantify ventricular elastance since non-invasive methods would be preferable.[110] They found that left ventricular end-systolic diameter had a good linear relationship with end-systolic volume in a conscious canine model ($V = 32.D – 65$). Results demonstrated that the diameter-derived values of ventricular elastance were close approximations of invasive values in control states and in increased inotropy. However, only normal canine hearts were used and there was concern that the linear relationship may not hold true in dilated, failing ventricles. This work was extended to human testing in 11 subjects undergoing cardiac catheterisation at the Johns Hopkins Hospital.[110] These preliminary results showed a good correlation between diameter-derived E_{es} values and subjective grading of LV systolic function.

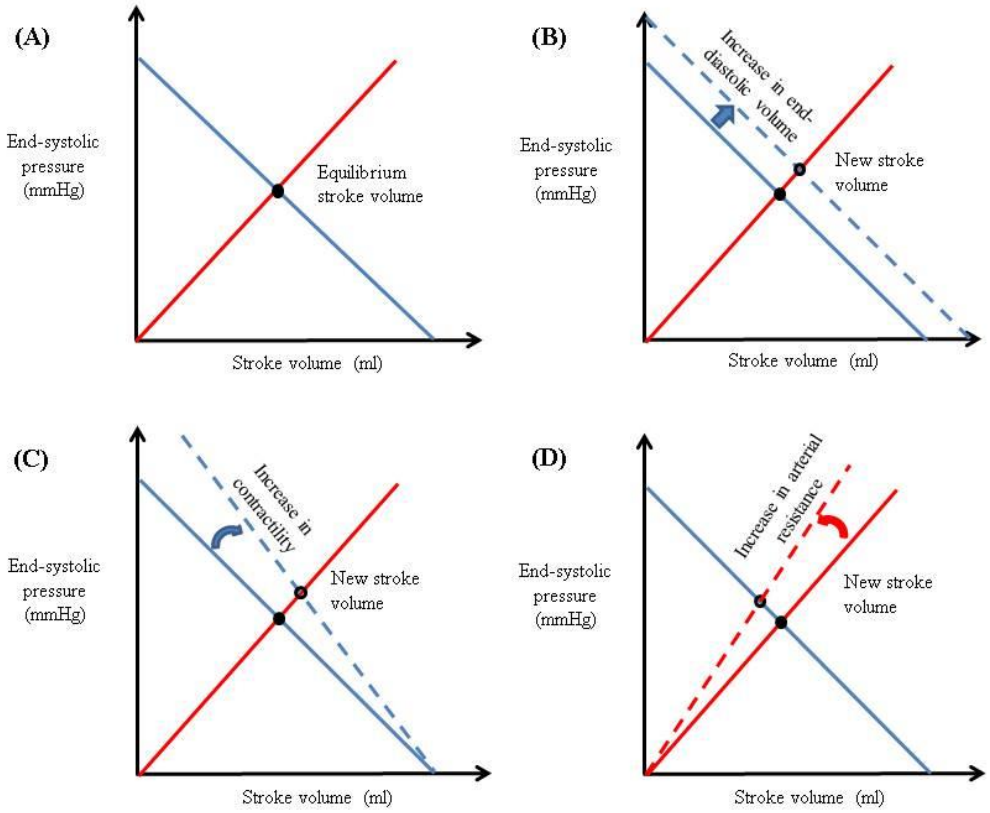

Figure 1-4. Diagrams showing how the intersection between arterial elastance (red) and ventricular elastance (blue) affect stroke volume. The equilibrium stroke volume is shown by the closed circle (A). The effect of an isolated increase in preload is shown in (B) – the ventricular elastance line moves to the right but retains the same slope resulting in an increase in stroke volume (open circle) at a higher end-systolic pressure. The effect of an isolated increase in contractility is shown in (C) – the ventricular elastance line has an increased slope resulting in an increased stroke volume at a higher end-systolic pressure. The effect of an isolated increase in arterial resistance is shown in (D) – the arterial elastance line has an increased slope resulting in a decreased stroke volume but a higher end-systolic pressure. Note that matched increases in arterial and ventricular elastance will result in a higher end-systolic pressure but no change in stroke volume. Adapted from Sunagawa *et al.*[109]

Validation of a non-invasive method of calculating arterial elastance

The same team of researchers led by David Kass at the Johns Hopkins Hospital in the U.S. validated the simplified equation for arterial elastance ($E_a = P_{es}/SV$) in 10 subjects (9 men) across a wide age range (19-60 years).[111] Six of the older subjects had hypertension and their monotherapy was withheld for 10 days before the study. Pressure-volume loops were derived from invasive measures at baseline, during stages of preload reduction with transient IVC balloon obstruction and during pharmacological afterload changes. The simplified elastance calculation ($E_a(PV)$) was almost identical to that calculated using traditional impedance ($E_a(Z)$) measurements ($E_a(PV) = 0.98 \times E_a(Z) + 0.17$, r^2 0.98, $p<.0001$).

In order to facilitate non-invasive estimation of arterial elastance, the authors also tested the use of two equations which could estimate central aortic end-systolic pressure from brachial cuff pressure. These were

$$P_{es} \approx \frac{(2 \times systolic\ BP + diastolic\ BP)}{3}$$

$$P_{es} \approx 0.9 \times systolic\ BP$$

Regardless of which equation was used in the calculation of $E_a(PV)$, it remained closely correlated with $E_a(Z)$; the latter equation yielded an r^2 of 0.97, $p<.0001$. The results of this study demonstrated that the simplified calculation of $E_a(PV)$ could be used to index pulsatile load and that non-invasive means of estimating central end-systolic pressure were acceptable. In addition, the similarity of the equation to that for ventricular elastance (E_{es}) made assessment of ventricular-arterial coupling more attractive and feasible in a non-invasive environment.

While generalisation of these results is hampered by the small study size and lack of diverse pathology, these methods have been used in other studies.[112-117]

Validation of a non-invasive single-beat calculation of LV elastance

The slope of ventricular elastance is more challenging to assess by non-invasive means since it requires knowledge of the volume intercept (V_0) which cannot be measured in an intact circulation. The alternative is to undertake interventions that alter loading (without affecting inotropy) and to plot the change in end-systolic pressure. In experimental studies this has frequently been achieved by transient occlusion of the IVC using a balloon. The invasive and time-consuming nature of the intervention makes it unsuitable for use in large-scale clinical studies.

The first study to validate echocardiographic elastance calculations in humans is believed to have been undertaken by Asanoi *et al.* in 1989.[118] This Japanese study comprised 28 subjects categorised by ejection fraction (EF) into three groups: (i) Normal LV function, EF ≥60%; (ii) Mild LV impairment, EF 40-59%; and (iii) Significant LV impairment, EF <40%. Loading interventions included administration of phenylephrine (a vasoconstrictor) and nitroprusside (a vasodilator).

In an effort to discover a non-invasive means of estimating ventricular elastance, echocardiographic measures of left ventricular volume were calculated using the Teicholz method and indexed for body surface area; these data were gathered simultaneously with arterial pressure waveforms and compared with contrast ventriculography. Echo derived volumes correlated strongly with ventriculography ($r=0.92$, $p<.01$) but echo consistently underestimated the volumes (echo volume = 0.77*Angio +13.70). The authors postulated that this may be because of an underestimated long axis dimension. As expected, ventricular elastance, a measure of contractility, declined with ejection fraction (those with normal EF had E_{es} of 4.5 ± 2.0mmHg/ml/m^2, those with mild impairment had E_{es} of 2.5 ± 1.1mmHg/ml/m^2, and those with significant impairment had E_{es} of 1.5 ± 0.7 mmHg/ml/m^2). Heart rate and end-systolic pressure were similar between groups. In contrast, E_a was noted to increase as EF decreased, largely because of the decrease in stroke volume without an accompanying change in end-systolic pressure. Ventricular-arterial coupling was expressed by the ratio of arterial to ventricular elastance and the ratio was found to progressively increase as ejection fraction decreased. However, only the difference between the normal and significantly impaired group was statistically significant. In the normal group (i), E_a was always less than E_{es} and resulted in a smaller stroke

work for a given contractility and preload. In group (ii) the ratio was near unity and ventricles were performing at almost maximal stroke work for any given preload. The ratio in group (iii) was substantially higher indicating decreased mechanical efficiency. Their aim was to alter loading without changing contractility in order to derive ventricular elastance, but the drugs used in this study could have altered contractility through the baroreflex.

It is difficult to perform loading interventions in large-scale studies and, since the volume at a pressure of zero is small in comparison with left ventricular end-systolic volume, some authors have simplified the calculation of ventricular elastance by assuming that V_0 is effectively zero i.e. that it passes through the origin.[112] This is appealing because it means that the slope of ventricular elastance can be calculated from a single blood pressure and LV volume measurement. However, this assumption can lead to overestimation of ventricular elastance with negative values of V_0 and underestimation with positive values of V_0.[119]

In addition, there is an assumption that the line can only pivot from a fixed volume intercept, whereas it has been shown to shift along the volume axis in disease conditions such as in regional ischaemia [120] and in patients with severely impaired left ventricular function.[118]

To overcome this problem the group of researchers led by Kass developed a more comprehensive method of assessing elastance which sought to calculate the volume intercept.[121] The hypothesis is based on the earlier finding that ventricular stiffening and relaxation through a single cardiac cycle cause predictable changes in the time-course of elastance. The method (Chen *et al*.) establishes two single-point elastance calculations within a single cardiac cycle and assumes that the volume intercept will be the same for both.

The calculation of the first elastance point at end-systole was described above. The authors selected the second elastance measurement to be at the end of the isovolumic contraction period. At this point the pressure in the LV would be similar to the aortic diastolic pressure (since this is necessary for the aortic valve to open) and the volume would be the same as the end-diastolic LV volume since the blood

has yet to be ejected from the ventricle. From these it was possible to calculate an end-diastolic elastance.

The ratio of diastolic to end-systolic elastance was described as amplitude and time-normalised elastance (E_{Nd}) for a single cardiac cycle. Therefore,

$$E_{Nd} = \frac{E_d}{E_{es}}$$

or,

$$E_d = E_{Nd} \times E_{es} \qquad (1)$$

The normalised elastance could then be used to calculate E_{es} without making assumptions about the volume intercept. Full details of the mathematical derivation from the original paper by Chen *at al*.[121] are included below in order to facilitate understanding of the methods used in this thesis. The method has been adapted by the inclusion of additional explanatory mathematical steps.

If,

$$E_{es} = \frac{P_{es}}{V_{es} - V_0} \qquad (2)$$

and

$$E_d = \frac{P_d}{V_d - V_0} \qquad (3)$$

Equations (1) and (3) can be combined to give

$$E_{Nd}\, x\, E_{es} = \frac{P_d}{V_d - V_0}$$

or

$$V_d = \frac{(P_d/E_{Nd})}{E_{es}} + V_0 \qquad (4)$$

and equation (2) can be rearranged to a similar format

$$V_{es} = \frac{P_{es}}{E_{es}} + V_0$$

The influence that a change in volume will have on pressure is therefore given by

$$V_d - V_{es} = \left(\frac{(P_d/E_{Nd})}{E_{es}} + V_0\right) - \left(\frac{P_{es}}{E_{es}} + V_0\right)$$

In this way, V_0 is removed from the equation and E_{es} becomes the common denominator

$$V_d - V_{es} = \frac{(P_d / E_{Nd}) - P_{es}}{E_{es}}$$

Multiplying all terms on the right of the equation by E_{Nd} removes the double division

$$V_d - V_{es} = \frac{P_d - (P_{es} \times E_{Nd})}{E_{es} \times E_{Nd}}$$

Finally, re-arranging for E_{es} gives

$$E_{es} = \frac{P_d - (P_{es} \times E_{Nd})}{E_{Nd} \times (V_d - V_{es})}$$

where P_d was the diastolic blood pressure, P_{es} was calculated by 0.9 x brachial systolic blood pressure and Vd-Ves was the stroke volume by the Doppler method.

This theory allowed the slope of ventricular elastance to be calculated without assuming a volume intercept at the origin. The original work by Suga documented a stable elastance during the isovolumic contraction period with limited variations caused by contractility and afterload.[104, 105] However, in the non-invasive method measurement needs to be taken at the onset of ejection so that the diastolic blood pressure can be used in the calculation of elastance (since an invasive measure of pressure is not available). As a result there was more variation in individual elastance using this method. The authors sought to overcome this by using data from their

invasive experimental work to derive a regression formula based on individual ejection fraction (contractility) and the ratio of systolic to diastolic pressure (afterload). Thus, the estimated normalised elastance became

$$E_{Nd(est)} = 0.0275 - (0.165 \times EF) + \left(0.3656 \times \frac{P_d}{P_{es}}\right) + \left(0.515 \times E_{Nd(avg)}\right)$$

where the group E_{Nd} average was given by a polynomial function

$$E_{Nd(avg)} = \sum_{i=0} ai \times T_{ND}i$$

and $T_{ND} = \frac{Time\ from\ R\ wave\ to\ AV\ opening}{Time\ from\ R\ wave\ to\ AV\ closure}$

and values of ai were 0.35695, -7.2266, 74.249, -307.39, 684.54, -856.92, 571.95 and -159.1 for i=0 to 7, respectively.

The authors showed that average normalised elastance from the polynomial equation had a strong correlation with the normalised elastance measured from the invasive data (r=0.88 and p<.0001, SEE 10% about the mean). Results showed that the estimated slope E_{es} tended to overestimate the invasively measured value in 43 comparisons with regression equation $E_{es} = 0.78 \times E_{es(est)} + 0.55$ (r=0.81, p<.0001, SEE 0.50). There was no systematic bias and 80% of the errors were <0.6mmHg/ml.

This way of calculating ventricular elastance was termed a single-beat method and was compared with the simple measurement which assumes a volume intercept with the origin (P_{es}/V_{es}). The simple measurement correlated less well with the invasive values (r=0.56) than did the single-beat method and consistently over-estimated the slope of ventricular elastance, with the error becoming worse at higher values.

Reproducibility of the single-beat method was assessed by repeating non-invasive measurements over 3 months in 7 subjects. The coefficient of variation was 20 ± 6% with variability related predominantly to changes in stroke volume (14 ± 6.6%).

Elastance measurements and obesity

There are many studies of the effects of obesity on ventricular or arterial function but few have an integrated approach and fewer still include non-invasive measurements of elastance.

Arterial and ventricular end-systolic elastance are positively related to body size in a healthy population. Therefore, it may be necessary to scale these measures before interpreting between-group differences associated with obesity. These and other echocardiographic variables are frequently scaled by dividing by a measure of body size which assumes a linear relationship between the two; this is called ratiometric scaling. Ratiometric scaling is inappropriate if the relationship with body size is non-linear and can result in both over- and under-estimation of results, depending on the true relationship. In such cases allometric scaling may be more appropriate; the physiological variable should be scaled by a measure of body size raised to some power of a scalar exponent.

One comprehensive study by the Asklepios investigators has confirmed a positive association of arterial (E_a) and ventricular end-systolic elastance (E_{es}) with height, weight and body surface area in a large reference population (n=612) of healthy, lean volunteers aged 35-55yrs.[122] The relationships with height and weight were non-linear suggesting that allometric scaling by these variables is necessary. The relationship between arterial and ventricular elastance and body-surface area was approximately linear so ratiometric scaling might be suitable for this measure of body size.

In the same study these scaling methods were used to assess whether obesity affected arterial and ventricular elastance beyond the normal relationship with body size. The authors chose to use allometric rather than ratiometric scaling by body surface area (the reason for this is unclear). The unscaled arterial elastance *decreased* with overweight (n=901) and obesity (n=316), while the unscaled ventricular elastance appeared unaffected. These findings are counterintuitive given the established links between obesity, hypertension and increased left ventricular mass. In contrast, scaled values of arterial and ventricular elastance significantly *increased* with each category of obesity [mean E_a ($mmHg/mL/m^{2.4}$) was 2.89 in lean subjects

(n=1151), 3.1 in overweight subjects (n=901) and 3.89 in obese subjects (n=316); mean E_{es} (mmHg/mL/$m^{2.1}$) was 3.28 for lean subjects, 3.55 for overweight subjects and 3.89 for obese subjects; all differences were statistically significant ($p<.0001$)]. The ratio of elastances was unchanged in obesity but this should not be mistaken for a 'normal' finding. In this case it occurred because the increase in arterial elastance was matched by an increase in ventricular end-systolic elastance. This may have the effect of preserving ejection fraction but at the expense of elevated systolic pressure, reduced ventricular contractile reserve and more labile blood pressure.

Since the study above was cross-sectional in nature it was not possible to say with certainty that obesity was the cause of the increased elastance. Therefore, the same authors sought to establish this in a longitudinal study.[116] Subjects were drawn from a randomly selected, community-based population of 1,402 U.S. subjects who underwent comprehensive echocardiography on two occasions separated by 4 years. Only 56% of subjects had adequate paired data for determining arterial and ventricular end-systolic elastance, and while this suggests that the feasibility of the technique may be low in a general population, the results are likely affected by the absence of elastance measurements among the primary outcome measures in the initial study design. In the four years between study visits, arterial elastance decreased by 3% because of improved blood pressure control in the sample population which negated the effect of ageing. In contrast, ventricular end-systolic elastance increased by 14%. Weight loss between examinations was associated with a greater decrease in arterial elastance than was explained by blood pressure control. However, central obesity was not an independent predictor of the change in arterial elastance. In women, central obesity resulted in greater age-related changes in ventricular end-systolic elastance (after adjustment for arterial elastance) but this finding was not evident in men. From this the authors speculate that measures to control central obesity are particularly important in preventing heart failure in women.

While these data provide an insight to the relationship between obesity and ventricular-arterial coupling, interpretation of the findings is complicated by the inclusion of a significant number of subjects with hypertension as well as those taking cardiovascular medication. For example, the improved blood pressure control

between visits may have masked any age related increase in arterial elastance and the anti-hypertensive medication may well have affected several key contributors to arterial and ventricular elastance.

Therefore, there is some uncertainty about the effects of uncomplicated obesity on unscaled and scaled measures of arterial and ventricular end-systolic elastance. It is also unclear whether central obesity and insulin resistance explain more of the variation in elastance than general obesity. In addition, the mechanisms linking increased elastance with obesity are unclear; relative contributions of altered geometry, structure, heart rate, resistive and pulsatile load to elastance are not well defined in an uncomplicated obese population. More detailed physiological information is needed to better understand the complexities of these simplified measures of stiffness and to inform potential therapeutic targets.

Elastance and pre-diabetic states

I have been unable to find conclusive data relating to the independent effects of pre-diabetic states on elastances and ventricular-arterial coupling. There are also limited studies of ventricular-arterial coupling in patients with diabetes.

Data from the Asklepios study suggest that impaired fasting glucose is associated with higher mean arterial blood pressure without a change in aortic stiffness or pulsatile indices, whereas diabetes was associated with increased aortic stiffness reflected by increased characteristic impedance, and increased pulse wave velocity.[123] These data suggest that arterial elastance may be increased in the later stages of abnormal glucose handling but it is unclear whether ventricular elastance will be affected.

One small but comprehensive cross-sectional study has compared elastances and their ratio in a group of middle-aged subjects with metabolic syndrome.[115] All subjects were free from hypertension, diabetes and overt cardiovascular disease so the authors were better able to examine the effects of metabolic syndrome on cardiovascular function. Resting measures of arterial and ventricular end-systolic elastance were *not* different between those with metabolic syndrome and controls. The authors found no relation between body surface area and elastances in their very small sample population so compared unscaled values despite a significant between-

group difference in body size (BMI 36 ± 1 vs. 25 ± 0.8 Kg/m^2, p <.0001). Given the findings of Chirinos *et al.*,[122] this might have masked any difference related to the obesity. In addition, the study was powered to look for differences in elastance on exercise and the sample size may be too small to detect resting changes in the absence of significant LV dysfunction (n= 47 including 20 controls).

There is currently insufficient evidence to ascertain whether the pre-clinical levels of abnormal glucose handling associated with obesity directly affect arterial and ventricular end-systolic elastance and cardiac energetics.

Elastance and polycystic ovary syndrome

To the best of my knowledge there are no studies of arterial and ventricular elastance in women with PCOS. There is evidence that PCOS may be associated with altered arterial structure,[93] increased arterial stiffness,[87, 90] altered ventricular geometry and function [99, 100] but this frequently comes from older subjects, those with the most severe reproductive phenotype, or from studies where there are confounding factors.

There is evidence for sex-differences in elastance values in a healthy population,[124] so it is tempting to speculate that the altered hormonal profile in PCOS may affect elastance measures. It is unclear whether elastance data will enhance our understanding of the effects of the syndrome on cardiovascular risk.

Reproducibility and sensitivity of non-invasive measures of elastance

The reproducibility of non-invasive measures of elastance are infrequently reported but appear to be ~8-23% (coefficient of variation) for arterial elastance and 8-29% for ventricular elastance depending on the methods used to calculate elastance as well as whether inter-observer, inter-session data were collected.[115, 121, 125-127]

For example, Gayat *et al.* established intra-observer coefficients of variability of 16 (±17) % for arterial elastance, 22 (±17) % for ventricular end-systolic elastance and 13 (±10) % for the ventricular-arterial coupling ratio.[127] Inter-observer coefficients of variability were 23 (±24) % for arterial elastance, 29 (±22) % for ventricular end-systolic elastance and 15 (±12) % for ventricular arterial coupling. This variability is higher than for many standard echocardiographic measures and

may reduce the ability of the method to identify between-group differences precisely in a sub-clinical setting.

In the same study the authors demonstrated that the methods used to derive ventricular end-systolic elastance and the coupling ratio were sufficiently sensitive to detect small positive changes in inotropy associated with dobutamine stress and negative changes associated with left ventricular systolic dysfunction.[127] It is worth noting that the study included modifications to the original validated method.[121] LV volumes were calculated using 3D echocardiography, and end-systolic pressure was estimated from 0.9 x central systolic BP by tonometry (rather than brachial arm cuff pressure). While these modifications should result in more precise measures they were not used in the original validation study of the single-beat ventricular elastance method.[121]

It is unclear whether the unmodified non-invasive methods described in the methods of this thesis will be feasible in a predominantly obese female population and whether they are sufficiently sensitive to detect what may be subtle differences in sub-clinical cardiovascular function in relatively young subjects.

Limitations of the current evidence-base

It is difficult to interpret the combined published data on elastance confidently, because of significant between-study differences in methods. These differences relate to surrogate measures of end-systolic pressure, whether methods assumed a volume intercept of the end-systolic pressure-volume relation (ESPVR) at the origin, method of measuring of ventricular volumes, and methods of scaling for body size or use of unscaled data. In the majority of non-invasive studies of elastance, the variables were not scaled for body size even though obesity was sometimes a feature of the sample population or there were between-group differences in sex (and therefore size).

Several studies include older subjects with positive family history or comorbid conditions which confounds the results and their interpretation. In addition, there are very few studies where patients and matched controls are comprehensively characterised in terms of metabolic, anthropometric and cardiovascular function.

Method 2: Arterial wave physiology

The Windkessel effect is the term used to describe how large elastic arteries store a proportion of the stroke volume in systole and discharge that volume in diastole.[128] This ensures that ventricular ejection into the systemic circulation does not generate an excessively high pressure (which opposes emptying), as well as smoothing the pulsatile blood flow to ensure more even distal flow in arterioles.

Blood ejected from the LV has kinetic energy and causes the muscular aorta to stretch, converting kinetic into potential energy in the wall of the aorta. Elastic recoil of the artery causes transfer of potential energy back to kinetic energy in the blood, displacing the blood particles. This cyclical energy transfer results in a wave which moves through blood more quickly than the blood flows.[129]

Waves in arteries tend to be longitudinal in nature so that blood particles are displaced in a direction that is parallel to wave propagation. Longitudinal waves are characterised as compression or expansion depending on their effect on the sign of pressure change. A wave that causes an increase in pressure is described as a 'compression' wave while that causing a decrease in pressure is an 'expansion' wave. Since blood is considered a relatively incompressible liquid, an arterial compression wave that is generated by LV contraction will cause expansion of the arterial diameter. A reduction in the rate of LV contraction at end-systole causes an expansion wave to be generated which has the effect of decelerating aortic flow and assisting aortic closure.[129]

Waves propagated from the LV travel forward through the arterial circulation and a fraction of their power may be reflected backward from sites of impedance mismatch, such as at bifurcations or at sites of high resistance vessels. The magnitude of the reflection (reflection co-efficient) appears to be determined by the properties of the artery either side of the junction.[130] In addition, a backward wave may not be transmitted through the circulation in the same way as a forward wave. There is experimental evidence that bifurcations may be poorly matched for backward waves resulting in significant re-reflections and 'wave trapping' in the distal circulation so that the magnitude of backward wave is significantly reduced at the heart.[130] The systemic circulation is considered to be a 'closed-end' circuit and

this has the effect of the reflected wave being of the same type as the forward wave e.g. a forward compression wave will be reflected as a backward compression wave having the effect of increasing pressure but *decelerating* flow velocity.

The kinetic energy in ejected blood depends on LV function, while the mechanical properties of the arterial wall are determined by the collagen and elastin deposited by smooth muscle cells in the medial layer. Thin, fenestrated sheets of elastin are connected by elastic fibres and transfer stress throughout the wall of the vessel. The artery wall acts as a 'two-phase' material because bundles of collagen between these layers do not have a particular arrangement at low pressures, resulting in compliance that is predominantly determined by the properties of elastin. At high pressure, the collagen fibres become circumferentially aligned so that the vessel becomes much less compliant, restricting aortic distension. These properties account for the non-linear nature of vascular elasticity.[131] As a result, the speed of the arterial wave has a non-linear relationship with blood pressure and the artery is less compliant at high pressures as a result of a greater number of collagen fibres being recruited. The aorta also demonstrates dynamic compliance which depends on the rate of volume change during ejection. A more rapid change in volume, as occurs at higher heart rates with increased ejection velocity, is associated with lower compliance (a stiffer artery).[132]

Therefore, the arterial wave contains information about both ventricular and arterial function which may provide valuable insights to the effects of pathophysiology on haemodynamics and to assess cardiovascular risk.

There are two main methods of studying arterial waves. The first is an impedance method that presumes a linear relationship between pressure and flow. The relationship is described by an equation that is analogous to Ohm's Law (V = I.R):

$$P = Z.Q$$

where P is pressure (analogous to voltage), Q is flow rate (analogous to current) and Z is impedance.

This method is used to derive complex electrical networks designed to represent the resistance, capacitance and inertance of different parts of the vasculature.[133] Each

pulse is considered to be one of a number in a series generated repetitively from the heart. The characteristics of this series of pulses can be described by Fourier analysis in an approach that centres on the frequency-domain.[134] The method assumes that the circulation is in a steady state of oscillation and that waves have an additive effect. Some researchers have questioned the assumption of steady state oscillation because of the effect of a ventricular ectopic (VE) on arterial pressure. A very early VE does not result in an arterial pressure wave and there is usually a smooth exponential decline of pressure until the next sinus beat. The pulse pressure immediately following the VE may be increased due to the Frank-Starling mechanism but the mean pressure is often decreased for ~4-5 beats before the oscillation returns to the same state as before the VE. This behaviour suggests that the cardiovascular system is over-damped and not in steady-state oscillation.[135]

These assumptions, coupled with the fact that results presented in the frequency-domain may be more difficult to interpret by clinical researchers, have led to the development of time-domain techniques.[129]

The time-domain approach applies the method of characteristics based on the work of Riemann to solve the nonlinear form of conservation of mass equations derived by Euler. These inspired the development of wave intensity (WI) analysis by Parker and Jones.[136] This method considers each arterial pulse to be an individual event and does not make assumptions about linearity or periodicity. Since results are presented in the time-domain they have the potential of being more readily understood by clinical researchers. Further detail regarding the method can be found in the following section.

Whilst there has been debate about which method is best, there are similarities in that both are used to separate measured pressure and flow into forward and backward components of waves. Both methods are dependent on the determination of wave speed and, while wave speed is calculated differently in each method, the magnitude of difference between calculations does not appear to affect the results of wave separation.[137]

Wave intensity

Wave intensity is the flux of energy per unit area carried by the wave as it moves past a point in the circulation; it has the units of power (W/m^2). Wave intensity analysis was introduced in the late 1980s and early 1990s to overcome the limitations of frequency-domain measurements which had dominated studies of arterial haemodynamics since the 1960s. WI has its origins in gas dynamics and is based on the solution of equations describing the conservation of mass and momentum in an elastic tube. Detailed explanations of the mathematics are described in an article by Kim Parker.[135]

While the mathematical solution of equations is difficult, the process of obtaining WI signals is relatively simple and requires simultaneous measurements of arterial blood pressure and velocity from the same site in the circulation. Wave intensity at any given point in time, $dI_{(t)}$, is the product of the change in pressure ($dP_{(t)}$) and change in velocity ($dU_{(t)}$) at the same point in time.

$$dI(t) = dP(t).dU(t)$$

Since WI is a time-domain measurement it can be used to determine the timing of wave arrival as well as their magnitude and direction. This is not easily achieved with Fourier methods because the wave-train is always present.

The net WI (d*I*) indicates whether forward or backward waves are dominant and how big they are at a point in the cardiac cycle. Table 1-3 demonstrates the relationship between different waves and d*I*. The main purpose of WI analysis is to provide information about the net direction of wave travel at a point in time. However, in an arterial wave any change in pressure will necessarily result in a change in velocity because wave propagation relies on the transfer of energy between kinetic and potential forms. The relationship between pressure and velocity in a wave is given by the water hammer equations and these can be used to separate the signal into forward and backward components, if the wave speed can be calculated.

$dP+ = \rho.c.dU +$ for forward waves arising from the heart

$dP- = \rho.c.dU -$ for backward waves reflected from the arteries

where ρ is the density of blood and c is the wave speed. When these equations are used to separate waves the results are very similar to those derived from impedance methods.[137]

Table 1-3. The relationship between wave types and wave intensity. Forward waves will always result in a positive wave intensity signal and backward waves will result in a negative wave intensity. Adapted from Parker. [135]

	d*P*	**d*U***	**d*I***
Forward	>0 compression	>0 acceleration	positive
	<0 expansion	<0 deceleration	positive
Backward	>0 compression	<0 deceleration	negative
	<0 expansion	>0 acceleration	negative

While net WI signals are useful, the separation of waves provides more detailed information about the contributions of forward and backward waves to the signal. For example, a small positive WI signal may be due to a forward wave of low amplitude or the simultaneous arrival of a forward and backward wave of similar magnitude.

Calculation of local wave speed is necessary for separation of waves and this also gives independent information about local distensibility of the artery. The standard measurement of wave speed is PWV and this relies on measurement of a transit time between two sites to give an average wave speed over this section of artery. It is typically performed between carotid and femoral artery sites. Local wave speed is more difficult to measure clinically because it requires invasive assessment of elastic properties of the arterial wall. However, WI analysis can be used to derive local wave speed using the water hammer equation. In the event of a situation when

there are only forward waves present, the relationship between pressure (P) and velocity (U) should be linear with a slope that is equal to the product of the density of blood (ρ) and wave speed (c). Since the density of blood is known, the value of wave speed can be calculated. Pressure-velocity loops from experimental and clinical data have shown that there is a short time period in early systole when the relationship is linear and this period is selected to calculated local wave speed.

Key invasive studies of wave intensity

In 1998, Kim Parker's group were the first to demonstrate the use of WI analysis to examine arterial wave reflections in man.[138] The study population comprised 14 middle-aged or older subjects who were undergoing coronary artery bypass surgery. The aim of the study was not to evaluate the effects of disease, but to study the relationship between features of the invasively recorded aortic pressure and flow with the amplitude and timing of wavefronts obtained through WI analysis. The results confirmed that the first positive peak in WI was the largest and coincided with acceleration of blood in the aorta and an increase in aortic pressure (FCW). The second positive peak in late systole was smaller and associated with deceleration of blood and a decrease in aortic pressure (FEW). A backward mid-systolic wave consistently occurred at the time of the inflection point of the aortic pressure tracing and was therefore associated with an increase in aortic pressure. This BCW was the smallest wave and its amplitude strongly correlated with augmentation index ($r=.63$, $p<.001$).

In a later study, data from a canine model confirmed that the compression and expansion waves generated by the LV had a significant impact on the acceleration and deceleration of blood in the ascending aorta. Furthermore, the intensity of the FCW was related to LV inotropy and chronotropy (increased by dobutamine and decreased by propranalol) and was decreased by vasoconstriction (methoxamine). In contrast, the FEW was not affected by pharmacological changes in inotropy or vasoconstriction, but was reduced during vasodilation by nitroglycerin.[139] This confirmed that WI signals could be altered by manipulations of cardiovascular performance.

Non-invasive studies of wave intensity

Two main methods of non-invasive measurement are evident in the literature. The first involves *sequential* measurement of carotid pressure using applanation tonometry and measurement of carotid flow using Doppler.[140-144] The second method was developed by a group in Japan and allows *simultaneous* measurement of changes in carotid artery diameter (as a surrogate measure of pressure) using echo-tracking software, and flow using Doppler.[145-153]

Regardless of the method used, the pattern of WI peaks appears to be consistent among non-invasive studies and is comparable with that found in invasive studies. The first positive peak in net WI (sometimes called W_1) tends to be the largest and coincides with acceleration of blood in the aorta and an increase in aortic pressure (dominant forward compression wave, FCW). This is followed by a smaller negative peak reflected from the circulation which has the effect of increasing pressure and decelerating velocity (dominant backward compression wave, BCW). A second positive peak (W_2) occurs in late systole associated with deceleration of blood and a decrease in aortic pressure (dominant forward expansion wave, FEW).

Sequential measurement of pressure and flow

The sequential method was first described in 2002 by Kim Parker's group.[141] The purpose of the study was to establish the reproducibility of the method in deriving WI measures in the right common carotid, brachial and radial arteries. Twelve subjects with hypertension who were enrolled in the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT) underwent applanation tonometry and pulsed Doppler measurements of the three arteries on two occasions. Applanation tonometry was applied to the artery so that the force acting on the transducer represented the intra-arterial pressure. Once pressure waves had been recorded, ultrasound was applied to the same site to obtain Doppler measurements of velocity. Acquisition data were recorded alongside a real-time ECG and ensembled using MatLab software. The intra-observer intra-session coefficients of variation for local wave speed and WI ranged between 3.7% and 19.6%. Local wave speed had the best reproducibility and the reflected WI (a relatively small signal) had the poorest reproducibility. The authors concluded that the method was therefore sufficiently reproducible to be used

in other research studies. This study also demonstrated that while the pattern of WI signals was similar in predominantly elastic or muscular arteries, the local wave speed and the magnitude of WI signals was not uniform through the arterial tree with higher values in muscular arteries. In addition, a mid-systolic expansion wave, largest in the muscular arteries, was described; this was attributed to re-reflection of the backward compression wave at the brachial bifurcation.

In 2005, the same group applied this method to a small cohort of healthy adults (n=21, 14 men, mean age 44±6 years).[140] The study confirmed that the pattern of WI signals was similar in the carotid, brachial and radial arteries (Figure 1-5). Again the local wave speed was higher in the muscular brachial artery compared with the elastic carotid artery (p=.01). In addition, this study was the first to report separated wave analysis at each arterial site using non-invasive methods. The finding that the FCW was higher in the muscular arteries (p<.01) despite similar heart rate and contractility, suggested that it was attributable to the differences in wave speed due to local structure, geometry and branching. This was expected because of the relation between wave speed and pressure in the waterhammer equation ($dP \pm = \rho c dU \pm$).

The relative timing of the FEW to the FCW was similar in all arteries supporting the theory that it originated from the LV. The amplitude of the FEW became smaller with distance from the heart and the authors suggested that this was due to arterial damping of the signal. Differences in impedance mismatching between arterial beds may account for the higher pressure reflected waves that were observed from the hand and forearm compared with the head; increased amounts of reflection from the hand may be due to vasoconstrictor tone or the anatomy of the circulation in the hand. Finally, the study confirmed the presence of a peripheral mid-systolic forward expansion wave in normal subjects which had not been documented in the ascending aorta in invasive studies.

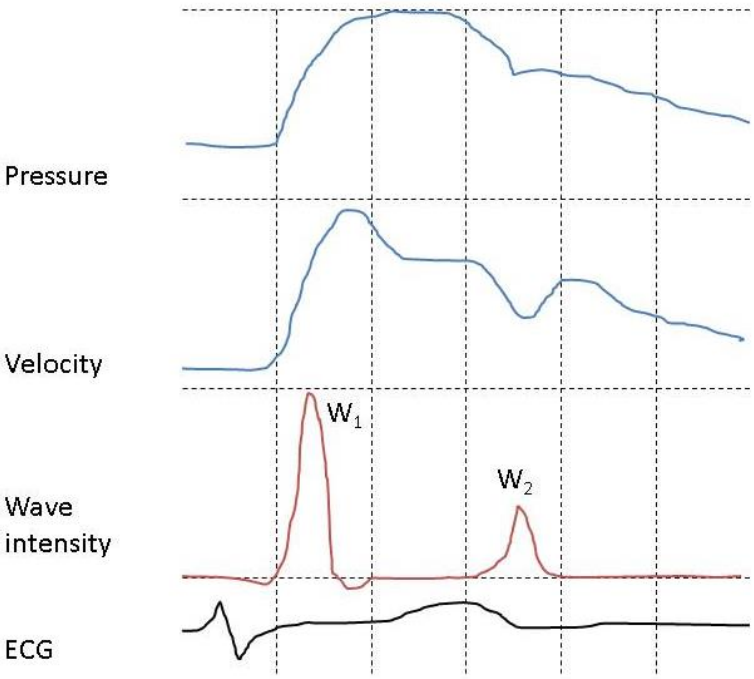

Figure 1-5. Drawing showing the timing of simultaneous pressure, flow velocity, wave intensity and ECG signals. W_1 occurs in early systole and is dominated by a forward compression wave. A small negative area can be seen following W_1 because of a backward compression wave. W_2 occurs towards the end of systole and is dominated by a forward expansion wave. Adapted from Hughes *et al.* [140]

Having described the haemodynamic variables obtained from WI, the authors applied the same non-invasive methods to a chronic heart failure population.[142] Using impedance methods, others had proposed that increased wave speed due to vasoconstriction and endothelial dysfunction in heart failure would result in earlier reflection of waves so that they arrived in systole, attenuating ejection,[154] rather than in diastole. However, there was no conclusive evidence of this to date in the literature. Therefore, the aim of the study was to determine the effect of heart failure on the amplitude and timing of forward and backward components of waves. The study population comprised 67 patients with New York Heart Association (NYHA) class II or III heart failure and 29 healthy controls. The heart failure patients were older (66±10 years vs. 60±9 years, p=.004) and were all medicated with diuretics and an angiotensin-converting enzyme inhibitor or angiotensin-receptor blocker but the

groups were otherwise well matched. Heart failure patients had similar carotid pressure but reduced carotid flow velocity compared with controls. This was explained by a significantly reduced energy in the FCW (467±29 vs. 745±46 mJ/m^2, p <.001). In addition, the time to peak of the wave was delayed in heart failure (0.15±0.01 vs. 0.12±0.01s, p<.001). The wave amplitude was progressively reduced in relation to NYHA class. Perhaps surprisingly, the wave amplitude was not associated with LV mass or ejection fraction.

Although the FCW had reduced energy in heart failure, the proportion of energy reflected back from the periphery was higher. Since this reflection was in the form of a BCW it may contribute to the maintenance of mean arterial pressure at the expense of flow velocity. There was no difference in local wave speed or in the timing of the reflected wave in heart failure. Despite the altered proportion of reflected energy the augmentation index, a measure of arterial stiffness, was not significantly different between-groups.

Simultaneous measures of diameter-derived pressure and flow

The simultaneous method of measuring arterial pressure and flow at a single site was first described in 1999 by Niki *et al*. [145] and the literature is predominantly from the research groups based in Japan who developed the technology. The method used an ultrasound system (Hayashi Denki, Kanagawa, Japan) capable of automatically tracking changes in the carotid artery diameter and of simultaneously measuring blood velocity by Doppler at the same site. Others had previously shown that the diameter waveform was similar to an arterial pressure waveform in animal models, so the authors calibrated the diameter waveform with brachial systolic and diastolic blood pressure to derive an estimated pressure waveform. This technique was used to characterise the WI signals of 24 normal volunteers and of 11 patients with mitral valve regurgitation (before and after corrective surgery). The pattern of WI signals in normal volunteers was as described by other invasive and non-invasive methods. However, the second positive peak, W_2, was significantly reduced or absent in the patients with mitral regurgitation but became significantly larger after corrective surgery. W_2 is dominated by a forward expansion wave generated by the LV and the authors interpreted a very small or absent W_2 as reflecting an inability of the LV to efficiently decelerate aortic blood at the end of ejection because of continued

emptying into the left atrium across the incompetent valve. This theory was supported by an increase in W_2 after surgery.

The same group validated the theory that carotid artery pressure could be derived from the arterial diameter waveform in 6 patients with heart disease.[155] Invasive pressure in the left common carotid artery was compared with simultaneous arterial diameter changes at the same site, assessed using an ultrasound echo-tracking system. The relationship between pressure and diameter was relatively linear throughout the cardiac cycle ($r^2 \geq 0.97$). While there was slight non-linearity and hysteresis, the effects were considered to be minimal. There are few human studies to support this data and it is limited by the small sample size of diseased patients. However, the authors compared many data points on each wave through the cardiac cycle so that a significant volume of data was generated for statistical analysis.

Normal values of WI measurements using the Aloka ultrasound system were established in 2002 by Niki *et al*. who studied 135 healthy volunteers (74 men) across a wide age range (25 years and over).[146] The results can be found in Table 1-4 and demonstrated that W_1 was larger than W_2 with neither of these parameters being correlated with age. The reproducibility of measures was reported in the same study and the mean coefficients of variation for intra-observer intra-session measures were reported to be 10% for W_1 and 13% for both W_2 and the negative area. As expected, the intra-observer inter-session reproducibility was poorer with confidence limits of 17% for W_1 and up to 36% for W_2 and the negative area. While the values for W_1 are clinically acceptable, those for the smaller waves (particularly the negative area) are large and this might preclude widespread adoption of the technique in the clinical environment.

The physiological significance of W_1 and W_2 using this method was confirmed in a study of 64 patients with suspected coronary artery disease who underwent cardiac catheterization.[147] The amplitude of W_1 was strongly associated with the rate of LV pressure increase ($r = .74$, $p<.001$) and therefore it was confirmed as a measure of contractile performance. The amplitude of W_2 was inversely associated with the time-constant of LV relaxation ($r = -.77$, $p<.001$) suggesting that it provided information the transition between the end of systole and the beginning of diastole.

Table 1-4. Wave intensity values derived from healthy volunteers in a small study using the Aloka system. Adapted from Niki et al.[146] Only the stiffness index, β, was correlated with age ($r = 0.66$, $p<.0001$ in men and $r = 0.81$, $p<.0001$ in women).

Wave intensity parameters	**Mean ± SD**
W_1 (mmHg ms^{-3})	8940 ± 3790
W_2 (mmHg ms^{-3})	1840 ± 880
Negative area (mmHg ms^{-3})	27 ± 13
Time from R wave to W_1 (ms)	104 ± 14
Time from W_1 to W_2 (ms)	270 ± 19
Stiffness, β	10.4 ± 4.8

Few studies have used WI to examine the effects of CV disease on ventricular-arterial coupling. The Japanese group of Sugawara *et al*. have briefly reported the effects of dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and hypertension on WI signals compared with 170 controls.[150] In control subjects (demographics not presented), neither W_1 nor W_2 depended on age. In the DCM group (n=36) W_1 was significantly lower than in controls (mean ± SEM: 4,320 ± 420 vs. 9,060 ± 330 mmHg ms^{-3}, $p<.001$), and deteriorated with age. The smaller amplitude of W_1 in DCM was expected since this is predominantly a disease which affects systolic function.

In the HCM group (n=27), the values of W_1 were similar to controls but declined with age. The values of W_2 were significantly lower in subjects with HCM (875 ± 98 vs. 1,793 ± 57 mmHg ms^{-3}, $p<.001$). These results may be expected given that the hypertrophied LV in HCM causes ventricular stiffening and a significantly impaired ability to relax.

Separated wave intensity signals

The commercially-available ultrasound system which can measure WI (Aloka) presents the *net* signals of W_1, W_2 and a negative area. These are summations of forward and backward waves arriving at an arterial site at a moment in time and show the dominant direction, energy and timing of the waves across the cardiac cycle. Some authors have suggested that a more precise assessment of ventricular-arterial interaction is achieved by analyzing the separated forward and backward components of WI signals.[156] This can be achieved by constructing a loop of the relationship between pressure and velocity across a cardiac cycle, then determining the local wave speed from the slope of the loop in early systole when the relationship is linear. The local wave speed can subsequently be used to calculate the separate forward and backward components of waves as described in the methods section of Chapter 5. This separation of waves is possible with both sequential and simultaneous methods of deriving WI signals.

Separated WI signals appear to be sufficiently sensitive to detect changes in physiology associated with caffeine (increased FCW and wave speed) [153] and high dietary salt-intake (increased wave reflections) [157] in healthy volunteers. This suggests that the technique may be suitable for the detection of sub-clinical pathophysiology associated with obesity and pre-diabetic states.

Wave intensity and obesity

To the best of my knowledge there are no published papers detailing the effects of uncomplicated obesity on net WI or separated waves. There are brief reports from conference proceedings that obese subjects have higher carotid artery stiffness as measured by WI techniques [158] and that expansion wave reflections are associated with artery stiffness as well as measures of general and central obesity.[159]

Given that elastance measures are related to body size in a lean, healthy population [122] and that augmentation index (a surrogate measure of wave reflection) is related to height,[144] it would seem prudent to establish whether such relationships exist for WI measures, but such data are lacking. One study has shown that a wave reflection coefficient derived using different methods is not related to height [144] but it

is unclear whether WI variables should be scaled in cross-sectional studies where there are between-group differences in size.

Wave intensity and pre-diabetic states

I have been unable to find any published papers of WI in subjects with pre-clinical dysglycaemia or insulin resistance. One study has used measures of net WI to examine the relationships between type 2 diabetes and ventricular-arterial coupling.[148] Sixty-five patients with diabetes were compared with 57 controls. All were free from overt coronary or peripheral vascular disease. Those with diabetes had higher amplitude W_1 (11785±7491 versus 9191±4299 mmHg m/s^3 in controls, p=.04) and greater beta stiffness index (11.5±4.6 versus 9.2±2.7, p=.002). There was no between-group difference in W_2 despite evidence of worse diastolic function (e′ 7.8±1.2 versus 9.5±1.6, p=.001). An increase in W_1 is usually associated with increased ventricular inotropy but in this study those with diabetes also tended to have reduced systolic longitudinal function (s' 7.5±1.1 versus 7.9±1.2 cm/s, p=.053). The authors suggest that the increase in W_1 in diabetes was due to increased regional arterial stiffness since aortic pulse wave velocity was increased in those with the condition.

Wave intensity and polycystic ovary syndrome

To the best of my knowledge there are no published papers of WI, wave reflections or pulsatile loading in women with PCOS.

Limitations of the current evidence-base

WI analysis has not been widely adopted in clinical studies and while there is some evidence that the magnitude and timing of net WI signals may be altered in those with overt cardiovascular disease, it is unclear whether the technique is sufficiently sensitive to detect sub-clinical changes associated with obesity and pre-diabetic states.

The separation of net WI signals into forward and backward components may provide more detailed information about the ventricular-arterial interaction, particularly in respect of the magnitude and timing of wave reflections which affect

the pulsatile load on the LV. The technique has the potential to add information about the mechanisms linking obesity, LV hypertrophy and diastolic information but this has yet to be studied in a young population with uncomplicated obesity or PCOS.

The two methods of studying the ventricular-arterial interaction in this thesis (the ratio of end-systolic elastances and arterial WI signals) tend not to be applied together in other published studies so it is unclear whether they provide similar information i.e. whether they are equal in characterising arterial load and its impact on the LV. Elastance measures provide an assessment of ventricular-arterial coupling at a single point in the cardiac cycle and there is evidence that arterial elastance is predominantly affected by heart rate and resistive (static) vascular load.[160] In contrast, arterial WI signals provide information about the time-course of loading, including pulsatile (dynamic) load across a cardiac cycle. These factors appear to be important in the development of left ventricular hypertrophy [161] which may ultimately lead to heart failure with preserved ejection fraction (HFpEF) [50] and increased mortality.[162]

Aims of this thesis

This thesis has been designed to study the effect of obesity and pre-diabetic states on cardiovascular structure and function in young women. The work tests the hypothesis that quantitative measures of ventricular-arterial coupling will provide information about subclinical cardiovascular dysfunction that complements standard clinical measures. This may lead to an improved understanding of the pathophysiology of left ventricular hypertrophy and diastolic dysfunction which are common in older obese women. Such information could inform potential intervention studies which seek to reverse abnormal cardiovascular function before the development of diabetes or overt cardiovascular disease.

The specific aims of the thesis are as follows.

1. To examine whether non-invasive measures of ventricular-arterial coupling are sufficiently sensitive to detect sub-clinical changes in cardiovascular function associated with uncomplicated obesity and polycystic ovary syndrome in young women.
2. To determine whether quantitative measures of ventricular-arterial coupling are more strongly associated with central obesity and insulin resistance than with general obesity.
3. To determine whether non-invasive measures of ventricular-arterial coupling independently explain some of the variation in left ventricular mass and diastolic function in obese women.

2. General Methods

The studies reported in this thesis were performed in collaboration with an MD student in endocrinology, Dr. Rose-Marie Coulson, who recruited the subjects and undertook their clinical evaluation, blood tests, obesity measurements and applanation tonometry. Computed tomographic measurements of abdominal fat areas were performed by the Medical Physics department at the University Hospital of Wales, Cardiff. I performed all echocardiograms and carotid ultrasound scans, whilst blind to the subjects' status as patients with PCOS or controls.

Detailed analyses of the associations between body composition and basic measures of cardiovascular function in women with PCOS are the subject of Dr. Coulson's MD thesis and we have co-authored a publication of these data.[163]

Subject recruitment

The study was sponsored by Cardiff University and received ethical approval from South East Wales Regional Ethics Committee B. Each subject gave informed written consent before participation.

Healthy volunteers were recruited from local staff and students of Cardiff University and University Hospital of Wales, Cardiff. In addition, advertisements were placed in the local media to recruit healthy women with dress size 14 or greater in order to match those with PCOS who tended to be obese. Two hundred and sixty nine healthy volunteers responded to advertisements. One hundred and seventy four were excluded because they failed to meet the inclusion criteria of regular menstrual cycles (27-32 days), normal history, clinical examination and hormone evaluation (testosterone, thyroid function, prolactin and 17-hydroxyprogesterone). The sample comprised 95 healthy control subjects.

Five hundred and seventy women with potential PCOS were identified from a departmental database or from consecutive patients attending the endocrine clinic at the University Hospital of Wales Cardiff. A diagnosis of PCOS was made according to the Rotterdam criteria.[71] Congenital adrenal hyperplasia, Cushing's syndrome, androgen-secreting tumours, hyperprolactinaemia and thyroid disease were excluded

by biochemical testing. Those who were pregnant, breastfeeding and those who had used glucocorticoids, lipid-lowering agents, antihypertensives, antidiabetics or anti-obesity drugs within three months of the study were also excluded. All women were free from overt cardiovascular disease and hypertension.

Women with PCOS, mild oligomenorrhoea or eumenorrhoea were studied in the early follicular phase of their menstrual cycle but no temporal limitations were applied to women with severe oligomenorrhoea or amenorrhoea.

Two hundred and twenty nine patients were excluded because of a different final diagnosis, co-existing diabetes, hypertension or hypercholesterolaemia, treatment with metformin, statins or anti-hypertensive medication. A further 257 were not contactable or declined to take part. The sample comprised 84 women with PCOS defined by the Rotterdam criteria; 62 had polycystic ovaries on ultrasound, 75 had oligo-/anovulation and 75 had clinical or biochemical hyperandrogenism.

Study protocol

All women attended the Clinical Research Facility at the University Hospital of Wales, Cardiff at 08:00 following an overnight fast. Dr. Coulson performed a pregnancy test on each woman and recorded a full medical history and physical examination prior to other investigations being performed. The study protocol comprised a number of investigations to be performed during the same visit in the order presented below in accordance with the study protocol (Figure 2-1). The oral glucose tolerance test was performed on a second separate visit.

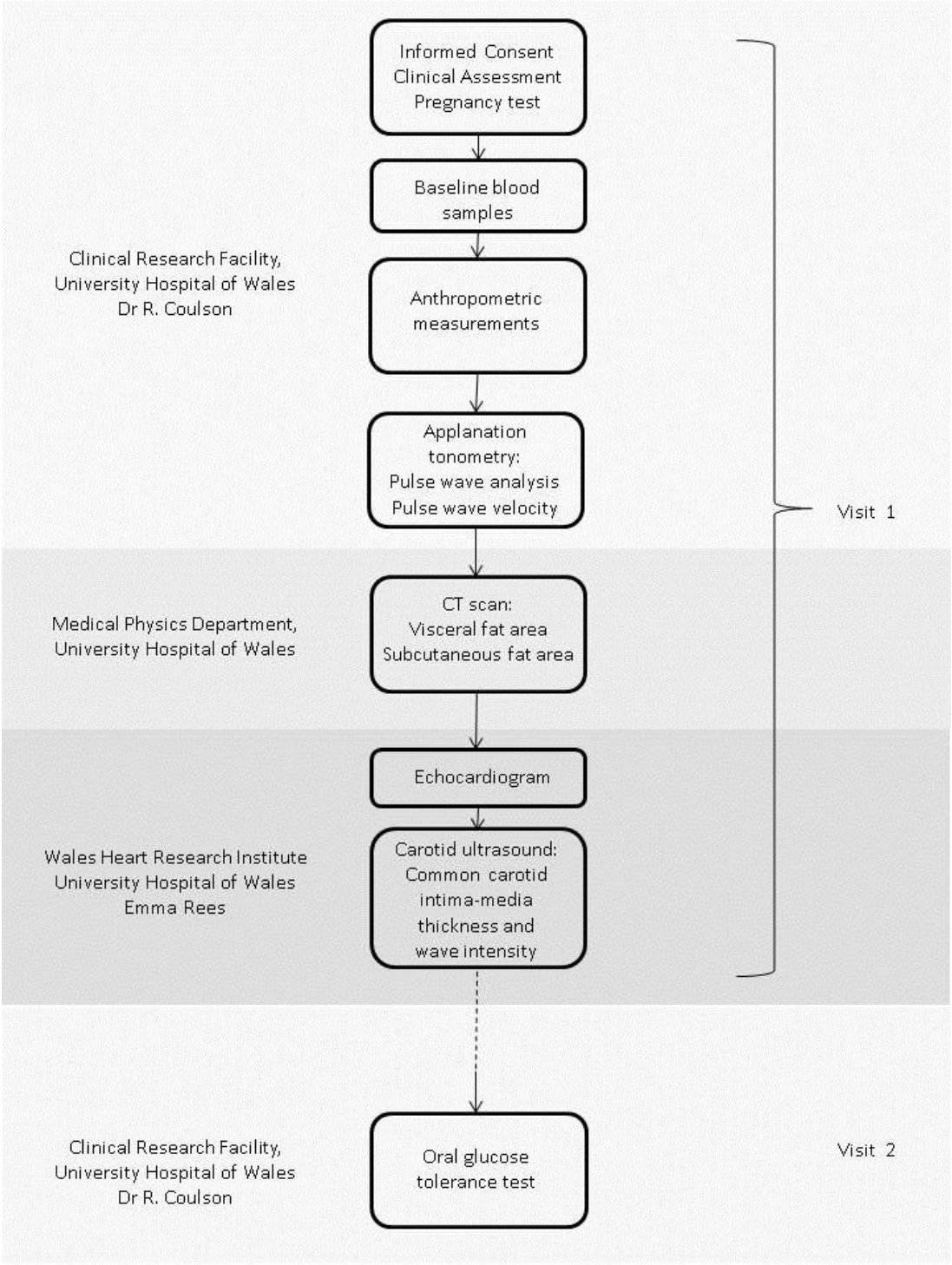

Figure 2-1. Study protocol.

Body composition measurements

For all subjects, anthropometric measurements included height to the nearest 0.1cm without shoes using a stadiometer, and weight to the nearest 0.5kg in light clothing without shoes (Omron Monitor BF500, Omron Corporation, Japan). Body mass index was calculated as weight divided by height in metres squared. Waist circumference was measured to the nearest 0.5cm from a position just above the iliac crest with a flexible tape (in a transverse plane) during minimal respiration. Hip circumference was measured in the same manner at the greatest protrusion of the buttocks.

Biochemical measurements

After basal sampling, subjects had a standard oral glucose tolerance test (113ml of Polycal® with 187ml of water). Blood samples were collected at 30 minute intervals until 120 minutes.

Fasting venous blood samples were prepared by centrifugation at 4000rpm for 8 minutes and serum was stored at -30°C prior to analysis. Table 2-1 details the method and equipment used to perform biochemical tests. Intra- and inter-assay coefficients of variation were all <9% according to laboratory data.

The trapezoid method was used to calculate insulin and glucose areas under the curve (AUC). Insulin resistance was also assessed using the homeostatic model assessment method (HOMA-IR) [164].

Table 2-1. Biochemical assays.

Variable (s)	**Method**	**Equipment**
Total cholesterol, HDL, triglycerides	Aeroset automated analyser	Abbott Diagnostics, Berkshire, UK
LDL	Calculated by Friedewald's formula	
Insulin	Immunometric assay specific for human insulin	Invitron, Monmouth, UK
Glucose	Aeroset chemistry system	Abbott Diagnostics, Berkshire, UK
High sensitivity c-reactive protein	Nephelometry	BN™ II System, Dade Behring, Milton Keynes, UK
Total testosterone	Liquid chromatography tandem mass spectrometer	Quattro™ Premier XE triple quadrupole tandem mass spectrometer, Waters Ltd., Watford, UK
High-molecular weight adiponectin	ELISA	EMD Millipore, Billerica, MA, USA

Abdominal fat area measurement by computed tomography

Abdominal subcutaneous and visceral fat areas were measured by x-ray computed tomography (CT) (Hawkeye, GE Medical Systems) on one cross-sectional scan at the level of L4-L5. Subjects were supine and standard acquisition parameters were used (140kV, 2.5mA, 10mm slice width, 13.6s rotation time, 256^2 pixel matrix).

A single operator, blind to the subject's status, analysed the image using MATLAB. Fat was identified by using a fixed range of CT numbers (-120 to -80) defined by Watson *et al*..[165] The visceral fat area was then calculated by segmenting the intraperitoneal region from the whole image. The subcutaneous fat area was calculated as the difference between the total and visceral fat areas (Figure 2-2).

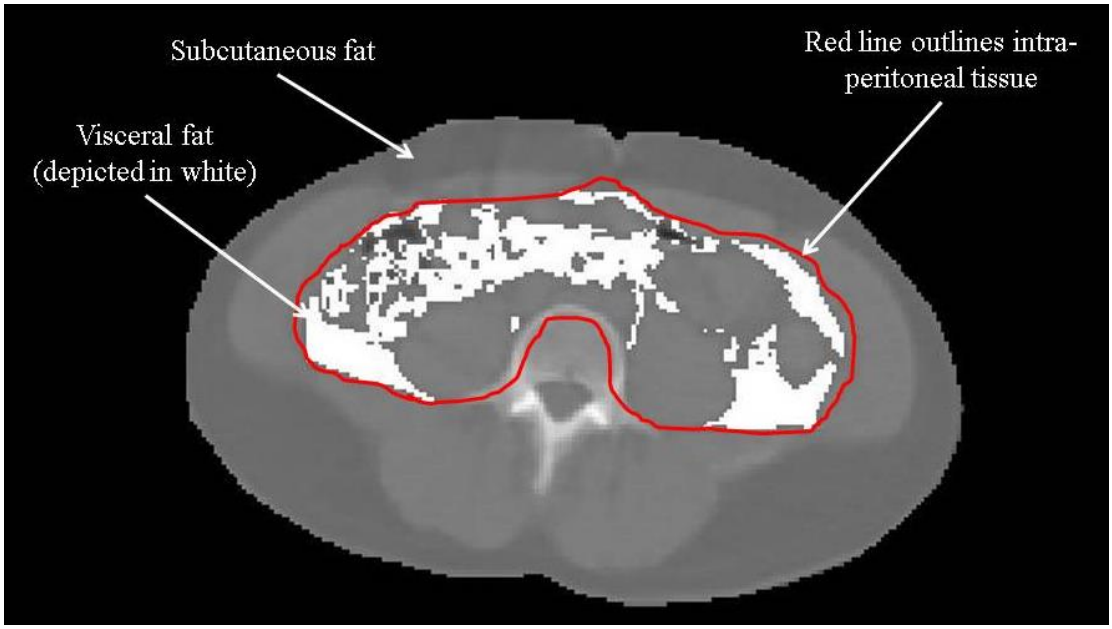

Figure 2-2. CT image demonstrating identification of visceral fat. The intraperitoneal region was defined by tracing its outline. Tissue within the intraperitoneal region with pixels having attenuation within the defined range of fat was depicted as white. Fat area was calculated by summation of the pixels in each area.

Applanation tonometry

Applanation tonometry was performed by Dr. Coulson after a short period of training. Before the study began, the reproducibility of intra-operator intra-session duplicate measurements was established in 15 volunteers (age 21-45 years). The coefficients of variation were 18% for augmentation index and 4% for carotid-femoral pulse wave velocity.

Brachial blood pressure was measured after at least five minutes of rest from the left arm using a validated, automated device (Omron 7051T, Omron Corp.). An average of three measurements was used. A high-fidelity micromanometer (SPC-301, Millar Instruments, Houston, Texas) attached to a SphygmoCor MM3 device (software version 8.0, AtCor Medical Ltd., Sydney, Australia) was used to obtain pulse wave analysis (PWA) of the radial artery waveform and carotid-femoral pulse wave velocity (PWV).

Pulse wave analysis

Subjects were asked to lie supine and allowed to rest for ten minutes. The wrist of the right hand was supported in a dorsi-flexed position to facilitate detection. The tonometer was placed on the wrist at a site where the radial pulse was best detected with the tonometer perpendicular to the artery. The tip of the tonometer was supported between two fingers to minimise movement. After 20 sequential waveforms the waveform was stored and a generalised transfer function was used to derive a central pressure waveform. Quality control indices were inspected and used to determine whether the recording was acceptable (Table 2-2). The recording was repeated and if the two measures of augmentation index (AIx) were within 5% the recordings were accepted and an average value noted for analysis. The augmentation index adjusted to a heart rate of 75 beats per minute was used in analyses. Central blood pressures were also derived from analysis of this waveform.

Table 2-2 Quality control indices used in pulse wave analysis.

	Value
Average pulse height	>80
Pulse height variation	<5
Diastolic variation	<5
Operator index	>80

Pulse wave velocity

Carotid-femoral pulse wave velocity (PWV) was obtained directly after PWA measurement. The method followed international recommendations.[59]

Electrocardiogram (ECG) leads were attached for continuous monitoring during acquisition of the arterial waveform. The carotid and femoral pulses were palpated and marked. The distance of each site from the suprasternal notch was measured in a direct line and entered into the software with the mean and diastolic brachial blood pressure. The carotid-femoral PWV (m/s) was calculated as the distance between measurement sites divided by the transit time. Transit time was the delay between the arrival of the pulse wave at the carotid artery and at the femoral artery. This was measured indirectly by subtracting the time from the R wave of the ECG to the peak pressure of the carotid recording from the same measurement taken at the femoral artery.

The average of two measurements was used in analyses. The intra-session intra-observer coefficient of variability for PWV was 4%.

Echocardiography

I performed all echocardiograms in the Wales Heart Research Institute at the University Hospital of Wales Cardiff. I was blind to each subject's status.

Left ventricular function was assessed by echocardiography (Vivid 7, GE Medical Systems, Horten, Norway) with a 2.5 MHz transducer and harmonic imaging. Images were acquired at passive end-expiration when possible. All measurements were performed during off-line analysis using EchoPAC PC software

version 110.0.0 (GE Healthcare). The mean measurement of three consecutive beats was used in analysis.

Standard echo measurements and calculations were performed according to the joint recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging.[166] Table 2-3 lists all echocardiographic measurements recorded in the study population. Detailed methods for the key echo variables used in statistical analysis can be found below.

Table 2-3. Resting echocardiographic measurements.

Time intervals	**Left ventricular systolic function**
Heart rate (bpm)	Fractional shortening (%)
R wave to aortic valve opening (ms)	Ejection fraction (Teicholz) (%)
R wave to aortic valve closure (ms)	Stroke volume by Doppler (ml)
R wave to mitral valve opening (ms)	Cardiac output by Doppler (L/min)
R wave to mitral valve closure (ms)	Mean of lateral and septal s′ (cm/s)
	Mitral annular plane systolic excursion (average of 4 sites) (cm)
	Biplane LV end-diastolic volume (ml)
	Biplane LV end-systolic volume (ml)
	Biplane ejection fraction (%)
Dimensions	**Left ventricular diastolic function**
Aortic root diameter (cm)	Biplane LA volume (ml)
LV outflow tract diameter (cm)	Flow propagation velocity (cm/s)
Ventricular septum (diastole) (cm)	E (cm/s)
LV internal dimension (diastole) (cm)	E deceleration time (ms)
Posterior wall (diastole) (cm)	A (cm/s)
Ventricular septum (systole) (cm)	E:A
LV internal dimension (systole) (cm)	Mean of lateral and septal e′ (cm/s)
Posterior wall (systole) (cm)	Mean of lateral and septal a′ (cm/s)
Left ventricular mass (ASE method) (g)	Mean of lateral and septal e′/a′

Left ventricular dimensions

Left ventricular wall thickness and internal dimensions were measured from m-mode recordings (leading-edge to leading-edge) (Figure 2-3) or from 2-dimensional images if a perpendicular alignment could not be obtained. Fractional shortening and ejection fraction (Teicholz method) were automatically calculated by EchoPac software using standard formulae.

Left ventricular mass and identification of hypertrophy

Left ventricular mass was automatically calculated by the Echopac software using the cube method which is recommended by the American Society of Echocardiography when there are no major distortions of ventricular geometry.[167] Mass was subsequently indexed by height allometrically scaled to the power of 2.7 since this has been shown to be the most accurate scaling method in obese subjects.[168] A partition value of $44 g/m^{2.7}$ was used to identify left ventricular hypertrophy.[169] Relative wall thickness (RWT) was calculated by

$$RWT = \frac{2 \times LV\ posterior\ wall\ in\ diastole}{LV\ internal\ dimension\ in\ diastole}$$

A relative wall thickness partition value of 0.42 was used to identify a concentric pattern of remodelling or hypertrophy as described by the American Society of Echocardiography (Table 2-4).[167]

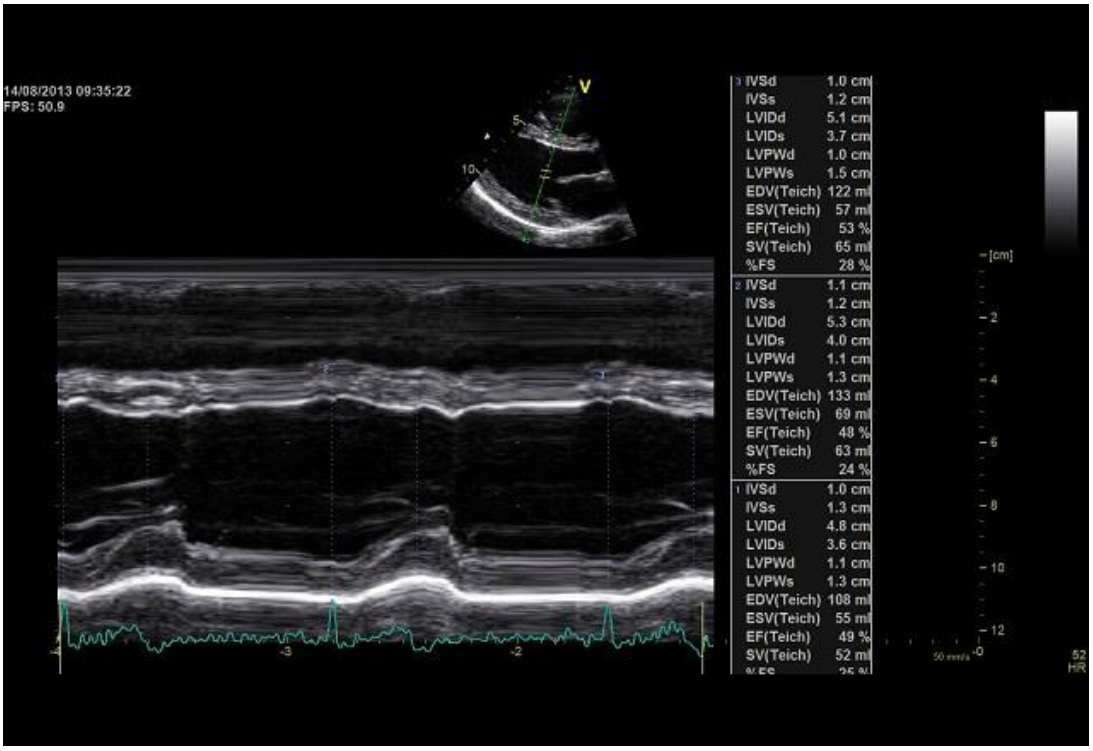

Figure 2-3. M-mode recording demonstrating measurement of left ventricular dimensions.

Table 2-4. Criteria used to identify patterns of left ventricular remodelling and hypertrophy.

	Relative wall thickness	**LV mass/height$^{2.7}$ (g/m$^{2.7}$)**
Normal	≤0.42	≤44
Concentric remodelling	>0.42	≤44
Eccentric hypertrophy	≤0.42	>44
Concentric hypertrophy	>0.42	>44

Stroke volume

Left ventricular stroke volume was measured using the Doppler method given by

$$Stroke\ volume\ =\ LVOT\ CSA\ \times\ LV\ vti$$

where LVOT CSA is the cross sectional area of the LV outflow tract found by

$$0.785\ \times\ LVOT\ diameter^2 \qquad \text{(Figure 2-4),}$$

and LV vti is the velocity time integral of flow in the LV outflow tract (Figure 2-5).

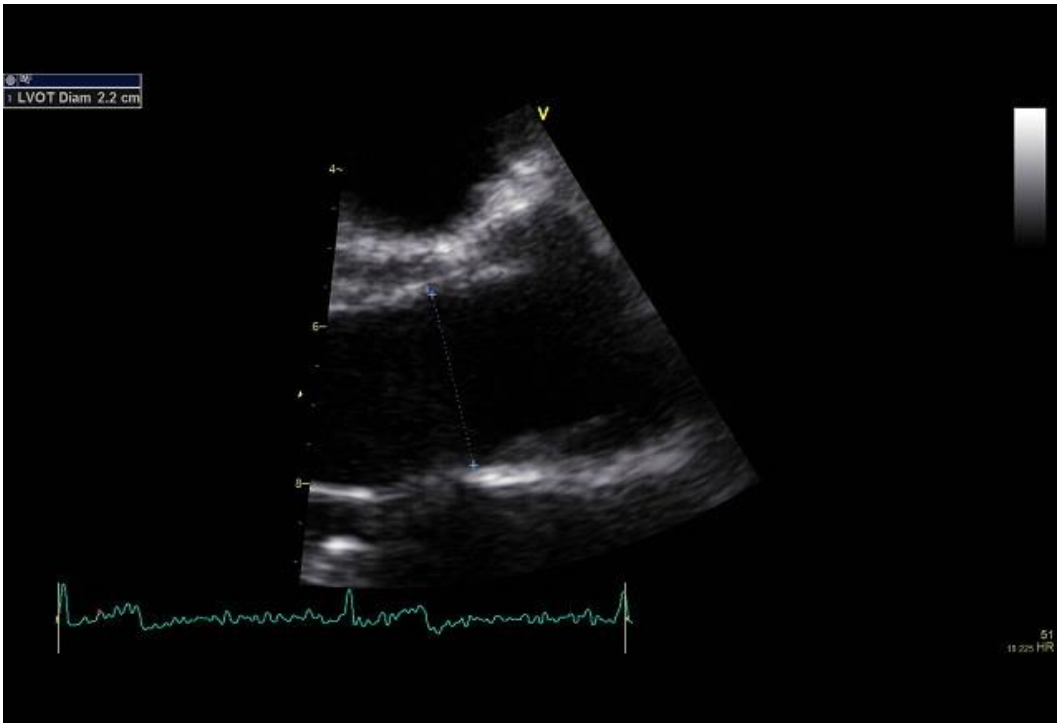

Figure 2-4. Two-dimensional left ventricular outflow tract measurement.

Myocardial velocities

Left ventricular longitudinal systolic function (s′) and diastolic function (e′, a′ and e′/a′) were assessed by pulsed myocardial velocity imaging. The mean of measurements from the lateral and septal mitral annulus over three consecutive cycles was used in analysis (Figure 2-6).

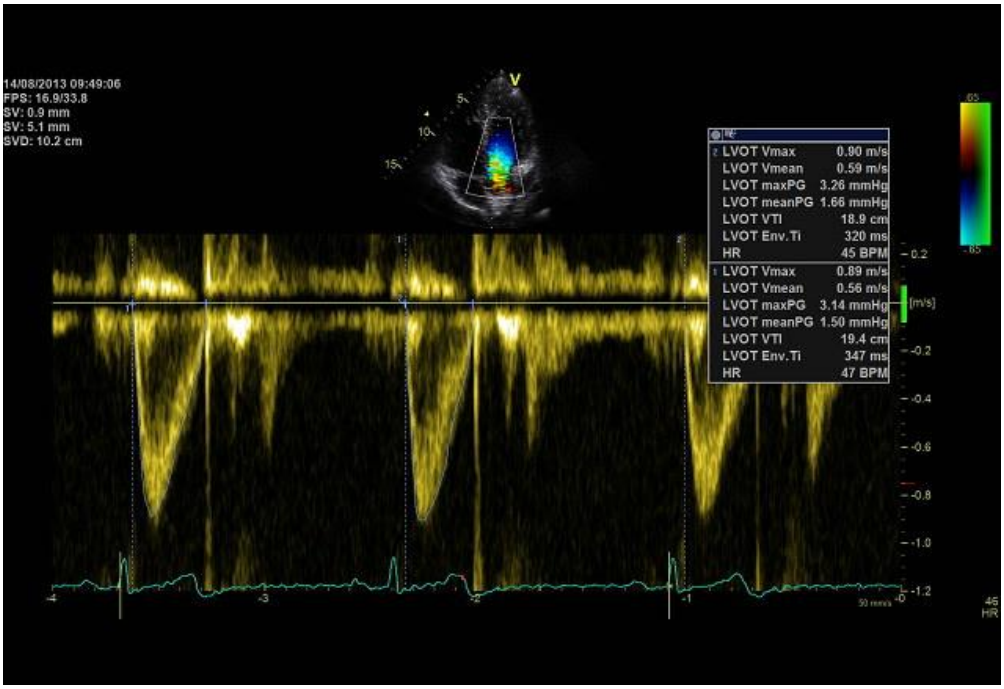

Figure 2-5. Pulsed-wave spectral Doppler recording showing measurement of the left ventricular outflow velocity time integral.

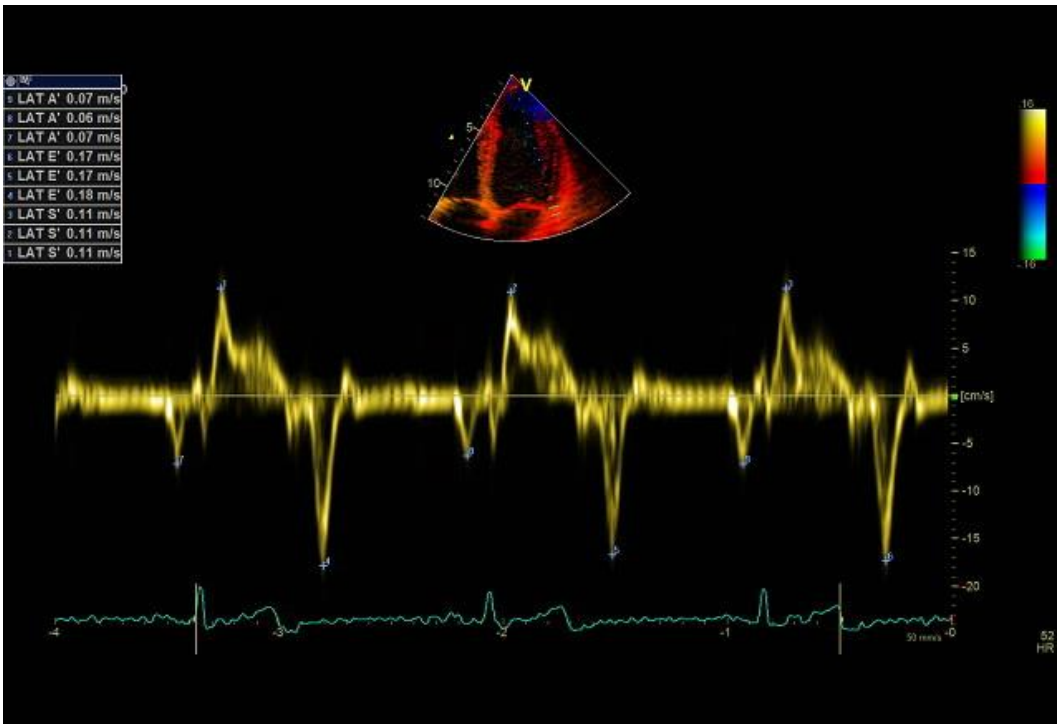

Figure 2-6. Measurement of longitudinal myocardial tissue velocities.

Global longitudinal strain and strain rate imaging

Global longitudinal strain was assessed by 2D strain analysis of the apical four-chamber, apical two-chamber and apical long-axis views.

The frame rate was typically 40-60fps. The endocardial border was defined by automated tracking software. The width of the region of interest was adjusted manually, where necessary, to include only the myocardium. The accuracy of automated wall tracking through the cardiac cycle was visually inspected. The aim was to include an 18 segment model in the analysis which included basal-, mid- and apical- aspects of the septum, lateral, inferior, anterior, posterior and anteroseptal walls. However, only walls with good border detection and accurate tracking were approved for inclusion in quantitative analysis.

Following approval of the segment, local strain curves were assessed for accuracy and artefact before use in analysis. The aortic valve closure time was detected automatically by the software but adjusted manually if necessary. The average global longitudinal strain was derived from bulls-eye plots.

Carotid ultrasound

I performed each carotid ultrasound scan immediately after the echocardiogram. The right and left common carotid artery (CCA) were scanned consecutively using an Aloka Prosound SSD-5500 with 7.5MHz linear array transducer.

Carotid intima media thickness

Common carotid intima-media thickness (ccIMT) of the far artery wall was assessed in a 1cm segment of the left and right common carotid artery 1cm proximal to the bulb (Figure 2-7). The method followed international recommendations.[170] Measurements were made offline using automated wall tracking and analysis software (Carotid Analyser, Medical Imaging Applications). The software measures ccIMT at each pixel within the 1cm region of interest at every end-diastolic frame (~140 measurement points). A minimum of five end-diastolic frames were studied and a mean of left and right ccIMT was used in analyses. The reproducibility of intra-observer analysis from stored images was 5% for the right and 6% for the left common carotid artery.

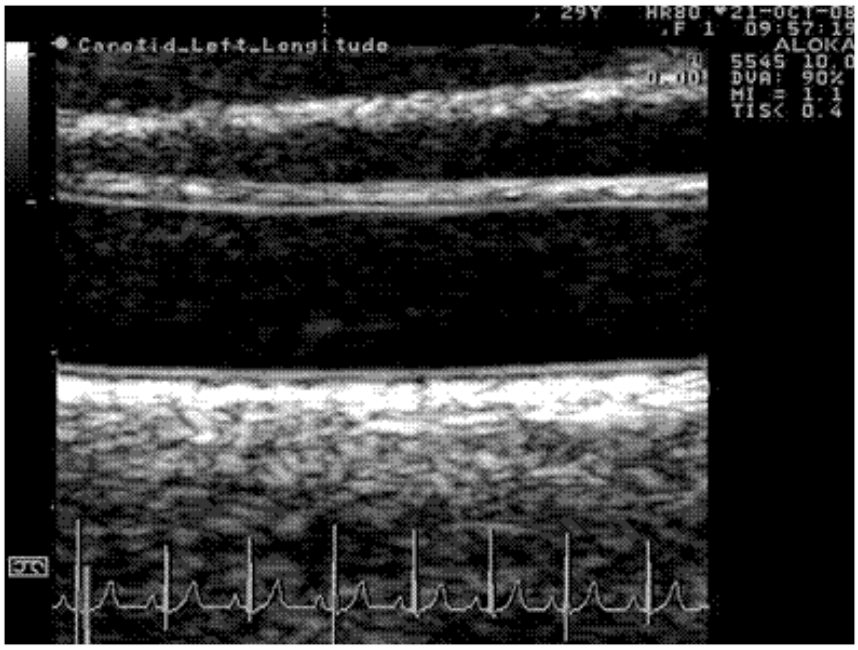

Figure 2-7 Longitudinal image of left common carotid artery.

Data analysis

In the study design phase, power calculations had suggested that recruiting 80 women with PCOS and 80 controls would yield an 80% power to detect a difference of 0.45m/s in PWV and 0.25 in e′/a′ - these were considered to be clinically different changes to key outcome measures based on the work by Cruickshank *et al.* [171] and Mogelvang *et al.* [172]. The likely power for the regression analyses was harder to estimate without detailed knowledge of the relationships but a statistician estimated there to be 80% power for detecting partial correlation coefficients of around 0.25. Despite attempts to match age and BMI during recruitment, women with PCOS enrolled in the study were younger and heavier than controls. Therefore, groups were matched for age and BMI after enrollment which resulted in a final sample of 86 controls and 73 women with PCOS.

Data are expressed as mean ± standard deviation unless otherwise stated. Distributions were checked for normality by visual inspection of histograms and use of the Kolmogorov-Smirnov statistic. Univariate correlations were assessed with Spearman's Rho because of the high number of skewed distributions; these were transformed by taking the natural logarithm prior to between-group comparisons. Between-group comparisons were assessed using one-way ANOVA with Welch's robust test of equality of means when group variances were significantly different (Levene's test) and with Tukey post-hoc correction. Between-group comparisons of categorical data were assessed using the chi-squared statistic.

The stepwise method of multivariate regression was used to derive basic predictive models for arterial and ventricular end-systolic elastance. These were followed by separate hierarchical models to assess whether measures of general and central obesity made significant independent contributions which improved the basic model. Only significant univariate correlates were used in multivariate models and care was taken to select independent variables that were not highly correlated e.g. multiple measures of central obesity were not included in the models. The β weights and standard errors were considered to be accurate i.e. not influenced by multicolinearity, when the tolerances were greater than .200. Tables report standardised β weights because of the inclusion of measures with significantly different units and standard deviations.

In all cases $p<.05$ was considered statistically significant and significant results have bold type-face in all tables.

3. Obesity, polycystic ovary syndrome and standard measures of cardiovascular function

There is considerable evidence of the effects of obesity on cardiovascular risk factors and on cardiovascular function, but the majority of studies include patients with confounding factors such as hypertension, diabetes or those who are taking cardiovascular medication. There is some evidence of the effects of uncomplicated obesity in children but very few studies of uncomplicated obesity in adults, particularly concerning the independent effects of general obesity, central obesity and insulin resistance.

There are also significant gaps in the literature concerning the effects of PCOS on cardiovascular function and it is unclear whether PCOS, with its associated high rates of obesity and insulin resistance, can be used as a pre-diabetic model for other groups of young women.

Aims of this chapter

The primary aim of this chapter is to present the clinical characteristics of the study population and to confirm the effects of general obesity, central obesity and insulin resistance on standard measures of cardiovascular function. The secondary aim is to establish whether women with PCOS have similar cardiovascular risk factors and function as age- and BMI-matched controls.

Hypotheses

In young women who are free from cardiovascular symptoms

1. uncomplicated obesity (regardless of PCOS status) will be associated with increased cardiovascular risk factors and altered cardiovascular structure and function;
2. there will be a higher prevalence of insulin resistance, impaired glucose tolerance and type 2 diabetes in those with PCOS compared with age- and BMI- matched controls; and

3. women with PCOS will have comparable cardiovascular risk factors and cardiovascular function as age- and BMI-matched controls.

Specific methods

Women with PCOS and control volunteers were recruited from local clinics and the general community. Full details of recruitment, exclusion criteria and general methods are described in Chapter 2.

Data analysis

To understand the independent and potential additive effects of obesity and PCOS on cardiovascular function, women were grouped according to PCOS status defined by the Rotterdam criteria, and by obesity status using a BMI partition value of $\geq 27 kg/m^2$ since this has been shown to identify abnormal glucose handling. There were four groups - lean control, lean PCOS, obese control, and obese PCOS.

Results

The sample sizes per group were as follows: (i) lean control = 44; (ii) lean PCOS = 20; (iii) obese control = 42; and (iv) obese PCOS = 53.

Abnormal glucose handling

Table 3-1 shows the proportions of women with undiagnosed insulin resistance, IGT and type 2 diabetes. Sixteen percent of lean control women had insulin resistance. As expected the prevalence was higher in lean women with PCOS than without (p=.003, odds-ratio 6:1 for women with PCOS). Obese women with PCOS had the highest prevalence of insulin resistance but the rate was not significantly higher than for obese controls (p= .230).

The prevalence of impaired glucose tolerance was higher in all other groups compared with lean controls. In this study, obese women with PCOS were not more likely to have IGT than lean women with PCOS or obese controls. Eight of the 11 patients with IGT were overweight or obese and the majority had waist circumference greater than 88cm.

There were no cases of impaired fasting glucose (IFG) but five cases of undiagnosed type 2 diabetes in obese PCOS subjects; all were excluded from subsequent analyses since the aim of this thesis was to establish the effect of pre-diabetic status on cardiovascular function. The remaining obese PCOS group comprised 48 women. Three of the cases of diabetes would have been missed had an oral glucose-tolerance test not been performed. All five patients had class II or III obesity (BMI 37-42.7 kg/m^2) and waist circumference significantly greater than 88cm (112-139cm).

Table 3-1. Proportions of women with undiagnosed abnormalities of glucose handling.

	Lean Control	Lean PCOS	Obese Control	Obese PCOS
Insulin resistance	7/44(16%)	11/20 (55%)	26/42 (62%)	40/53 (83%)
Impaired glucose tolerance	1/44 (2%)	2/20 (10%)	3/42 (7%)	5/53 (6%)
Type 2 diabetes	0/44	0/20	0/42	5/53 (6%)

Clinical characteristics of the study population

Table 3-2 shows the clinical characteristics of the final studied population. By design, age was similar across the groups and all measures of obesity were higher in obese compared with lean groups. In addition, the mean testosterone of lean women with PCOS was 34% higher than that of lean controls but this was not statistically significant. However, the result may be affected by the distribution of data in controls; the technique used to assess testosterone could not discriminate between values less than 0.7nmol/l. Therefore, a value of 0.69 was ascribed to all women having testosterone level categorised as <.7 nmol/l. Obese women with PCOS had the highest testosterone levels.

Body composition was similar in women with PCOS and BMI-matched controls. Obesity, regardless of PCOS status, was associated with higher levels of fasting insulin, insulin area under the curve, lower HDL cholesterol, higher triglycerides and higher hs-CRP. Obesity tended to be associated with reduced adiponectin but only the difference between lean and obese PCOS groups was statistically significant. The mean adiponectin for lean women with PCOS was 56% higher than the mean for lean controls although there was significant variation within the group and this did not reach significance after applying post-hoc corrections for multiple group comparisons[a].

[a] The distribution of adiponectin in lean controls was significantly different from that of lean women with PCOS when assessed using the non-parametric Man-Whitney U test (p=.022).

Table 3-2. Clinical characteristics (mean ± s.d.) of the final studied population.

	Lean Control (n=44)	**Lean PCOS (n=20)**	**Lean Control vs. Lean PCOS**	**Obese Control (n=42)**	**Lean Control vs. Obese Control**	**Obese PCOS (n=48)**	**Lean PCOS vs. Obese PCOS**	**Obese Control vs. Obese PCOS**
Age (years)*	30 ± 7	29 ± 7	.911	33 ± 7	.364	31 ± 7	.775	.559
BMI (kg/m^2)*	23 ± 2	23 ± 3	.840	33 ± 5	**<.0001**	35 ± 5	**<.0001**	.134
Weight (kg)*	61 ± 7	64 ± 10	.905	89 ± 15	**<.0001**	95 ± 19	**<.0001**	.381
Waist (cm)*	75 ± 6	77 ± 9	.784	96 ± 10	**<.0001**	102 ± 13	**<.0001**	.111
Waist/hip*	0.79 ± 0.05	0.80 ± 0.05	.780	0.82 ± 0.05	**.006**	0.84 ± 0.05	**.019**	.445
TFA (cm^2)*	185 ± 62	200 ± 75	.887	400 ± 72	**<.0001**	417 ± 75	**<.0001**	.925
SFA (cm^2)	169 ± 58	184 ± 69	.852	358 ± 65	**<.0001**	372 ± 70	**<.0001**	.758
VFA (cm^2)*	15 ± 8	16 ± 8	.994	42 ± 19	**<.0001**	44 ± 27	**<.0001**	.999
Cholesterol (mmol/l)	4.6 ± 0.9	4.4 ± 0.9	.884	4.7 ± 0.9	.921	4.9 ± 0.6	.096	.547
HDL (mmol/l)*	1.5 ± 0.3	1.6 ± 0.4	.961	1.3 ± 0.3	**.001**	1.3 ± 0.3	**.002**	1.0
LDL (mmol/l)	2.6 ± 0.8	2.5 ± 0.7	.802	2.9 ± 0.8	.322	3.1 ± 0.6	**.007**	.637
Triglycerides (mmol/l)*	0.9 ± 0.4	0.8 ± 0.2	.977	1.1 ± 0.5	**.023**	1.2 ± 0.5	**.005**	.858
Glucose (mmol/l)*	4.5 ± 0.3	4.6 ± 0.3	.901	4.7 ± 0.4	**.050**	4.7 ± 0.4	.443	1.0
Gluc AUC (mmol min/l)	660 ± 122	733 ± 135	.196	739 ± 135	**.038**	777 ± 137	.613	.533
Insulin (pmol/l)*	53 ± 28	72 ± 31	.145	88 ± 39	**<.0001**	134 ± 9	**<.0001**	**.010**
Ins AUC (pmol min/l)*	42789 ± 31999	58387 ± 35196	.312	61150 ± 37886	**.033**	107507 ± 65098	**.003**	**.001**
HOMA-IR*	1.7 ± 1.0	2.4 ± 1.1	.106	3.0 ± 1.5	**<.0001**	4.7 ± 3.5	**<.0001**	**.019**
hs-CRP (mg/l)*	1.3 ± 1.4	2.4 ± 5.8	.851	3.9 ± 3.9	**<.0001**	4.6 ± 4.9	**.001**	.679
Testosterone (nmol/l)†*	0.9 ± 0.3	1.2 ± 0.8	.194	0.9 ± 0.3	1.0	1.6 ± 0.8	**.047**	**<.0001**
Adiponectin (µg/ml)*	11 ± 6	18 ± 11	.120	9 ± 8	.105	9 ± 7	**.001**	.984

TFA: total fat area, SFA: subcutaneous fat area, VFA: visceral fat area, HDL: high-density lipoproteins, LDL: low-density lipoproteins, AUC: Area under the curve (during oral glucose tolerance test), HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high sensitivity c-reactive protein.
*† The mass spectrometry technique was unable to discriminate between results <0.7nmol/l. *Variables were transformed using natural log before use in ANOVA.*

Obese women with PCOS did not have lower levels of adiponectin than obese controls despite having higher levels of insulin.

In controls obesity was associated with higher fasting glucose and glucose area under the curve, but this finding was not evident in women with PCOS.

The mean fasting insulin of lean women with PCOS was 36% higher than the mean of lean controls, and a similar finding was evident in HOMA-IR, but the differences did not reach statistical significance. The combination of obesity and PCOS resulted in the highest levels of insulin resistance and hyperinsulinemia (p=.001 for HOMA-IR and fasting insulin). The between-group differences in HOMA-IR, testosterone and adiponectin are shown in Figure 3-1.

Cardiovascular function by study group

Table 3-3 shows standard measures of cardiovascular structure and function in the four groups. Obesity, regardless of PCOS status, resulted in higher peripheral and central systolic BP, LV mass and lower e′/a′. Obese subjects tended to have higher stroke volume than controls but this difference was not statistically significant. In controls, obesity was associated with increased PWV and LV relative wall thickness but this difference was not evident in obese compared with lean PCOS subjects.

Measurements of augmentation index, LV systolic function, global LV strain and strain rate (data not presented) were not different between groups in this study. Due to a technical problem with storage there was a small sample size for strain and strain rate measurements (n = 33 lean control, n = 9 lean PCOS, n = 31 obese control, n= 22 obese PCOS).

In lean women, a diagnosis of PCOS did not affect any standard measure of cardiovascular function. In addition, a combined diagnosis of PCOS and obesity did not result in worse cardiovascular function than obesity alone despite women with PCOS having worse endocrine function than controls.

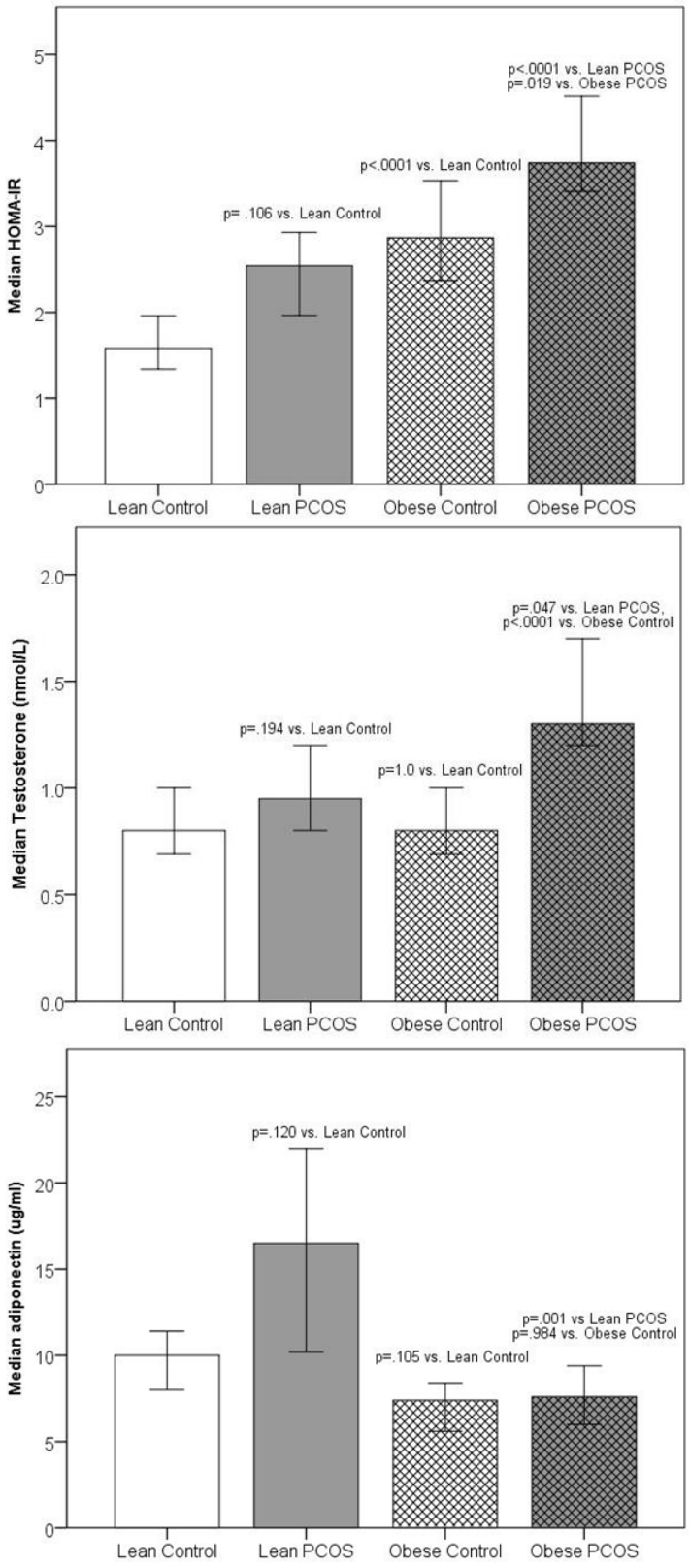

Figure 3-1. Median HOMA-IR, testosterone and adiponectin by subject group. Error bars give 95% confidence interval.

Table 3-3. Selected measures (mean ± s.d.) of cardiovascular function in the studied population.

	Lean Control	**Lean PCOS**	**Lean Control vs. Lean PCOS**	**Obese Control**	**Lean Control vs. Obese Control**	**Obese PCOS**	**Lean PCOS vs. Obese PCOS**	**Obese control vs. Obese PCOS**
Heart rate (bpm)	69 ± 10	71 ± 12	.895	72 ± 12	.715	75 ± 10	.604	.543
bSBP (mmHg)	108 ± 8	108 ± 6	.996	116 ± 11	**<.0001**	115 ± 9	**.026**	.759
bDBP (mmHg)	62 ± 9	63 ± 6	.942	66 ± 9	.083	67 ± 10	.472	1.0
cSBP (mmHg)	92 ± 9	91 ± 6	.998	99 ± 11	**<.0001**	98 ± 9	**.042**	.693
cDBP (mmHg)	63 ± 9	65 ± 6	.857	67 ± 9	.070	69 ± 10	.455	.989
ccIMT (mm)*	0.492 ± 0.048	0.473 ± 0.063	.529	0.524 ± 0.064	.100	0.507 ± 0.063	.102	.630
Aortic PWV (m/s)*	5.96 ± 0.75	5.91 ± 0.71	.997	6.74 ± 0.90	**<.0001**	6.42 ± 0.87	.133	.190
AIx-75 (%)	1.14 ± 14.73	1.75 ± 10.28	.998	7.63 ± 14.73	.124	4.31 ± 11.63	.891	.666
RWT*	0.35 ± 0.06	0.35 ± 0.06	1.0	0.39 ± 0.07	**.024**	0.35 ± 0.05	.997	**.047**
LVIDd (cm)	4.52 ± 0.37	4.51 ± 0.40	1.0	4.73 ± 0.39	.144	4.73 ± 0.36	.108	.962
LV mass ($g/m^{2.7}$)*	30 ± 6	30 ± 7	.998	39 ± 7	**<.0001**	36 ± 5	**.003**	.325
FS (%)	33 ± 5	34 ± 5	.916	35 ± 6	.140	36 ± 5	.294	.844
Stroke volume (ml)	61 ± 10	60 ± 12.3	.988	67 ± 12	.127	67 ± 12	.130	1.0
S’ (cm/s)*	9.8 ± 1.5	9.6 ± 1.1	.986	10 ± 2.1	.974	9.8 ± 1.6	.990	.970
MAPSE (cm)	1.3 ± 0.1	1.3 ± 0.1	.576	1.4 ± 0.2	.090	1.4 ± 0.1	.970	.996
e' (cm/s)*	15 ± 2	17 ± 2	.446	14 ± 3	.081	14 ± 3	**.014**	.999
a' (cm/s)	7.7 ± 1.5	8.1 ± 1.7	.922	9.2 ± 2.0	**.004**	9 ± 1.9	.408	.960
e'/a'* (cm/s)	2.1 ± 0.5	2.1 ± 0.5	.973	1.6 ± 0.6	**.001**	1.7 ± 0.5	**.024**	.914
IVRT (ms)	81 ± 8	80 ± 11	.896	87 ± 11	**.023**	82 ± 12	.879	.072
FPV (cm/s)	54 ± 8	56 ± 11	.996	60 ± 12	.130	58 ± 10	.884	.902

*b: brachial, c: central, SBP: systolic blood pressure, DBP: central diastolic blood pressure, ccIMT: common carotid intima-media thickness, PWV: pulse wave velocity, AIx-75: augmentation index corrected for a heart rate of 75bpm, RWT: relative wall thickness, LVIDd: left ventricular internal dimension in diastole, FS: fractional shortening, s': longitudinal systolic myocardial velocity, e'/a': ratio of early to late diastolic myocardial tissue velocity, IVRT: isovolumic relaxation time. *Variables were transformed using natural log before use in ANOVA.*

Left ventricular geometry by study group

Frequencies of concentric remodelling, concentric hypertrophy and eccentric hypertrophy are shown in Table 3-4. In controls, obesity was associated with an increased prevalence of ventricular remodelling and hypertrophy with evidence of both eccentric and concentric patterns. The prevalence of concentric remodelling was higher in lean women with PCOS than in lean controls but the numbers were small in both groups. The prevalence of hypertrophy in obese women with PCOS was significantly lower than in obese controls. The difference in frequency of hypertrophy was related to the study group ($\chi^2 = 32.32$, p=.000183) with an odds ratio of 18:1 for hypertrophy in obese control versus obese PCOS subjects.

Table 3-4. Frequency of left ventricular remodelling and hypertrophy by study group.

	Lean Control	**Lean PCOS**	**Obese Control**	**Obese PCOS**
Unable to determine	2/44 (4.5%)	1/20 (5%)	2/40 (5%)	4/47 (8.5%)
Normal geometry	39/44 (89%)	16/20 (80%)	22/40 (55%)	39/47 (83%)
Concentric remodelling	2/44 (4.5%)	3/20 (15%)	5/40 (12.5%)	3/47 (6.4%)
Concentric hypertrophy	1/44 (2%)	0/20 (0%)	4/40 (10%)	1/47 (2.1%)
Eccentric hypertrophy	0/44 (0%)	0/20 (0%)	7/40 (17.5%)	0/47 (0%)

Univariate correlates of key cardiovascular function variables

Table 3-5 shows univariate correlations between metabolic variables and key measures of cardiovascular structure and function in the pooled data.

The most important correlate of ccIMT was age. There were weaker but significant direct associations with measures of general and central obesity (except WHR), central systolic BP and aortic PWV, and an inverse association with adiponectin. There were no significant associations of ccIMT with measures of insulin resistance (except glucose AUC), cholesterol, testosterone or inflammation.

The most important correlate of aortic PWV was central systolic blood pressure (which can be a cause and effect of increased PWV, a pressure dependent measure of aortic stiffness). Associations also existed with age, measures of general and central obesity, fasting measures of glucose handling, diastolic blood pressure, inflammation, LDL and total cholesterol, ccIMT, mitral annular plane systolic excursion (all direct) and adiponectin (inverse). There was no association of aortic stiffness with testosterone.

The most important correlate of LV mass was weight. There were also associations with age, other measures of general and central obesity, stroke volume, inflammation, fasting measures of glucose handling, systolic and diastolic BP, aortic stiffness, LDL and triglycerides (all direct). LV mass was also inversely associated with adiponectin and HDL. There was no association of LV mass with testosterone. There was a direct association of LV mass with ccIMT which may be partly due to a shared association with body size, particularly with lean mass.

The most important correlate of the diastolic function measure, e′/a′, was age (inverse). There were also inverse associations with aortic PWV, ccIMT, LV mass, all measures of general and central obesity, all measures of glucose handling, cholesterol, inflammation, brachial and central BP, stroke volume, and fractional shortening. The ratio of e′/a′ was also directly associated with HDL. There was no significant association of e′/a′ with testosterone or with adiponectin.

Table 3-5. Univariate correlates [Spearman's Rho (p-value)] of key cardiovascular variables in the pooled data.

	ccIMT		**Aortic PWV**		**LV mass**		**e′/a′**	
Age	**.44**	**(<.0001)**	**.56**	**(<.0001)**	**.32**	**(.0001)**	**−.60**	**(<.0001)**
Weight	**.26**	**(.001)**	**.40**	**(<.0001)**	**.62**	**(<.0001)**	**−.45**	**(<.0001)**
BMI	**.26**	**(.001)**	**.47**	**(<.0001)**	**.58**	**(<.0001)**	**−.51**	**(<.0001)**
Waist	**.28**	**(.001)**	**.47**	**(<.0001)**	**.58**	**(<.0001)**	**−.57**	**(<.0001)**
Waist/hip	.10	(.243)	**.23**	**(.006)**	**.23**	**(.006)**	**−.38**	**(<.0001)**
TFA	**.24**	**(.004)**	**.41**	**(<.0001)**	**.47**	**(<.0001)**	**−.44**	**(<.0001)**
SFA	**.22**	**(.008)**	**.38**	**(<.0001)**	**.46**	**(<.0001)**	**−.40**	**(<.0001)**
VFA	**.27**	**(.001)**	**.42**	**(<.0001)**	**.50**	**(<.0001)**	**−.54**	**(<.0001)**
Cholesterol	.03	(.713)	**.17**	**(.049)**	.13	(.123)	**−.23**	**(.012)**
HDL	−.12	(.151)	−.15	(.069)	**−.26**	**(.003)**	**.25**	**(.006)**
LDL	.07	(.406)	**.19**	**(.022)**	**.20**	**(.021)**	**−.26**	**(.003)**
Triglycerides	.13	(.117)	.15	(.070)	**.25**	**(.003)**	**−.21**	**(.018)**
Glucose	.13	(.112)	**.20**	**(.018)**	**.18**	**(.031)**	**−.30**	**(.001)**
Gluc AUC	**.20**	**(.017)**	.17	(.051)	.11	(.188)	**−.37**	**(<.0001)**
Insulin	.02	(.807)	**.20**	**(.018)**	**.23**	**(.006)**	**−.20**	**(.030)**
Ins AUC	.03	(.724)	.15	(.097)	.01	(.875)	**−.29**	**(.003)**
HOMA-IR	.03	(.685)	**.24**	**(.004)**	**.25**	**(.001)**	**−.23**	**(.013)**
hs-CRP	.12	(.141)	**.26**	**(.001)**	**.30**	**(<.0001)**	**−.19**	**(.032)**
Testosterone	−.14	(.099)	.02	(.849)	.05	(.583)	−.05	(.605)
Adiponectin	**−.26**	**(.002)**	**−.18**	**(.039)**	**−.26**	**(.002)**	.16	(.091)
Heart rate	−.05	(.530)	.11	(.193)	.00	(.995)	**−.19**	**(.035)**
bSBP	.10	(.214)	**.43**	**(<.0001)**	**.36**	**(<.0001)**	**−.22**	**(.013)**
bDBP	.03	(.775)	**.48**	**(<.0001)**	**.22**	**(.008)**	**−.35**	**(<.0001)**
cSBP	**.20**	**(.015)**	**.62**	**(<.0001)**	**.36**	**(<.0001)**	**−.43**	**(<.0001)**
cDBP	.02	(.775)	**.46**	**(<.0001)**	**.25**	**(.003)**	**−.37**	**(<.0001)**
SV	**.27**	**(.001)**	.16	(.062)	**.44**	**(<.0001)**	**−.28**	**(.002)**
s′	−.01	(.951)	.06	(.515)	−.16	(.094)	.02	(.817)
MAPSE	.06	(.452)	**.18**	**(.033)**	.12	(.153)	−.14	(.115)
FS	.15	(.081)	.14	(.096)	.08	(.323)	**−.39**	**(<.0001)**
ccIMT	1		**.28**	**(.001)**	**.38**	**(<.0001)**	**−.36**	**(<.0001)**
aPWV			**1**		**.32**	**(<.001)**	**−.54**	**(<.0001)**
LV mass					**1**		**−.39**	**(<.0001)**

TFA: total fat area, SFA: subcutaneous fat area, VFA: visceral fat area, HDL: high-density lipoproteins, LDL: low-density lipoproteins, AUC: Area under the curve (during oral glucose tolerance test), HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high sensitivity c-reactive protein, b: brachial, c: central, SBP: systolic blood pressure, DBP: central diastolic blood pressure, SV: stroke volume, s': longitudinal systolic myocardial velocity, MAPSE: mitral annular plane systolic excursion, FS: fractional shortening, ccIMT: common carotid intima-media thickness, aPWV: pulse wave velocity, e′/a′: ratio of early to late diastolic myocardial tissue velocity.

Independent contributors of common carotid intima-media thickness

The variation in ccIMT was explained reasonably well by a basic model comprising age and a measure of body size. Separate regression models for each measure of body size are shown in Table 3-6. The combination of age and weight resulted in the best model.

Glucose AUC and adiponectin were added to the second block of a hierarchical model using a stepwise method. Adiponectin emerged as a significant explanatory variable and improved the model (r^2 change 4%, p=.003) while the measure of body size became non-significant.

In the next step central systolic blood pressure, aortic pulse wave velocity and LV stroke volume were added using the same method. Stroke volume emerged as a significant explanatory variable and improved the model (r^2 change 5%, p=.003).

The final model therefore included age, adiponectin and stroke volume which together explained 32% of the variation in carotid intima-media thickness (p<.0001) (Table 3-7).

Age explained most of the variation in ccIMT in this population but a significant proportion of the variation was independently explained by adiponectin, suggesting that the hormone may represent a link between obesity and increased intima-media thickening in the young women in this study. Left ventricular stroke volume, which tended to be increased in obese subjects, was also an independent explanatory variable for ccIMT.

Table 3-6. Separate basic multivariate regression models for common carotid intima-media thickness.

*Dependent variable = ccIMT**	**r^2 value**	**Model p-value**
Age* + weight*	.233	**<.0001**
Age* + BMI*	.223	**<.0001**
Age* + waist circumference*	.220	**<.0001**
Age* + visceral fat area*	.222	**<.0001**

**Variables were transformed by taking the natural logarithm prior to use in multivariate regression*.

Table 3-7. Final multivariate regression model for common carotid intima-media thickness.

*Dependent variable = ccIMT**	**Standardised β**	**P-value**	**Part correlation**
Age*	.383	**<.0001**	.373
HMW adiponectin*	−.286	**<.0001**	−.285
LV stroke volume	.231	**.005**	.226
Model r^2 = .323, p<.0001			

**Variables were transformed by taking the natural logarithm prior to use in multivariate regression*.

Independent contributors of aortic pulse wave velocity

The variation in aortic PWV was explained reasonably well by a basic model comprising age, central systolic blood pressure and a measure of body size. Although PWV is a measure of aortic stiffness and a rise in PWV would result in higher systolic blood pressure, PWV is also dependent on systolic blood pressure because the aorta becomes functionally stiffer at higher blood pressures. Separate regression models for each measure of body size are shown in Table 3-8. The use of waist circumference or BMI resulted in an improved model compared with visceral fat.

HOMA-IR, hs-CRP, adiponectin, ccIMT, cholesterol, LDL and MAPSE were added to the second hierarchical block using a stepwise method but these did not independently contribute to aortic PWV and did not improve the model.

The final model therefore included age, central systolic blood pressure and waist circumference which together explained 54% of the variation in aortic pulse wave velocity ($p<.0001$) (Table 3-9). Central systolic blood pressure and age explained most of the variation in aortic pulse wave velocity in this population with a small proportion of the variation independently explained by waist circumference.

Table 3-8. Separate basic multivariate regression models for aortic pulse wave velocity.

*Dependent variable = aortic PWV**	**r^2 value**	**Model p-value**
Age* + cSBP + weight*	.529	**<.0001**
Age* + cSBP + BMI*	.543	**<.0001**
Age* + cSBP + waist circumference*	.544	**<.0001**
Age* + cSBP + visceral fat area*	.517	**<.0001**

*cSBP: central systolic blood pressure. *Variables were transformed by taking the natural logarithm prior to use in multivariate regression.*

Table 3-9. Final multivariate regression model for aortic pulse wave velocity.

*Dependent variable = aortic PWV**	**Standardised β**	**P-value**	**Part correlation**
Age*	.353	**<.0001**	.322
Central systolic blood pressure	.399	**<.0001**	.337
Waist circumference*	.195	**.003**	.172
Model r^2 = .544, p<.0001			

**Variables were transformed by taking the natural logarithm prior to use in multivariate regression*.

Independent contributors of left ventricular mass

The variation in LV mass was explained reasonably well by a basic model comprising age, central systolic blood pressure and a single measure of body size. Separate regression models for measures of body size are shown in Table 3-10. Weight explained more of the variation in ventricular mass than did the other basic measures of body size.

Measures of glucose handling were added to the second block of a hierarchical model using a stepwise method but these did not independently explain a significant proportion of the variation in LV mass and did not improve the model.

Aortic pulse wave velocity and ccIMT were added to the second block of the hierarchical model using a stepwise method. ccIMT emerged as an independent contributor to LV mass and resulted in a small but significant improvement in the model (r^2 change 6%, $p < .0001$).

The final model for the dependent variable LV mass is shown in Table 3-11. The model explained 50% of the variation in LV mass and weight was the most important independent contributor. The introduction of ccIMT had the effect of reducing the beta value of age and central systolic blood pressure to a non-significant value.

Table 3-10. Separate basic multivariate regression models for left ventricular mass.

*Dependent Variable = LV Mass**	**r^2 value**	**Model p-value**
Age* + cSBP + weight	.466	**<.0001**
Age* + cSBP + BMI*	.401	**<.0001**
Age* + cSBP + waist circumference*	.381	**<.0001**
Age* + cSBP + visceral fat area*	.289	**<.0001**

*cSBP = central systolic blood pressure. *Variables were transformed by taking the natural logarithm prior to use in multivariate regression.*

Table 3-11. Final multivariate regression model for left ventricular mass.

*Dependent variable = LV mass**	**Standardised β**	**P-value**	**Part correlation**
Weight*	.558	**<.0001**	.490
ccIMT*	.214	**.002**	.188
cSBP	.096	.191	.080
Age*	.040	.583	.034
Model r^2 = .501, p<.0001			

*cSBP = central systolic blood pressure. *Variables were transformed by taking the natural logarithm prior to use in multivariate regression.*

Independent contributors of diastolic function

The variation in diastolic function (e′/a′) was explained reasonably well by a basic model comprising age, central systolic blood pressure and a measure of body size. Separate regression models for measures of body size are shown in Table 3-12. Among the measures of obesity, visceral fat area appeared to explain more of the variation in diastolic function. LV mass did not independently explain any of the variation in diastolic function when measures of obesity were included.

A hierarchical approach was used to test whether the model was improved by the addition of any of the following variables (in separate blocks); measures of glucose handling, ccIMT and aortic PWV. Insulin area under the curve emerged as an independent contributor of diastolic function explaining a further 7% of its variation (Table 3-13).

The final model is shown in Table 3-14. Age was the most important independent determinant of reduced diastolic function, followed by insulin area under the curve and visceral fat area. Central systolic blood pressure did not independently explain a significant proportion of the variation in e′/a′ in the final model.

Table 3-12. Separate basic multivariate regression models for the e′/a′.

*Dependent variable = e′/a′**	**r^2 value**	**Model p-value**
Age* + cSBP + weight*	.428	**<.0001**
Age* + cSBP + BMI*	.458	**<.0001**
Age* + cSBP + waist circumference*	.480	**<.0001**
Age* + cSBP + visceral fat area*	.511	**<.0001**

*cSBP = central systolic blood pressure. *Variables were transformed by taking the natural logarithm prior to use in multivariate regression.*

Table 3-13. Separate hierarchical multivariate regression block for e′/a′.

*Dependent variable = e′/a′**	**r^2 change**	**Cumulative r^2**	**F change statistic**
Basic model + insulin AUC*	.066	.577	F (1, 91) = 14.22, **p<.0001**

Basic model included age, central systolic blood pressure and visceral fat area. *Variables were transformed by taking the natural logarithm prior to use in multivariate regression.*

Table 3-14. Final multivariate regression model for e′/a′.

*Dependent variable = e′/a′**	**Standardised β**	**P-value**	**Part correlation**
Age*	−.491	**<.0001**	−.419
Insulin AUC*	−.262	**.001**	−.241
Visceral fat area*	−.209	**.011**	−.179
cSBP	−.147	.068	−.128
Model r^2 = .577, p<.0001			

*cSBP = central systolic blood pressure. *Variables were transformed by taking the natural log prior to use in multivariate regression.*

The effects of obesity and PCOS on the relationship between age and cardiovascular function

Common carotid intima-media thickness and aortic PWV are known to increase with age, while e′/a′ decreases. Scatterplots and linear regression were used to determine whether the relationship between these measures and age was affected by obesity and PCOS status (Figure 3-2).

The effect of obesity in controls: Comparison of the *solid* lines shows the effect of obesity on the relationship between age and cardiovascular function. The slope tended to be steeper in obese (red line) compared with lean (black line) controls for each of the three variables although the differences in regression coefficients did not reach statistical significance (p= .184 for ccIMT, p= .085 for PWV and p= .247 for e′/a′).

The effect of obesity in women with PCOS: Comparison of the *broken* lines shows the effect of obesity on the relationship between age and cardiovascular function in women with PCOS. Those who were obese (red) appeared to have higher PWV and lower e′/a′ than those who were lean (black) across the age-range because of a tendency for different intercepts but these differences were not statistically significant (p= .469 for PWV and p= .513 for e′/a′). The slopes were very similar between lean and obese women with PCOS (p= .689 for ccIMT, p= .793 for PWV and p= .877 for e′/a′).

The effect of PCOS in lean women: Comparison of the solid and broken *black* lines shows the effect of PCOS on the relationship between age and cardiovascular function in lean women. The intercept between age and ccIMT appeared lower in those with PCOS (broken line) (p=.150) but the slope tended to be increased compared with lean controls (solid line) (p=.225) so that the lines crossed at around age 35 years. A diagnosis of PCOS had no effect on the relationship between age and aortic PWV in lean women (p=.907 for differences in slope and p=.969 for differences in intercept). The decline in diastolic function with age appeared marginally steeper in those with PCOS (p=.463) so that the lines separated at higher ages.

The effect of PCOS in obese women: Comparison of the solid and broken *red* lines shows the effect of PCOS on the relationship between age and cardiovascular function in obese women. The age-related change in ccIMT tended to be steeper in obese women with PCOS compared to obese controls (p=.180). However, the intercept was lower (p=.160) so that only women with PCOS who were older than 35 years had higher ccIMT than controls. A higher intercept (p=.190) but shallower slope (p=.142) meant that after age 30 years obese women with PCOS tended to have lower PWV than obese controls. Similarly, the e′/a′ intercept and slope were more similar in obese women with and without PCOS (p=.559 and p=.696 respectively).

These trends, coupled with inconclusive evidence for altered cardiovascular outcome data in women with PCOS, led to the generation of a new hypothesis that HMW adiponectin may be more important in mitigating the effects of obesity and insulin resistance in PCOS than in healthy women.

HMW Adiponectin

Table 3-15 shows univariate correlations between HMW adiponectin and other cardio-metabolic variables by PCOS status. HMW adiponectin was negatively associated with obesity, fasting insulin and HOMA-IR, ccIMT and LV mass in subjects with and without PCOS. However, the correlation coefficients for measures of obesity were significantly greater in women with PCOS compared with controls. Furthermore, adiponectin was negatively associated with central systolic blood pressure in those with PCOS but not in controls.

There was no association of testosterone with HMW adiponectin when groups were split by PCOS diagnosis. However, in a separate analysis for lean subjects (regardless of PCOS status) there was a weak association of testosterone with HMW adiponectin (r=.270, p=.032).

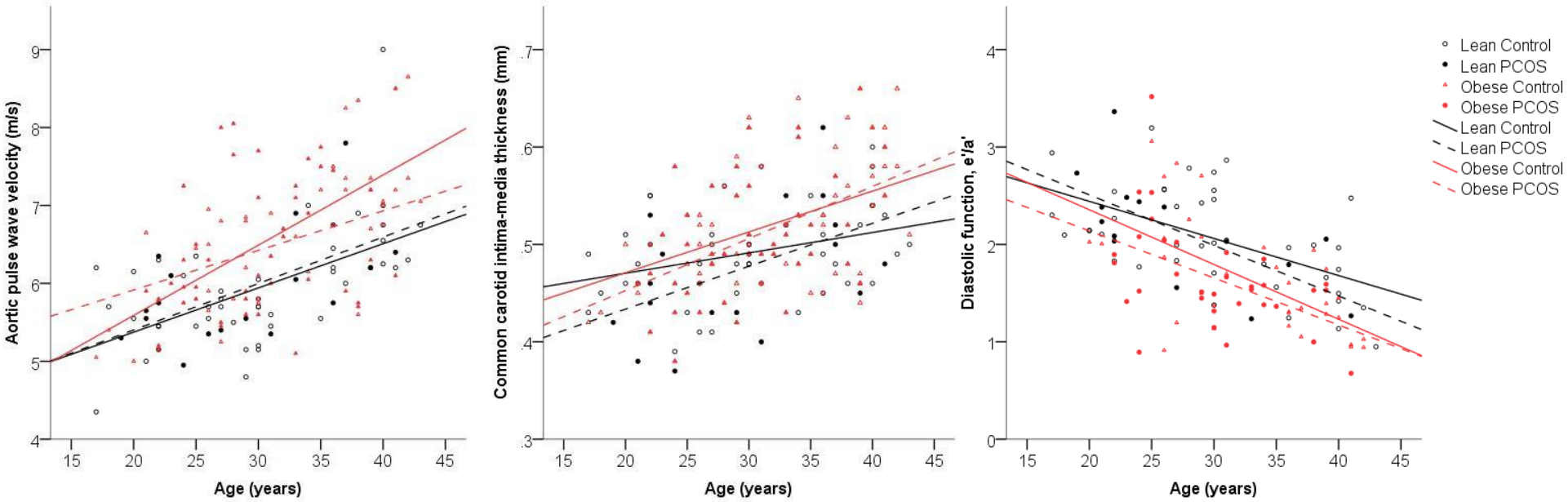

Figure 3-2. The relationship of age with pulse wave velocity, common carotid intima-media thickness and diastolic function by subject group.

Table 3-15. Univariate correlations [Spearman's Rho (p-value]) of HMW-adiponectin with cardio-metabolic variables.

	Control	PCOS	Fisher Z	p-value
Weight	−.22(.049)	−.56(<.0001)	**2.52**	**.006**
BMI	−.23(.035)	−.57(<.0001)	**2.55**	**.005**
Waist circumference	−.25(.028)	−.50(<.0001)	**1.81**	**.035**
Visceral fat area	−.21(.062)	−.56(<.0001)	**2.59**	**.005**
Glucose	−.03(.805)	−.09(.489)	0.37	.356
Glucose AUC	−.17(.132)	−.05(.683)	−0.75	.227
Insulin	−.38(<.0001)	−.43(.001)	0.37	.711
Insulin AUC	−.35(.002)	−.26(.056)	−0.61	.271
HOMA-IR	−.37(.001)	−.43(<.0001)	0.44	.330
Hs-CRP	−.17(.130)	−.32(.010)	0.99	.161
Testosterone	−.01(.996)	−.10(.439)	0.56	.288
Heart rate	−.14(.221)	−.08(.561)	−0.37	.356
Central SBP	−.05(.681)	−.34(.006)	**1.87**	**.031**
Central DBP	−.02(.854)	−.18(.144)	1.0	.159
ccIMT	−.23(.044)	−.29(.020)	0.40	.345
Aortic PWV	−.13(.260)	−.22(.084)	0.57	.284
LV mass	−.24(.035)	−.30(.020)	0.40	.345
e′/a′	.17(.154)	.17(.268)	0	.500

AUC: area under the curve, HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high sensitivity c-reactive protein, SBP: systolic blood pressure, DBP: diastolic blood pressure, ccIMT: common carotid intima-media thickness, PWV: pulse wave velocity.

Discussion

Undiagnosed abnormalities of glucose metabolism

Impaired fasting glucose and type 2 diabetes: There were no cases of IFG in any group and no cases of undiagnosed type 2 diabetes in control subjects. In contrast, five women with PCOS had undiagnosed type 2 diabetes. These figures are comparable to the 4-10% quoted by other studies,[173-175] which is 7.5-10 times higher than the prevalence for women of a similar age without PCOS.[173] All those with diabetes in my study were drawn from the obese PCOS group which may also be more likely to contain those with the most severe reproductive forms of the syndrome. Three of the five cases of diabetes were only evident when the post-prandial glucose response was assessed. This finding is not unique to this study and the Androgen Excess Society has recommended screening women with PCOS for diabetes using an oral glucose tolerance test.[176]

Impaired glucose tolerance: There were very small numbers of women with IGT in all categories. Perhaps surprisingly, the rate was not higher in women with PCOS who were obese than for those who were lean. The prevalence of IGT in women with PCOS in my study (6-10%) is lower than the 20-35% quoted by others.[173-175, 177]. This may be related to the use of the Rotterdam criteria although 79% of women with PCOS in my study also met the NIH criteria that requires a diagnosis of hyperandrogenism and chronic anovulation.

Insulin resistance: Insulin regulates blood glucose levels by stimulating glucose uptake by tissues, adipocytes, skeletal and cardiac muscle. It also suppresses glucose production in the liver. The term insulin resistance is used to describe a decreased ability of insulin to mediate these actions so that more insulin is required to achieve any given effect. Therefore, insulin resistance results in elevated basal insulin and elevated post-prandial insulin when pancreatic β-cells are intact. The hyperinsulinaemic-euglycaemic clamp technique is considered the gold standard for assessing insulin resistance in vivo but this method is complex and expensive, rendering it unsuitable for many larger studies. I used fasting insulin and glucose measures, the HOMA-IR index, and OGTT-derived parameters as surrogate

measures of insulin resistance. While these are less precise assessments of insulin sensitivity, they do correlate with clamp methods.[178, 179]

Two-thirds of obese controls had undiagnosed insulin resistance according to a HOMA-IR result of 2.5 or greater. It is unclear whether this partition value represents the most sensitive and specific threshold for the diagnosis of insulin resistance but I used it because of the absence of a clear definition in guidelines and because it allowed a direct comparison between my results and those of another group who also studied cardiovascular function in women with and without PCOS.[98]

As expected, the rates of insulin resistance were higher in women with PCOS. Insulin resistance affected around half of those who were lean and 83% of those who were obese. Others have reported a prevalence of 44-95% for insulin resistance in women with PCOS, with rates likely affected by the diagnostic method, partition value and reproductive phenotype.[74, 76]

In 1980 Burghen *et al*. were the first to report that women with PCOS had increased insulin responses during oral glucose-tolerance testing that were not solely attributable to obesity.[180] This triggered an exponential rise in the number of studies on insulin resistance associated with the syndrome. While there is a general consensus that *obese* women with PCOS are insulin resistant,[72] some studies using the clamp method in lean women with PCOS have failed to demonstrate insulin resistance.[181]

I found a trend towards an intrinsic level of basal hyperinsulinemia and elevated HOMA-IR in lean women with PCOS but the differences were not statistically significant after corrections for multiple post-hoc comparisons. Fasting insulin levels are affected by insulin production, insulin clearance and insulin sensitivity, but our study was not designed to determine the precise mechanism for these findings. Others have demonstrated, using clamp studies, that lean women with PCOS have hyperinsulinemia due to increased insulin secretion and not as a consequence of insulin resistance.[181] However, postprandial dysglycaemia was also evident in our study and this suggests peripheral, mainly skeletal muscle, insulin resistance.[182] Fasting glucose levels were normal reflecting endogenous glucose production (in the liver) that was similar to controls.

Insulin resistance and body composition

Visceral fat is more metabolically active than subcutaneous fat and is strongly associated with insulin resistance. In my study visceral fat area was measured from CT scans and was similar among controls and age- and BMI-matched women with PCOS. These results are similar to other studies using MRI which is considered the gold-standard method of quantifying visceral and subcutaneous fat.[183, 184]

There is speculation in the literature that the insulin resistance in PCOS is attributable to increased visceral fat rather than global obesity. This is based on an increased frequency of upper body obesity (the so-called 'android shape') in those with hyperandrogenemia,[185] and a positive association between exogenous androgen levels (nandrolone) and visceral fat.[186] The data in the present study suggest that body composition and in particular visceral fat levels, are not different in women with PCOS and controls so this is unlikely to be the cause of higher rates of insulin resistance in the former.

The addition of obesity to PCOS was associated with insulin resistance beyond the 'intrinsic' level of hyperinsulinemia. The relationship between visceral fat area and insulin resistance was similar in PCOS and in controls suggesting that obesity is linked to extrinsic insulin resistance in a similar manner in both groups. This is relevant to management strategies since therapies designed to target insulin resistance (such as metformin) may be more successful in treating the extrinsic insulin resistance in PCOS.[187] Given this, it would be useful to have a better understanding of the associations between obesity, insulin resistance and cardiovascular function in women with PCOS.

Metabolic phenotype mirrors reproductive phenotype in PCOS

Not all women with PCOS defined by the Rotterdam criteria have insulin resistance. There are two reproductive phenotypes with normal or near normal insulin sensitivity; these are (i) anovulatory women with polycystic ovaries but normal androgen levels (ii) women with hyperandrogenaemia and polycystic ovaries but normal ovulation.[177, 188] These women are often leaner than those diagnosed by NIH criteria who have both anovulation and hyperandrogenemia. Since I was interested in the effects of insulin resistance and obesity on cardiovascular function I did not

characterize subjects by reproductive phenotype. However, lean women with PCOS in my study had better insulin sensitivity and lower testosterone levels than obese women with PCOS i.e. the metabolic phenotype appeared to mirror the reproductive phenotype as noted by others.[76] There is evidence for a mechanistic link between the reproductive phenotype and insulin since it decreases hepatic production of the sex hormone binding globulin (SHBG) which results in elevated levels of circulating testosterone.[189]

There are no detailed longitudinal studies which explore the natural history of PCOS and it is unclear whether the milder reproductive phenotypes are an intermediate stage between normality and the more severe form of the syndrome with progression triggered by weight gain.

Cardiovascular function

Common carotid intima-media thickness: Obesity tended to be associated with increased ccIMT but the differences were not significant. In addition, women with PCOS had similar ccIMT to age- and BMI-matched controls.

The study population was aged 16 to 45 years and it may be that significant increases in ccIMT are not evident until later in life. One study has demonstrated that only women aged >45 years with PCOS had thicker ccIMT than controls after adjustment for BMI.[190] Subtle differences in a small measurement are also difficult to detect without very large sample sizes. A systematic review and meta-analysis in 2012 combined 19 studies for meta-analysis (1123 women with PCOS and 923 controls in total) and found that ccIMT was increased by 0.072 - 0.084mm ($p<.0001$) for good and high quality studies.[93] The authors suggested that the difference in ccIMT equates to ~7 years of ageing since the average change in ccIMT for women is reported to be 0.009 – 0.015mm per year.[191, 192] The review was unable to determine which cardiovascular risk factors or reproductive phenotypes were associated with increased ccIMT but many of the studies used NIH criteria to diagnose PCOS.

A key finding of this study is that age, adiponectin and LV stroke volume were the only independent contributors to ccIMT in this young population who were free from overt cardiovascular disease.

This suggests that obesity and increased ccIMT may be linked through a reduction in adiponectin (which has anti-inflammatory and anti-atherogenic effects) and an increase in body size and circulating volume. The data support a recent large study by Kozakova *et al.* who found that the increased thickening of the common carotid wall in an uncomplicated obese population may be predominantly due to adaptive remodelling to normalise arterial wall stress.[94] Wall stress increases in obesity because the persistently increased circulating volume causes an increased luminal diameter. This suggests that the increase in ccIMT in subjects with uncomplicated obesity may be reversible.

Despite the lack of between-group difference in mean ccIMT in the present study, I found a potentially different relationship between age and ccIMT in those with and without PCOS. Even lean women with PCOS appeared to have increased age-related thickening of the ccIMT than did controls. The results did not reach statistical significance and this could be due to the small sample size in each group. Since ccIMT was not associated with measures of insulin resistance it is unlikely that the basal hyperinsulinemia in PCOS is influencing the relationship. In contrast, adiponectin was a significant negative independent predictor of ccIMT and others have suggested that it is selectively reduced in PCOS diagnosed by NIH criteria regardless of BMI and insulin resistance.[193] I did not find lower adiponectin associated with PCOS in this study but it is tempting to speculate that reduced adiponectin may be a link between PCOS and accelerated ageing of the ccIMT.

Aortic stiffness (pulse wave velocity): My data confirm that young women with uncomplicated obesity have increased aortic PWV even in the absence of hypertension. Others have reported similarly increased PWV in obese young adults [194] and in obese children.[56] In my study central obesity (waist circumference) was an independent predictor of the variation in aortic PWV. These data are similar to the study of Wohlfahrt *et al.* who found that central obesity was a more important determinant of PWV than BMI in a large study of the general population.[195] I did not find stronger associations of PWV with visceral fat than with waist circumference as others have reported,[196] but the subjects in my study were considerably younger and it may be that obesity has different short- and long-term effects on arterial stiffness.

The mechanisms linking obesity to increased PWV are not well defined but may include insulin resistance. In healthy individuals an infusion of insulin within the physiological range decreases arterial stiffness with vessel tone altered via actions on endothelial nitric oxide synthesis. However, this does not appear to happen in those with obesity and insulin resistance, reflecting an impaired large conduit artery response to insulin.[197] Basal hyperinsulinemia also promotes vascular smooth muscle hyperplasia [198] and collagen production,[199] which may also contribute to increased arterial stiffness.

While insulin resistance was associated with PWV in my study, it was not an independent contributor to multivariate regression. This may explain why the relationship between arterial stiffness and age was very similar for lean women with and without PCOS, despite the latter having evidence of insulin resistance. It may be that the mechanism linking obesity and increased aortic PWV is not related to hyperinsulinemia.

A key finding of this study is that women with PCOS had similar aortic stiffness (PWV) to age- and BMI-matched controls.

This finding is comparable with other small studies of aortic PWV in young women with PCOS [88, 90, 91] but differs from Meyer *at al*. who found increased PWV in 100 overweight young women with PCOS compared with 20 overweight controls.[87] In the latter study, PCOS was diagnosed by NIH criteria and therefore all subjects had the more severe reproductive phenotype of hyperandrogenemia and anovulation; it may be that increased arterial stiffness is most evident in this phenotype which is associated with a constellation of cardiovascular risk factors including dyslipidemia and inflammation. I did not classify subjects by reproductive phenotype because I was interested in the effects of metabolic presentation on cardiovascular disease. While I have evidence of hyperandrogenemia in the obese PCOS group, I am unable to retrospectively identify who had anovulatory cycles.

I found that the relationship between age and PWV may be different for *obese* women with and without PCOS. According to my data, obese women with PCOS younger than ~30 years old may have higher PWV than BMI- and age-matched controls. However, the relationship with age was less steep and the lines crossed

such that older obese women with PCOS had increasingly lower PWV than comparable controls. The differences did not reach statistical significance and this is probably because of the small sample size in each group. Nevertheless, by including women across a wide age range in the study I may have unwittingly negated any difference between PCOS and controls.

It is unclear why obese control women tended to have a steeper rise in PWV with age than did obese women with PCOS. There was a weak negative association between HMW adiponectin and aortic PWV but the former was not an important predictor in multivariate analysis.

Left ventricular mass: The data from my study confirm that young women with obesity have increased LV mass compared with lean controls, even in the absence of hypertension. This finding is similar to other published studies in obese young women [200] and obese children.[61] In addition, I found that LV mass was more strongly associated with general obesity (BMI) than with central obesity. The Strong Heart Study also reported that LV mass was more strongly related to general obesity (particularly relative fat-free mass deficiency) than to central obesity and that this effect was particularly evident in women.[46] In contrast, data from the Multi-Ethnic Study of Atherosclerosis showed that insulin resistance and waist-hip ratio were associated with concentric LV remodelling after adjusting for BMI, suggesting that body size (haemodynamic load) and metabolic health may both be important contributors to the development of LV hypertrophy.[47] Therefore, it will be interesting to discover whether the quantification of ventricular-arterial coupling can provide additional information about the mechanisms liking uncomplicated obesity and increased left ventricular mass.

A key finding of this study is that women with PCOS had similar LV mass to age- and BMI-matched controls. However, obese women with PCOS had a significantly lower relative wall thickness and lower prevalence of LV hypertrophy compared with obese controls (2% vs. 28% respectively, $p<.001$, odds ratio of 18:1 for controls).

These findings differ from other studies which have reported a 10-40% higher LV mass in women with PCOS compared with age- and BMI-matched controls.[99, 100]

The difference in magnitude may be due to the inconsistent use of indexing methods. There is considerable debate in the literature regarding the best method of scaling echocardiographic dimensions and volumes for body size. I used the allometric scaling of LV mass by height$^{2.7}$. This has been shown to identify the pathophysiological increase in LV mass associated with obesity [168] and has been used in other studies of women with PCOS.[98, 99]

The studies reporting increased LV mass associated with obesity cited above used the NIH criteria for defining PCOS and it may be that only the most severe reproductive phenotype has evidence of increased LV mass beyond that associated with obesity. Like me, Kosmala *et al.* used the Rotterdam criteria and found that PCOS had no effect on LV mass in obese women compared with BMI matched controls.[98]

The lower relative wall thickness or lower rate of hypertrophy in PCOS did not appear to be explained by other differences in standard measures of cardiovascular function. This suggests that there may be elements of ventricular loading which are not fully described by the basic dataset.

Diastolic function: As expected, obesity was associated with impaired ventricular relaxation (a lower passive filling tissue velocity and higher atrial component to filling). These data are comparable with Wong *et al.* [201] and Peterson *et al.*[200] who studied subjects of a similar age with uncomplicated obesity and also found a reduction in e′ across categories of obesity. This finding has prognostic significance since tissue Doppler measurements, particularly the early diastolic velocity (e′), are significantly associated with outcome providing information that is independent from and incremental to clinical data even after adjustment for left ventricular hypertrophy.[202]

I found that visceral fat area was a stronger independent associate of diastolic function than was BMI. Libhaber *et al.* reported that waist circumference was an independent associate of E/A in a community sample with a high prevalence of obesity,[203] but this ratio of early to late diastolic flow is more sensitive to the changes in loading which are found obesity than is e′/a′. In addition, waist circumference includes lean mass and subcutaneous fat so is less specific a measure

of ectopic fat accumulation than visceral fat measured by CT or MRI. Data from the Baltimore Longitudinal Study of Ageing (n=843) measured visceral fat by CT and e′/a′ and also found that visceral fat was more strongly associated with diastolic function than other measures of obesity.[204]

A key finding of this study is that women with PCOS had similar diastolic function (e′/a′) to age- and BMI-matched controls.

My results are similar to those of Kosmala *et al.* [98] and Wang *et al.* [99] but differ from those of Erdogan [205] who found worse diastolic function in a small group of Turkish women with PCOS defined by Rotterdam criteria, and Orio *et al.* [100] who found worse diastolic function in a small group of women with PCOS defined by NIH criteria. Once again, discrepant results may be influenced by the reproductive phenotype and by the age of subjects.

The mechanistic links between insulin resistance or diabetes and diastolic dysfunction are multiple, complex and the subject of active research. Myocytes demonstrate reduced glucose uptake when glucose levels are higher in insulin resistant states leading to the theory that the heart, like skeletal muscle, becomes insulin resistant. There is evidence from animal models of intramyocardial effects such as altered calcium handling (reduced deactivation of cross-bridges), altered myocyte substrate metabolism, impaired bioenergetics, myocyte steatosis, pro-inflammatory and pro-fibrotic states, increased myocyte necrosis and apoptosis. In addition, hyperglycaemia can cause endothelial dysfunction, oxidative stress, altered collagen metabolism and ultimately increased artery stiffness which would lead to hypertrophy and altered diastolic function.[206]

Is there a specific phenotype of PCOS that is associated with hyperadiponectinaemia?

The finding of hyperadiponectinaemia in some lean women with PCOS was unexpected. This has not been reported by other studies and it is unclear whether I have unintentionally uncovered a phenotype with a compensatory response to the intrinsic insulin resistance associated with the syndrome. There was evidence of a weak negative association of adiponectin with ccIMT, aortic PWV and LV mass in

my data and adiponectin emerged as an independent predictor of ccIMT in multivariate analysis.

I would have liked to explore whether reproductive phenotype was relevant to adiponectin levels but the study was not designed to collect this data and it was not available retrospectively. It may be that women with PCOS who have normal length ovulatory cycles have higher levels of adiponectin. There is evidence that ~20% of women with PCOS defined by the Rotterdam criteria report a cycle length of fewer than 35 days and these do not have overt insulin resistance on oral glucose-tolerance testing.[207] It would be interesting to evaluate the HMW adiponectin in these women to determine whether the lack of overt insulin resistance is as a result of elevated levels of this insulin-sensitising adipokine.

Limitations

The main limitation of this study is that I did not adequately record details about reproductive phenotype. I was primarily concerned with the cardio-metabolic effects of PCOS and therefore focused my attention on comprehensive cardiovascular measure and detailed measures of obesity, as well basic measures of insulin resistance and androgen levels. At the time of study design, there was less evidence of the importance of reproductive phenotype on cardiovascular risk factors. It is clear from my data and from other publications since the inception of the study, that information relating to ovarian ultrasound, ovulation status and cycle length may have improved my understanding of the effects of PCOS on the cardiovascular system. Future studies should routinely record this information and analyse results by reproductive phenotype.

The case-control, observational nature of the study means that I cannot determine causal relationships between obesity, insulin resistance and cardiovascular function. Furthermore, there were complex relationships between key variables with many of them demonstrating inter-dependence. This rendered statistical analysis difficult and it may have been beneficial to approach a statistician to explore interaction effects particularly in respect of androgen levels and HMW adiponectin.

Given the lack of difference between fat distribution in women with PCOS and controls, I controlled for obesity by BMI. However, this does not take into account

the effects of aberrant adipocyte function which has been shown in women with PCOS.[183] Nevertheless, those with PCOS did not have significantly worse cardiovascular function than controls.

I did not analyse lean muscle mass in this study and this could have been considered to accurately assess insulin action since androgens lead to increased muscle mass and muscle is a major site of insulin-mediated glucose use.[54] Women with PCOS may have worse levels of insulin resistance than my data suggest if they have higher levels of lean muscle mass than controls. Furthermore, increases in LV mass and ccIMT may simply reflect increases in lean mass rather than pathologic effects of obesity and insulin resistance.

Echocardiographic measures of strain and strain rate were not among the primary outcome measures when the study was designed. I attempted to measure these retrospectively from stored data but this was not possible for all subjects and this limits the power to detect between-group differences in these variables. In addition, the high levels of obesity meant that the apical echo views were frequently not of sufficient quality to accurately measure ventricular volumes or perform the Simpson's biplane calculation of ejection fraction. Nevertheless, the parasternal views used to estimate LV mass were typically of sufficient quality and the myocardial velocity measures of systolic and diastolic function were feasible in the majority of subjects. These measures are well-validated and proven to add prognostic information to clinical data.

Conclusions

Young women with uncomplicated obesity had increased aortic stiffness (PWV), increased LV mass and worse diastolic function (e′/a′) even in the absence of hypertension or diabetes. There was a tendency for ccIMT to be increased in those who were obese but this did not reach statistical significance.

General obesity (weight) was more important than central obesity in the development of increased LV mass which suggests that haemodynamic factors related to whole body size are among the key mechanisms. This means that even those who are obese but metabolically healthy are likely to have increased LV mass.

In contrast, central obesity (waist circumference) was more important in the development of increased aortic stiffness, and visceral fat was more important in the development of impaired diastolic function. These findings suggest that those who are obese with abnormal glucose handling may be more likely to have altered arterial stiffness and diastolic dysfunction.

Stroke volume was more important than measures of obesity in explaining the variation in ccIMT which supports a theory put forward by others that this might represent vascular remodelling to reduce arterial wall stress associated with a persistently increased circulating volume. However, adiponectin was also an independent predictor suggesting that low adiponectin levels might also be a link between obesity and increased ccIMT, perhaps because of inflammation.

The trend for basal hyperinsulinemia and insulin resistance which is considered intrinsic to PCOS was not associated with significant subclinical cardiovascular dysfunction in lean women below age 45 years. Furthermore, the combination of PCOS and obesity did not have an additive effect on cardiovascular dysfunction beyond obesity alone. In fact, obese women with PCOS had a significantly *lower* LV relative wall thickness and lower prevalence of left ventricular hypertrophy than obese controls; there may be an as yet unappreciated element of PCOS which mitigates the effects of obesity on LV geometry.

The influence of PCOS reproductive phenotype on subclinical cardiovascular function requires further study. I found that lean women with PCOS had a trend towards higher adiponectin levels than controls and that obese women with PCOS had similar adiponectin levels as obese controls despite worse insulin resistance and similar levels of obesity. In addition, adiponectin was a significant univariate correlate (inverse) of ccIMT, aortic stiffness and LV mass. Furthermore, the relationship between obesity and adiponectin was significantly stronger in those with PCOS than in controls. Together, these findings suggest that there may be a phenotype of PCOS with hyperadiponectinaemia and that this might be among the mechanisms which mitigate the effects of obesity on the cardiovasculature in those with PCOS. This could have direct relevance to the lack of evidence for altered cardiovascular outcomes in women with PCOS despite increased risk factors.

It is clear that the measures of cardiovascular structure and function in this chapter are affected differently by general obesity and central obesity. However, it is less clear whether LV mass and impaired diastolic function occur mainly as a consequence of haemodynamic factors such as increased sympathetic nervous system activity and loading, or as a direct result of non-haemodynamic factors such as hyperinsulinemia. Subsequent chapters will focus on integrated measures of ventricular-arterial coupling to define the effects of obesity, insulin resistance and PCOS on static and pulsatile loading, in an effort to understand the pathophysiology.

4. Obesity, polycystic ovary syndrome and end-systolic elastance

Measurements of arterial and cardiac function are frequently considered in isolation but the interaction between the two is also an important determinant of effective cardiovascular performance.[102]

This interaction, called ventricular-arterial coupling, can be simply and non-invasively assessed using pressure-volume analysis derived from echocardiographic and blood pressure measurements. A detailed description of the development of this method is given in the general introduction. In essence, the method involves calculating the end-systolic elastance of the arteries and LV and expressing these as a ratio. Elastance is the reciprocal of compliance and considers how a given change in volume affects blood pressure. Higher values of elastance represent a stiffer system.

It is unclear whether non-invasive pressure-volume analysis is feasible in an obese, female population, or whether these methods are sufficiently sensitive to detect any subclinical cardiovascular dysfunction which may be associated with obesity and PCOS prior to the development of hypertension or clinical dysglycaemia.

Aims of this chapter

The primary aim of this chapter is to determine whether uncomplicated obesity and PCOS affect unscaled and scaled non-invasive measures of arterial elastance, ventricular end-systolic elastance, and the ratio of these measures (ventricular-arterial coupling).

The secondary aim is to establish whether central obesity and insulin resistance are more important contributors to arterial and ventricular elastance than general obesity.

The final aim is to explore potential mechanisms linking obesity and altered elastance by examining the components which determine these measures.

Hypotheses

In young women who are free from diabetes, hypertension and overt cardiovascular disease

1. obesity will be associated with increased arterial elastance and a matched increase in ventricular end-systolic elastance so that the coupling ratio will be similar to lean controls, but at the expense of elevated systolic blood pressure;
2. visceral fat area and/or insulin resistance will independently explain more of the variation in arterial or ventricular end-systolic elastance than will BMI
3. women with PCOS will have similar arterial and ventricular elastance to BMI- and age-matched controls; and
4. increases in arterial and ventricular elastance will be predominantly driven by the increased circulating volume and sympathetic nervous system activity associated with obesity, rather than intrinsic structural changes.

Specific methods

Women with PCOS and control volunteers were recruited from local clinics and the general community. Full details of recruitment, exclusion criteria and general methods are described in Chapter 2.

Single beat method of estimating ventricular end-systolic elastance

Ventricular end-systolic elastance (E_{es}) was calculated according to the single-beat method described by Chen as detailed in Chapter 1.[121] Briefly, the calculation is as follows

$$E_{es} = \frac{P_d - (E_{ND} \times P_{es})}{E_{ND} \times (V_d - V_{es})}$$

where P_d is the brachial diastolic blood pressure which is often similar to the central diastolic pressure, and P_{es} is the estimated central systolic blood pressure given by

$$P_{es} = 0.9 \times brachial\ systolic\ blood\ pressure$$

$V_d - V_{es}$ is the stroke volume of the LV which is measured by echocardiography using the Doppler method, given by

$$Stroke\ volume = LVOT\ CSA \times LV\ vti$$

where LVOT CSA is the cross sectional area of the LV outflow tract found by

$$0.785 \times LVOT\ diameter^2 \qquad \text{(Figure 4-1)}$$

LV vti is the velocity time integral of flow in the LV outflow tract (Figure 4-2).

Finally, the normalised elastance, $E_{ND,}$ is found by

$$E_{ND} = 0.0275 - (0.165 \times EF) + (0.3656 \times Pd/Pes) + (0.515 \times E_{ND}AVG)$$

where ejection fraction (EF) is derived using the Teicholz formula below to estimate ventricular volumes from internal dimensions measured by m-mode echocardiography (Figure 4-3).

$$LVEDV = \left[\frac{7}{2.4 + LVIDd}\right] \times LVIDd^3$$

$$LVESV = \left[\frac{7}{2.4 + LVIDs}\right] \times LVIDs^3$$

$$EF = \frac{LVEDV - LVESV}{LVEDV}$$

E_{ND}AVG is an empirically derived polynomial function found by

$$ENDAVG = 0.35695 + (-7.2266 \times T_{ND}) + (74.249 \times {T_{ND}}^2) + (-307.39 \times {T_{ND}}^3) + (684.54 \times {T_{ND}}^4) + (-856.92 \times {T_{ND}}^5) + (571.95 \times {T_{ND}}^6) + (-159.1 \times {T_{ND}}^7)$$

where $T_{ND} = \frac{Time\ from\ R\ wave\ to\ AV\ opening}{Time\ from\ R\ wave\ to\ AV\ closure}$

Arterial elastance

Arterial elastance (E_a) was calculated by

$$E_a = \frac{P_{es}}{SV}$$

Normalisation of elastances for body size

E_a and E_{es} were scaled allometrically for body surface area (BSA) following the method of Chirinos *et al*. [122]

$$E_aI = \frac{E_a}{BSA^{-1.18}} \qquad E_{es}I = \frac{E_{es}}{BSA^{-1.05}}$$

$$BSA\ (Gehan\ method) = 0.0235 \times height\ (cm)^{0.42246} \times weight\ (kg)^{0.51456}$$

Coupling ratio

The ratio of ventricular-arterial coupling (VAC) was found by

$$VAC = \frac{E_a}{E_{es}}$$

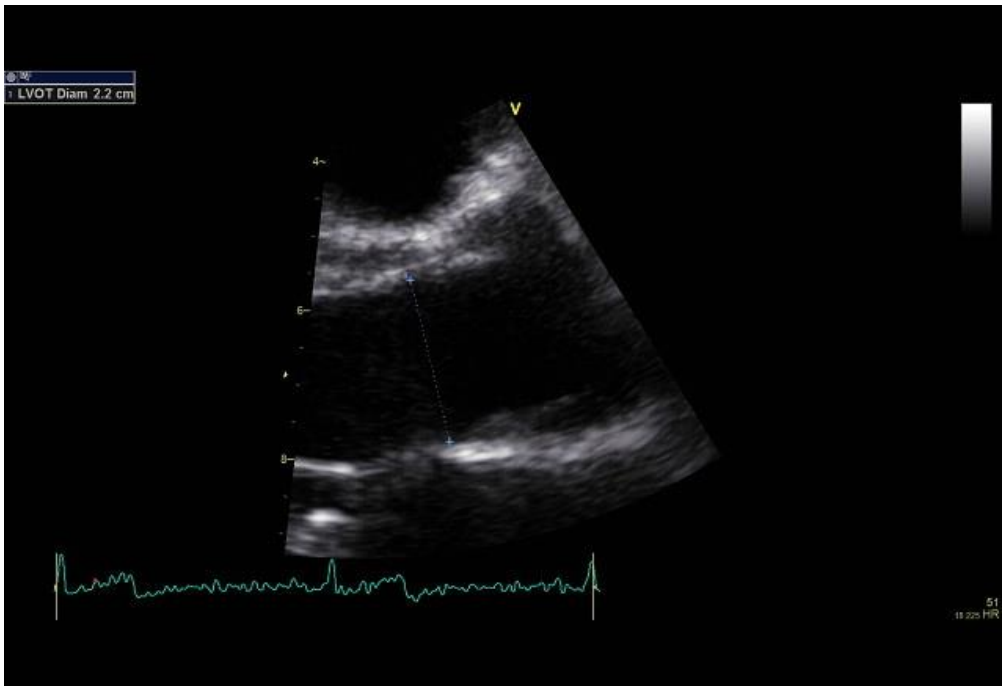

Figure 4-1. Two-dimensional image demonstrating measurement of left ventricular outflow tract diameter in mid-systole (used in the calculation of stroke volume).

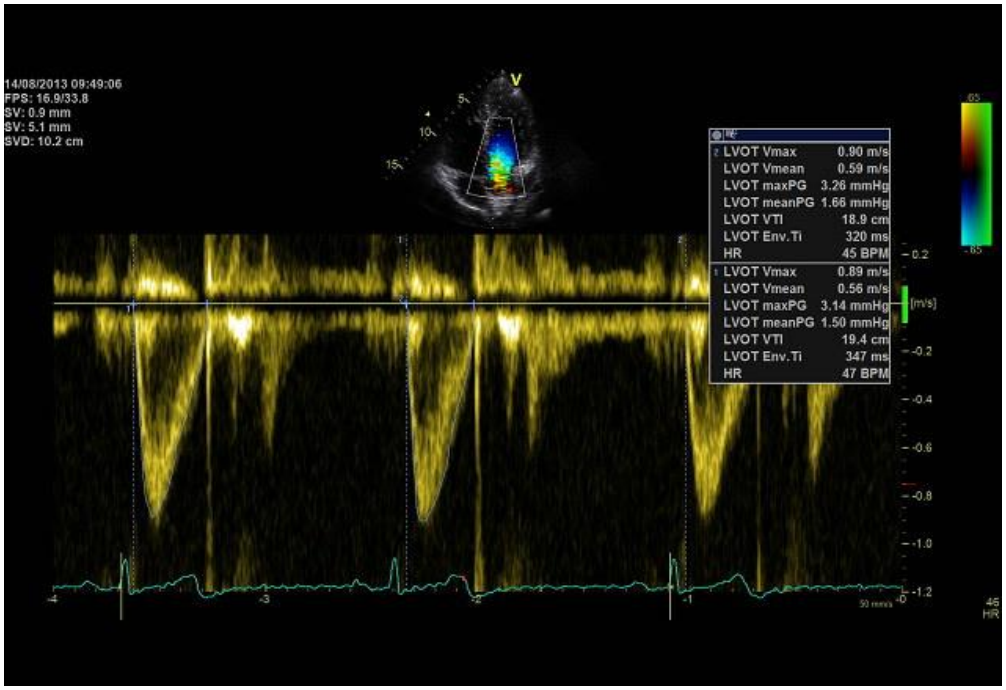

Figure 4-2. Spectral Doppler recording demonstrating measurement of left ventricular outflow tract velocity-time integral (used in the calculation of stroke volume).

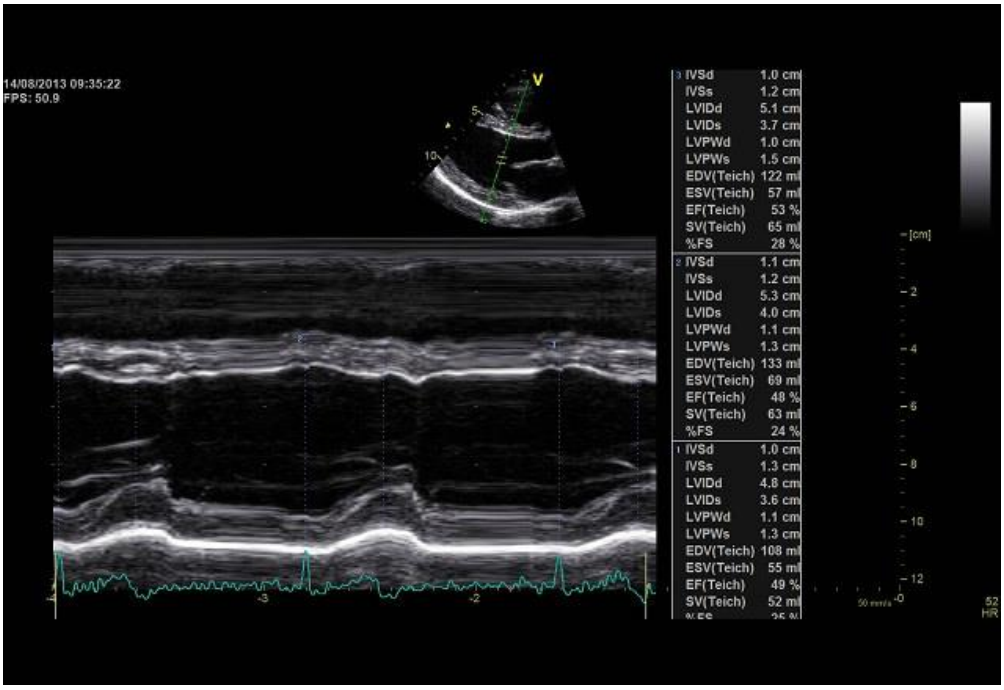

Figure 4-3. M-mode recording demonstrating measurement of left ventricular wall thickness and chamber dimensions used in the calculation of mass, fractional shortening and ejection fraction (Teicholz method).

Reproducibility of elastance methods

Reproducibility of elastance methods was established prior to the study by inviting six healthy volunteers to attend for testing on two occasions, under similar conditions, separated by at least 10 weeks. Subjects were aged 23-49 years (5 male) with BMI 20-27. The coefficient of variation (CV) was used to quantify the intra-observer inter-session reproducibility according to the formula

$$CV\ (\%)\ = \frac{S.D.of\ within\ subject\ differences}{Group\ mean\ of\ measurements}$$

The intra-observer inter-session CV was 11 % for E_a and 16% for $E_{es,}$ which is similar to other published studies (detailed in Chapter 1).

Systemic arterial compliance

Systemic arterial compliance was used as a surrogate of pulsatile afterload and was found by

$$SAC = \frac{Stroke\ volume}{Systolic\ BP - Diastolic\ BP}$$

Systemic vascular resistance

Systemic vascular resistance (SVR) was used as a measure of static afterload and was estimated by

$$SVR = \frac{MAP}{C.O.}$$

where mean arterial pressure (MAP) was found by

$$MAP = \frac{2.bDBP + bSBP}{3}$$

and cardiac output (C.O.) was calculated using the Doppler method. The result was converted into Dynes/s/cm^5 by multiplying by 80.

Results

Feasibility of elastance calculations

Measurement of arterial elastance was feasible in 97% of subjects and depended on whether the left ventricular outflow tract could be measured. There was no difference in feasibility between lean and obese subjects.

Ventricular elastance was obtained in 95% of lean subjects and 88% of obese subjects. Feasibility was limited by the ability to accurately measure left ventricular dimensions in the parasternal long-axis view (Teicholz method).

Calculation of ventricular-arterial coupling was therefore feasible in 94% of lean and 88% of obese subjects.

Unscaled elastances and the coupling ratio

Table 4-1 shows the *unscaled* values of arterial elastance, ventricular elastance and coupling ratio by group. There were no significant between-group differences.

Elastances scaled for body size

Table 4-2 shows the mean arterial and ventricular elastance after allometric scaling for the normal relationship with body surface area. In control subjects, obesity resulted in higher scaled arterial and ventricular end-systolic elastance but increases were matched so that there was no significant effect on ventricular-arterial coupling (Figure 4-4).

In women with PCOS, obesity resulted in a higher scaled arterial elastance but a non-significant trend for increased ventricular elastance. This appeared to be because lean women with PCOS had marginally higher ventricular elastance than lean controls. As a result, women with PCOS tended to have lower ventricular-arterial coupling ratios than controls but this difference was not statistically significant. The combination of obesity and PCOS did not have an additive effect on arterial or ventricular elastance.

Table 4-1. Arterial elastance, ventricular end-systolic elastance and ventricular arterial coupling (mean ± s.d.) by subject group.

	Lean Control	**Lean PCOS**	**Obese Control**	**Obese PCOS**	**ANOVA p-value**
E_a (mmHg/ml)*	1.65 ± 0.27	1.61 ± 0.34	1.65 ± 0.34	1.57 ± 0.29	.533
E_{es} (mmHg/ml)*	1.49 ± 0.35	1.55 ± 0.37	1.45 ± 0.32	1.49 ± 0.34	.873
VAC (E_a/E_{es})	1.15 ± 0.23	1.07 ± 0.16	1.16 ± 0.26	1.08 ± 0.20	.412

*E_a: Arterial end-systolic elastance, E_{es}: Ventricular end-systolic elastance, VAC: Ventricular-arterial coupling. *Variables were transformed with natural logarithm prior to use in ANOVA.*

Table 4-2. Scaled arterial and ventricular end-systolic elastance (mean ± s.d.) by subject group.

	Lean Control	**Lean PCOS**	**Obese Control**	**Obese PCOS**	**ANOVA P-value**
E_aI (mmHg/ml/$m^{2.4}$)*	3.03 ± 0.43	3.06 ± 0.53	3.80 ± 0.81	3.75 ± 0.86	**<.0001**
$E_{es}I$ (mmHg/ml/$m^{2.1}$)*	2.55 ± 0.55	2.69 ± 0.49	3.03 ± 0.74	3.18 ± 0.81	**<.0001**

*E_aI: Allometrically scaled arterial end-systolic elastance, EesI: Allometrically scaled ventricular end-systolic elastance. *Variables were transformed with natural logarithm prior to use in ANOVA.*

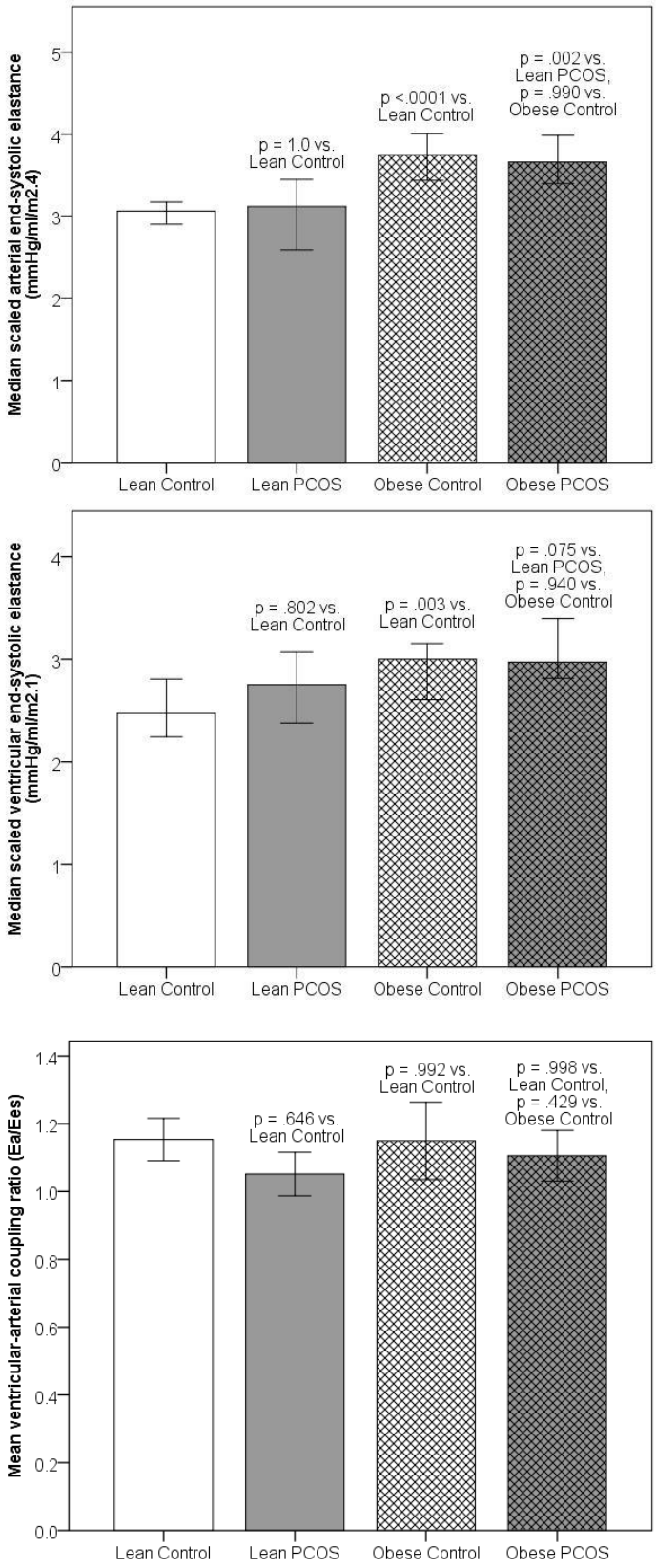

Figure 4-4. Median scaled arterial elastance, ventricular elastance and mean ventricular-arterial coupling ratio by subject group. Error bars show 95% confidence intervals.

Table 4-3. Univariate correlates [Spearman's rho (p-value)] of arterial elastance, LV end-systolic elastance and ventricular-arterial coupling.

	Arterial elastance, E_a		**Scaled arterial elastance, E_aI**		**Ventricular end-systolic elastance, E_{es}**		**Scaled ventricular end-systolic elastance, $E_{es}I$**		**Ventricular-arterial coupling, E_a/E_{es}**	
Age	−.15	(.079)	.03	(.767)	−.04	(.632)	.07	(.431)	−.09	(.285)
BMI	−.11	(.182)	**.54**	**(<.0001)**	−.04	(.629)	**.38**	**(<.0001)**	−.05	(.531)
Waist	−.09	(.282)	**.56**	**(<.0001)**	<.01	(.987)	**.42**	**(<.0001)**	−.09	(.276)
Waist/hip	.13	(.116)	**.35**	**(<.0001)**	.12	(.170)	**.26**	**(.002)**	−.05	(.573)
TFA	−.09	(.298)	**.52**	**(<.0001)**	−.05	(.558)	**.34**	**(<.0001)**	<.01	(.952)
SFA	−.09	(.286)	**.50**	**(<.0001)**	−.06	(.526)	**.32**	**(<.0001)**	<.01	(.975)
VFA	−.08	(.364)	**.47**	**(<.0001)**	−.02	(.860)	**.37**	**(<.0001)**	−.05	(.540)
Glucose	**−.16**	**(.050)**	.05	(.564)	−.06	(.495)	**.18**	**(.038)**	**−.20**	**(.019)**
Gluc AUC	.05	(.558)	**.18**	**(.032)**	**.26**	**(.003)**	**.33**	**(<.0001)**	**−.27**	**(.001)**
Insulin	.04	(.625)	**.43**	**(<.0001)**	.14	(.100)	**.40**	**(<.0001)**	−.13	(.131)
Ins AUC	**.18**	**(.036)**	**.41**	**(<.0001)**	**.31**	**(.001)**	**.45**	**(<.0001)**	**−.18**	**(.045)**
HOMA-IR	<.01	(.966)	**.40**	**(<.0001)**	.14	(.112)	**.41**	**(<.0001)**	−.16	(.058)
hs-CRP	.08	(.338)	**.39**	**(<.0001)**	.04	(.634)	**.24**	**(.004)**	.02	(.806)
Testosterone	.03	(.757)	.12	(.174)	.11	(.230)	**.20**	**(.018)**	−.13	(.129)
Adiponectin	**−.18**	**(.029)**	**−.40**	**(<.0001)**	.02	(.860)	**−.18**	**(.034)**	**−.20**	**(.025)**
Cholesterol	.10	(.219)	.16	(.056)	.01	(.895)	.09	(.286)	.13	(.131)
HDL	.03	(.736)	**−.38**	**(<.0001)**	<.01	(.975)	**−.20**	**(.018)**	−.06	(.464)
LDL	.12	(.157)	**.26**	**(.002)**	−.02	(.835)	.13	(.146)	**.18**	**(.043)**
Triglycerides	−.01	(.951)	**.23**	**(.006)**	<.01	(.931)	**.17**	**(.048)**	.04	(.615)

TFA: total fat area, SFA: subcutaneous fat area, VFA: visceral fat area, AUC: Area under the curve (during oral glucose tolerance test), HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high sensitivity c-reactive protein, HDL: high-density lipoproteins, LDL: low-density lipoproteins.

Associations of elastance measurements with cardiovascular risk factors

Table 4-3 shows univariate correlates of unscaled and scaled elastances and the dimensionless ratio of ventricular-arterial coupling. None of the measures was significantly associated with age in this young population.

Unscaled elastances: There were few significant associations of cardiovascular risk factors with unscaled elastance measures. Unscaled arterial elastance was inversely associated with fasting glucose and adiponectin, and directly associated with insulin area under the curve. Unscaled ventricular elastance was directly associated with glucose and insulin area under the curve.

Scaled elastances: Scaled measures of elastance were associated with many of the cardiovascular risk factors in this study including direct associations with all measures of obesity and many of the measures of glucose handling. The strongest associations were with waist circumference and insulin area under the curve. In addition, scaled arterial and ventricular elastance were inversely associated with adiponectin and HDL and directly associated with hs-CRP and triglycerides. Interestingly, there was a weak direct association of scaled ventricular elastance with testosterone and this may account for the trend towards higher values (and lower coupling ratio) in women with PCOS.

Ventricular-arterial coupling ratio: There were few significant associations with the ventricular-arterial coupling ratio. Fasting glucose, glucose area under the curve and insulin area under the curve were all inversely associated with the coupling ratio. This suggests that only obesity associated with significant dysglycaemia will result in altered ventricular-arterial coupling. In addition, there was an inverse association of coupling with adiponectin and a direct association with low-density lipoproteins.

Relationship of arterial and ventricular end-systolic elastance

There was a linear relationship between arterial and ventricular end-systolic elastance (regardless of whether the variables were scaled) (Figure 4-5). Arterial elastance accounted for 34% of the variation in ventricular end-systolic elastance.

Given this finding I repeated the testing for univariate associations of risk factors with scaled ventricular elastance, adding an adjustment for scaled arterial elastance (Table 4-4). The relationships with obesity became weaker, with the association of visceral fat changing the least. The positive association of glucose with ventricular elastance remained largely unchanged suggesting that this relationship is not significantly driven by an effect on arterial elastance.

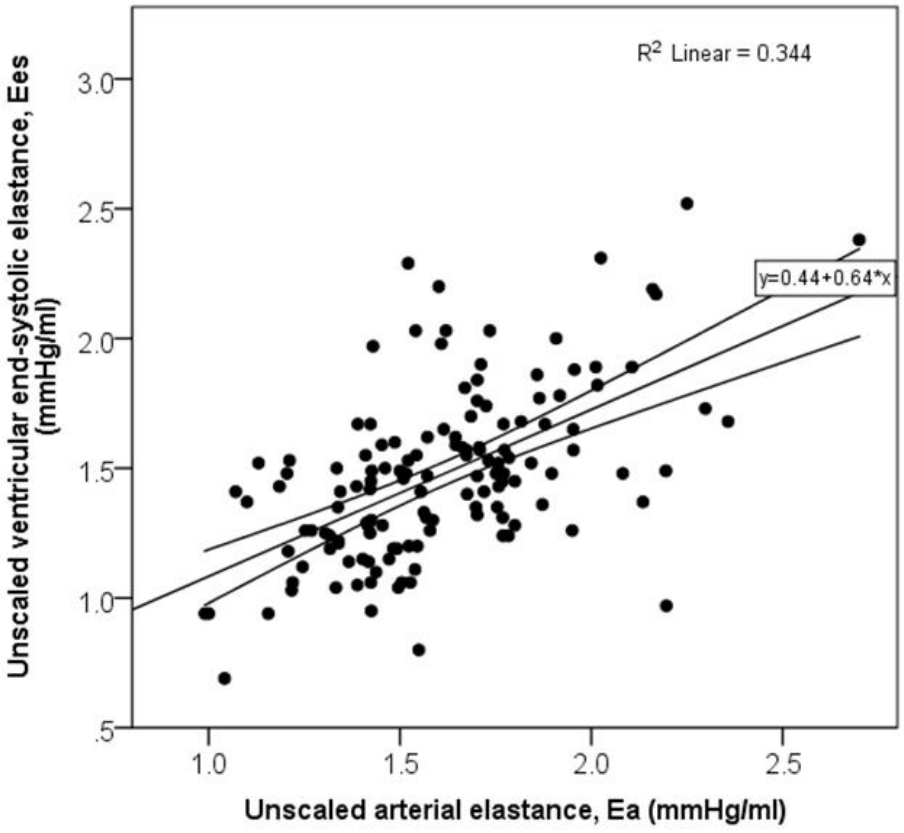

Figure 4-5. Relationship between unscaled arterial and ventricular end-systolic elastance.

Table 4-4. Univariate correlates [Pearson's R (p-value)] of allometrically scaled LV end-systolic elastance after adjustment for scaled arterial elastance.

	Ventricular end-systolic elastance, $E_{es}I$
Age*	.17 (.059)
BMI*	.14 (.128)
Waist*	**.19 (.037)**
Waist/hip*	.11 (.241)
TFA*	.12 (.195)
SFA	.09 (.311)
VFA*	**.20 (.021)**
Glucose*	.16 (.100)
Gluc AUC	**.29 (.003)**
Insulin*	.11 (.257)
Ins AUC*	**.25 (.010)**
HOMA-IR*	.13 (.195)
hs-CRP*	.03 (.728)
Testosterone*	.13 (.189)
Adiponectin*	.07 (.448)
Cholesterol	−.18 (.065)
HDL*	−.02 (.866)
LDL	−.16 (.098)
Triglycerides*	−.02 (.816)

*TFA: total fat area, SFA: subcutaneous fat area, VFA: visceral fat area, AUC: Area under the curve (during oral glucose tolerance test), HOMA-IR: homeostasis model assessment of insulin resistance, hs-CRP: high sensitivity c-reactive protein, HDL: high-density lipoproteins, LDL: low-density lipoproteins. *Variables were transformed using the natural logarithm before use in partial correlation.*

Table 4-5. Univariate echocardiographic and tonometric correlates [Spearman's Rho (p-value)] of unscaled and scaled elastances.

	Unscaled arterial elastance, E_a	**Scaled arterial elastance, E_aI**	**Unscaled ventricular elastance, E_{es}**	**Scaled ventricular elastance, $E_{es}I$**	**Ratio of arterial to ventricular elastance, E_a/E_{es}**
Heart rate	**.24 (.003)**	**.36 (<.0001)**	**.20 (.016)**	**.27 (.001)**	.02 (.852)
SVR	**.67 (<.0001)**	**.38 (<.0001)**	**.45 (<.0001)**	**.29 (.001)**	.11 (.194)
SAC	**−.70 (<.0001)**	**−.49 (<.0001)**	**−.33 (<.0001)**	**−.21 (.013)**	**−.32 (<.0001)**
ccIMT	−.15 (.064)	.07 (.372)	−.11 (.187)	.03 (.737)	−.01 (.935)
AIx-75	.03 (.743)	.13 (.115)	.15 (.086)	**.21 (.016)**	−.17 (.053)
aPWV	.04 (.599)	**.32 (<.0001)**	.07 (.405)	**.29 (.001)**	−.03 (.690)
RWT	**.22 (.010)**	**.35 (<.0001)**	**.15 (.071)**	**.27 (.001)**	**.01 (.866)**
LV mass	**−.26 (.002)**	**.22 (.009)**	**−.36 (<.0001)**	−.01 (.873)	**.18 (.040)**
FS	−.16 (.052)	.02 (.775)	**.32 (<.0001)**	**.41 (<.0001)**	**−.53 (<.0001)**
s′	.01 (.902)	.09 (.300)	**.21 (.024)**	**.29 (.002)**	**−.30 (.001)**
MAPSE	**−.35 (<.0001)**	−.10 (.239)	−.06 (.496)	.12 (.163)	**−.35 (<.0001)**
e′	−.05 (.581)	**-.25 (.006)**	<.01 (.969)	−.14 (.140)	−.08 (.382)
a′	**−.19 (.035)**	.06 (.507)	.14 (.131)	**.34 (<.0001)**	**−.37 (<.0001)**
e′/a′	.11 (.233)	−.17 (.058)	−.09 (.318)	**−.29 (.002)**	**.20 (.037)**
FPV	.08 (.397)	.17 (.073)	.03 (.740)	.12 (.219)	.01 (.940)
IVRT	<.01 (.934)	.11 (.198)	**−.23 (.008)**	−.10 (.246)	**.21 (.018)**

SVR: systemic vascular resistance, SAC systemic arterial compliance, ccIMT: common carotid intima-media thickness, AIx-75bpm: augmentation index corrected for heart rate of 75bpm, aPWV: aortic pulse wave velocity, RWT: left ventricular relative wall thickness, FS: fractional shortening, MAPSE: mitral annular plane systolic excursion, FPV: flow propagation velocity, IVRT: isovolumic relaxation time.

Relationship of elastance with other measures of cardiovascular structure and function

Table 4-5 shows the association of elastances with heart rate, measures of systemic vascular resistance and compliance, and echocardiographic and tonometric measures of structure and function.

Unscaled elastances: unscaled arterial and ventricular elastance was associated with heart rate, systemic vascular resistance, LV mass (all direct) and systemic arterial compliance (inverse). In addition, unscaled arterial resistance was associated with the mitral annular plane systolic excursion and the late diastolic tissue velocity (both inverse), brachial but not aortic pulse wave velocity. Unscaled ventricular elastance was associated with fractional shortening and the systolic myocardial velocity (direct) as well as the isovolumic relaxation time (inverse). The strongest associations of arterial elastance were systemic vascular resistance and arterial compliance. The strongest association of ventricular elastance was systemic vascular resistance. There were no associations of unscaled ventricular elastance with global strain and strain rate (data not presented).

Scaled elastances: The associations of elastance measures with heart rate, systemic vascular resistance and arterial compliance remained significant after scaling, as did the association of arterial elastance with LV mass and relative wall thickness. In contrast, the association of ventricular elastance with LV mass was no longer evident after scaling, but the association with relative wall thickness increased in strength. The associations of ventricular elastance with measures of contractile function remained after scaling but new associations emerged with the late diastolic tissue velocity (direct) and the ratio of early to late diastolic tissue velocities (inverse).

Ventricular-arterial coupling: ventricular-arterial coupling was negatively associated with systemic arterial compliance, LV mass (but not relative wall thickness), measures of contractile function, the late diastolic tissue velocity and the ratio of early to late diastolic tissue velocities.

Independent contributors of scaled arterial elastance

Arterial elastance has been proposed as a composite measure of afterload which integrates arterial stiffness. I tested whether arterial elastance could be explained by a basic model that included heart rate, systemic vascular resistance (a measure of resistive load), systemic arterial compliance (a basic measure of pulsatile load) and aortic pulse wave velocity (a measure of regional arterial stiffness). Table 4-6 shows the results of the basic model; systemic vascular resistance and heart rate contributed most towards the variation in arterial elastance. Aortic stiffness (PWV) and pulsatile load (systemic arterial compliance) were also independent contributors but explained less of the variation in arterial elastance.

Separate hierarchical multivariate regression models were used to determine whether adding measures of general and central obesity resulted in an improved ability to explain arterial elastance (Table 4-7). The addition of waist circumference resulted in a significant improvement in the model with this measure of central obesity independently explaining a further 33% of the variation in arterial elastance. In separate models, general obesity (BMI) resulted in a similar but a marginally smaller improvement (31%), while visceral fat area and insulin resistance each explained to 19% and 13% of the variation in arterial elastance.

Table 4-8 shows the final multivariate regression model for scaled arterial resistance. The model was able to explain 84% of the variation in arterial elastance. The inclusion of waist circumference in the model supplanted the effect of aortic pulse wave velocity.

Table 4-6. Initial stepwise multiple regression model for scaled arterial elastance (E_aI).

*Dependent variable: E_aI**	**Standardised β**	**P-value**	**Part correlations**
Systemic vascular resistance	.410	**<.0001**	.329
Heart rate	.382	**<.0001**	.314
Aortic PWV *	.276	**<.0001**	.264
Systemic arterial compliance	−.260	**.001**	−.214
Model r^2 = .505, p<.0001			

*PWV: pulse wave velocity. *Variables were log-transformed prior to use in inferential statistics.*

Table 4-7. Separate hierarchical multiple regression models for scaled arterial elastance (E_aI) and measures of obesity.

*Dependent variable: E_aI**	**Change in r^2**	**Cumulative r^2**	**F change statistic**	**P-value for model**
Basic model		.505		<.0001
Basic model + BMI*	.313	.818	**F (1, 131) = 225.14, p<.0001**	<.0001
Basic model + waist circumference	.334	.839	**F (1, 130) = 272.93, p<.0001**	<.0001
Basic model + visceral fat area*	.187	.692	**F (1, 123) = 77.41, p<.0001**	<.0001
Basic model + HOMA-IR	.127	.632	**F (1, 131) = 37.21, p<.0001**	<.0001

*HOMA-IR: homeostasis model of assessment for insulin resistance. *Variables were log-transformed prior to use in inferential statistics.*

Table 4-8. Final multiple regression model for scaled arterial elastance (E_aI).

*Dependent variable: E_aI**	**Standardised β**	**P-value**	**Part correlations**
Waist circumference*	.681	**<.0001**	.578
Systemic vascular resistance	.482	**<.0001**	.385
Systemic arterial compliance	-.340	**<.0001**	-.277
Heart rate	.292	**<.0001**	.238
Aortic PWV *	-.043	.304	-.036
Model r^2 = .841, p<.0001			

*PWV: pulse wave velocity. *Variables were log-transformed prior to use in inferential statistics.*

Independent contributors of scaled ventricular end-systolic elastance

Ventricular end-systolic elastance reflects systolic stiffness which, in steady states, is affected by ventricular geometry, diastolic stiffness, biochemical properties and contractile function. Multiple regression was used to test whether ventricular elastance could be explained by a model that included fractional shortening (a measure of circumferential contractile function), heart rate, systemic vascular resistance, systemic arterial compliance, relative wall thickness and a′ (a measure of late diastolic function). The stepwise model identified that, from these variables, fractional shortening, systemic vascular resistance, heart rate and late diastolic function were the largest independent contributors of scaled ventricular elastance, together accounting for 64% of its variation (Table 4-9). Systemic arterial compliance and LV geometry were not independent predictors of ventricular systolic stiffness in this population.

Table 4-10 shows the results of multivariate regression analysis when systemic vascular resistance and heart rate were replaced by scaled arterial elastance. The use of this composite measure of afterload resulted in a small but significant improvement in the model, explaining 3% more of the variation in ventricular systolic elastance.

Separate hierarchical multivariate regression models were used to test whether adding measures of obesity or hyperinsulinaemia to the basic model resulted in a significant improvement (Table 4-11). The addition of waist circumference resulted in a small but significant improvement, explaining 3% more of the variation in ventricular elastance. The use of BMI or visceral fat resulted in smaller or non-significant changes. Insulin resistance had no effect on the model.

The final model is shown in Table 4-12 and explained 70% of the variation in scaled ventricular elastance. Waist circumference had a *negative* independent association with ventricular elastance after adjustment for arterial elastance, fractional shortening and late diastolic function. This contrasts with the positive univariate association.

Table 4-9. Initial stepwise multiple regression model for scaled ventricular elastance ($E_{es}I$).

Dependent variable: $E_{es}I$	**Standardised β**	**P-value**	**Part correlations**
Fractional shortening	.520	**<.0001**	.478
Systemic vascular resistance	.605	**<.0001**	.316
Heart rate	.446	**<.0001**	.292
a′	.190	**.005**	.342
Model r^2 = .639, p<.0001			

**Variables were transformed using the natural logarithm prior to use in inferential statistics.*

Table 4-10. *S*tepwise multiple regression model for scaled ventricular elastance ($E_{es}I$) using scaled arterial elastance in place of heart rate and systemic vascular resistance.

Dependent variable: $E_{es}I$	**Standardised β**	**P-value**	**Part correlations**
E_aI*	.673	**<.0001**	.636
Fractional shortening	.370	**<.0001**	.343
a′	.175	**.003**	.163
Model r^2 = .672, p<.0001			

*E_aI: scaled arterial elastance. *Variables were transformed using the natural logarithm prior to use in inferential statistics.*

Table 4-11. Hierarchical separate multiple regression models for scaled ventricular elastance ($E_{es}I$).

Dependent variable: $E_{es}I$	**Change in r^2**	**Cumulative r^2**	**F change statistic**	**P-value for model**
Basic model		.672		<.0001
Basic model + BMI*	.015	.687	**F (1, 110) = 5.41, p=.022**	<.0001
Basic model + visceral fat area*	.009	.681	F (1, 103) = 2.63, p=.108	<.0001
Basic model + waist circumference*	.026	.698	**F (1, 110) = 9.45, p=.003**	<.0001

**Variables were transformed using the natural logarithm prior to use in inferential statistics.*

Table 4-12. Final multiple regression model for scaled ventricular elastance ($E_{es}I$).

Dependent variable: $E_{es}I$	**Standardised β**	**P-value**	**Part correlations**
E_aI*	.746	**<.0001**	.618
Fractional shortening	.421	**<.0001**	.375
a′	.259	**<.0001**	.217
Waist circumference*	−.229	**.003**	−.161
Model r^2= .698, p<.0001			

*E_aI: scaled arterial elastance. *Variables were transformed using the natural logarithm prior to use in inferential statistics.*

Independent contributors of ventricular-arterial coupling

Measures of obesity were not associated with ventricular-arterial coupling in this study but there were significant negative univariate correlations with other cardiovascular risk factors (see Table 4-3). Hierarchical multivariate regression was used to test which factors were the most important independent determinants of ventricular-arterial coupling.

The first block of the model included the variables glucose AUC, adiponectin, insulin AUC and LDL; these were inserted using the stepwise method. The second block of the model included measures of cardiovascular function. These were fractional shortening, MAPSE, s′, a′ and IVRT (Table 4-13).

Ventricular-arterial coupling is inversely related to contractile function and the final model (Table 4-14) confirmed inverse independent contributions of fractional shortening and mitral annular plane systolic excursion. Increased LV mass is a consequence of increased afterload (and therefore a higher coupling ratio). A direct association of LV mass and ventricular-arterial coupling was evident in this population. Finally, dysglycaemia, reflected by glucose AUC, independently explained some of variation in ventricular-arterial coupling. The inverse association between glucose AUC and the coupling ratio (E_a/E_{es}) suggests that dysglycaemia may affect the ventricular elastance more than arterial elastance.

Table 4-13. Hierarchical multivariate regression for ventricular-arterial coupling.

Dependent variable: VAC	**Change in r^2**	**Cumulative r^2**	**F change statistic**	**P-value for model**
Fractional shortening		.399		<.0001
LDL	.033	.431	**F (1, 84) = 4.80, p=.031**	<.0001
Glucose AUC*	.036	.468	**F (1, 83) = 5.69, p=.019**	<.0001
MAPSE	.057	.525	**F (1, 82) = 9.82, p=.002**	<.0001
LV mass*	.071	.595	**F (1, 81) = 14.20, p<.0001**	<.0001

*LDL: low-density lipoproteins, AUC: area under the curve, MAPSE: mitral annular plane systolic excursion. *Variables were transformed using the natural logarithm prior to use in inferential statistics.*

Table 4-14. Final multivariate regression model for ventricular-arterial coupling.

Independent variables	**Standardised β**	**P-value**	**Part correlation**
Fractional shortening	−.485	**<.0001**	−.439
MAPSE	−.292	**<.0001**	−.277
LV mass*	.274	**<.0001**	.266
Glucose AUC*	−.250	**.002**	−.228
LDL	.126	.085	.123
	Model r^2= .595, p<.0001		

*LDL: low-density lipoproteins, AUC: area under the curve, MAPSE: mitral annular plane systolic excursion. *Variables were transformed using the natural logarithm prior to use in inferential statistics*

Discussion

Feasibility

According to published data, non-invasive measurement of arterial and ventricular elastance is feasible in 56-95% of subjects depending on the characteristics of the sample population and whether there was a prospective aim to collect this data.[115, 116, 122, 125-127] My study was designed to look for sub-clinical dysfunction in subjects who were likely to be obese and I chose not to exclude subjects with poor acoustic views since this would have introduced selection bias. Nevertheless, it was feasible to derive paired arterial and ventricular elastance in 94% of lean and 88% of obese female subjects.

The feasibility of arterial elastance was dependent on whether the left ventricular outflow tract could be adequately visualised to measure its diameter. Similarly, the feasibility of the ventricular elastance calculation was dependent on whether the LV cavity could be adequately visualised to measure dimensions. This method is considered to be less accurate at deriving left ventricular volumes and ejection fraction than is the Simpson's Biplane method but the latter requires excellent apical acoustic windows. If I had chosen to use the latter method the feasibility would have been reduced to 64% because the obese women in this study frequently had poor apical acoustic windows.

My data confirm that non-invasive assessment of arterial and ventricular elastance is feasible in the majority of young, obese women.

Elastance values in healthy subjects

Published unscaled elastance values in healthy subjects are 1.3-1.43 mmHg/ml for arterial elastance and 1.4-2.2 mmHg/ml for ventricular elastance, depending on the age and characteristics of the study population and the methods used to derive these measures.[116, 122, 124, 127, 160, 208]

The study of Chirinos *et al*. had a large sample size (n=612 in reference group) and a population with characteristics similar to subjects in this thesis, but they found a lower median arterial elastance than in my study (1.43 vs. 1.67 mmHg/ml for

unscaled arterial elastance and 2.89 vs. 3.10 mmHg/ml/$m^{2.4}$ for scaled arterial elastance).[122] It is possible that the higher values in this thesis may be influenced by the all-female population. There is evidence that women have higher arterial elastance than men due to increased heart rate and pulsatile arterial load.[124] However, between-study differences may also be related to different methods of estimating central end-systolic pressure rather than to true differences in elastance. While Chirinos *et al.* derived central end-systolic blood pressure from arterial tonometry, I used the equation 0.9*brachial cuff systolic pressure because it was used in the key validation paper describing the single-beat method.[121] This may have resulted in an overestimate of central end-systolic pressure and therefore a higher arterial elastance value.

The measure of end-systolic pressure is also used in the calculation of ventricular end-systolic elastance but the normal values published by Chirinos *et al.* were *higher* than for lean controls in my study (1.73 vs. 1.49 mmHg/ml for unscaled and 3.28 vs. 2.55 mmHg/ml/$m^{2.1}$ for scaled ventricular elastance).[122] There is evidence that unscaled ventricular elastance tends to be higher in females so it is unlikely that the all-female population explains this difference.[124, 208] Between-study differences may not necessarily reflect worse contractile function or lower ventricular stiffness but may be due to the different method of estimating ejection fraction (used in the calculation of systolic elastance using the single-beat method). Chirinos *et al.* used the area-length method but I used the Teicholz method used in the validation paper by Chen *et al.*[121] The Teicholz method uses linear dimensions to derive three-dimensional volumes and therefore makes certain geometric assumptions. This method is not recommended to derive ejection fraction in clinical practice [169] but sample population in my study were young, free from overt cardiovascular disease, and unlikely to have significantly distorted or asymmetric geometry. In addition, this study was designed to look for between-group differences associated with obesity and/or PCOS, rather than to establish normal values. Therefore, the method would not affect the outcome unless the methods of estimating end-systolic pressure and ejection fraction were less accurate for obese than for lean subjects.

Having higher arterial elastance and lower ventricular elastance necessarily resulted in subjects having higher mean ventricular-arterial coupling ratio than

published by Chirinos *et al*. (1.14 vs. 0.82).[122] Some reviews state that energy efficiency and stroke work are maximised at coupling ratios of 0.7 and 0.8 respectively, commenting that values above one represent 'ill-matched' ventricular and arterial systems.[209] However, there is evidence from animal and human studies that coupling ratios of 0.6-1.2 may be observed without evidence of pathology.[109, 116, 118, 210, 211] In addition, changes in loading which alter the coupling ratio within the range 0.3-1.3 seem to have little effect on stroke work or energy efficiency which remain at >90% of their optimal values.[210]

No association of age with elastances

There is evidence from invasive and non-invasive studies that arterial elastance and ventricular elastance increase in tandem with healthy ageing so that the coupling ratio remains unchanged.[113, 212] I did not find an association of age with arterial or ventricular end-systolic elastance but this may be because subjects were relatively young (16-45 years) and free from hypertension.

Obesity affects scaled arterial systolic elastance

A key finding of this study is that obesity was associated with a significantly increased scaled but not unscaled arterial elastance.

The data in this thesis confirm the findings of Chirinos *et al.* that obesity exerts adverse effects on arterial load beyond the normal relationship of elastance with body size.[122] Furthermore, this was evident in a younger population who were normotensive.

The relationship between obesity and arterial elastance is likely to be complex and may be influenced by haemodynamic factors such as altered resistive load, pulsatile load, or heart rate, as well as by non-haemodynamic factors such as adipokines, hyperinsulinemia, low-grade inflammation and lipids. Indeed, I found that these were all significant univariate correlates of scaled arterial elastance.

Fat location may not be important for altered arterial elastance

A key finding of this study is that visceral fat area was not a stronger correlate of arterial elastance than general obesity.

Adding waist circumference or BMI to the basic model for arterial elastance resulted in a greater improvement than did adding visceral fat area or insulin resistance. Waist circumference was the largest contributor to the final model, independently explaining 58% of the variation in arterial elastance. It is worth noting that waist circumference, while used as a measure of central obesity, is determined by visceral fat, subcutaneous fat and lean mass. Therefore, these data suggest that the increased arterial elastance in obese women may be driven by a general increase in lean and fat mass in all compartments, rather than specifically by visceral fat and insulin resistance. This interpretation is supported by evidence from the Olmsted study, which reported that weight loss was associated with a reduction in arterial elastance in an older, mixed population (n=788, mean age 60±9 years, 48% men) but that central obesity (waist circumference) was not an independent predictor of the change.[116]

This thesis adds to current data by demonstrating that even young women with obesity, who are free from hypertension and dysglycaemia, have an increase in arterial elastance. In addition, the inclusion of a detailed measurement of visceral fat area helped to identify that fat location was not an important factor for altered arterial elastance.

Contributions of heart rate, resistive load and pulsatile load to arterial elastance

Arterial elastance was originally proposed as a useful composite measure of pulsatile and resistive afterload and in this thesis 51% of the variation in scaled arterial elastance could be predicted by a model comprising heart rate, systemic vascular resistance (a measure of resistive load), systemic arterial compliance (a measure of pulsatile load) and aortic pulse wave velocity (a measure of regional arterial stiffness). Resistive load and heart rate independently contributed more to the variation in arterial elastance than did pulsatile load or regional arterial stiffness (part correlations of .329 for systemic vascular resistance, .314 for heart rate, -.214 for systemic arterial compliance and .264 for pulse wave velocity).

Others have published invasive data demonstrating that arterial elastance was 2.5 times more sensitive to a change in the ratio of systemic vascular resistance and cardiac interval than to a similar change in the reciprocal of compliance.[213] In

addition, investigations of the sensitivity of arterial elastance to changes in pulsatile load associated with isometric exercise showed that a combination of systemic vascular resistance and heart rate predicted ~96% of the variation in elastance, with detailed measures of pulsatile load together accounting for <1%.[160] These authors have suggested that the interpretation of arterial elastance as a composite measure of pulsatile and resistive load should be reconsidered.

Taken together, these findings suggest that arterial elastance may not fully describe the influence of pulsatile load and the latter may be better represented by wave reflections and their timing.

Obesity affects ventricular end-systolic elastance

A key finding of this study is that obesity was associated with significantly increased scaled but not unscaled ventricular end-systolic elastance.

My data confirm the findings of Chirinos *et al*. that obesity exerts adverse effects on left ventricular systolic stiffening beyond the normal relationship with body size.[122] Furthermore, this was evident in our younger population, the majority of whom did not have altered left ventricular geometry.

The increase in ventricular elastance is largely driven by the increase in arterial elastance

Ventricular end-systolic elastance is considered to be a load-independent measure of contractility, but at rest it is also influenced by geometric and passive structural factors as well as diastolic function.[214] Therefore, the increase associated with obesity may be due to a change in one or more of these factors. I did not find a significant association of ventricular elastance with LV mass in this population of young women but there was an association with relative wall thickness which is a reflection of concentric remodelling. Ventricular elastance was positively associated with measures of arterial stiffness, contractile function and late diastolic function.

The associations of cardiovascular risk factors with ventricular elastance were similar to those for arterial elastance so I examined whether the increase in one was driven by an increase in the other; there was a linear relationship between the

elastance measures, with arterial elastance explaining one third of the variation in ventricular elastance. However, visceral fat, post-prandial glucose and insulin, and central blood pressures remained significant correlates of ventricular elastance after adjustment for arterial elastance.

Ventricular end-systolic elastance could be explained reasonably well with a multivariate model that comprised scaled arterial elastance, fractional shortening, and a′ (r-square .672, p<.0001). Scaled arterial elastance explained the majority of the variation in ventricular elastance (part correlation .636) but fractional shortening and a′ were independent contributors (part correlations .343 and .163 respectively). Adding BMI or waist circumference to this basic model made small but significant improvements but the visceral fat did not make a significant contribution despite having the largest univariate association. This suggests that visceral fat might exert its effects on ventricular elastance through changes in fractional shortening, diastolic function or both.

A key finding of this study is that uncomplicated obesity predominantly affects ventricular elastance through its effects on arterial elastance but there may be direct effects of visceral fat and abnormal glucose metabolism on ventricular systolic stiffness.

Obesity did not affect ventricular-arterial coupling

A key finding of this study is that the increase in arterial elastance associated with uncomplicated obesity is matched by an increase in ventricular elastance so that the system remains closely coupled.

These data support the work of Chirinos *et al*. who also reported matched increases in elastance associated with obesity.[122] This thesis adds to the current understanding by extending the work to a younger population who were free from hypertension and diabetes.

According to the experimental work of Sunagawa *et al*. (discussed in the introduction) an unchanged coupling ratio suggests that stroke volume is not significantly altered.[109] Instead, the stiffer ventricular-vascular system is operating at higher blood pressures. I confirmed this finding (see Table 3-3); while obese women

had a mean stroke volume which was ~10% higher (6ml) than the mean for lean women, there was a wide range of measures within each group so the difference was not statistically significant. In contrast, the 8% (7mmHg) increase in central systolic blood pressure was highly significant ($p<.0001$) because of a narrower data range within groups.

In this thesis, dysglycaemia (reflected by glucose area under the curve) was independently and inversely associated with the ventricular-arterial coupling ratio. This may suggest that, in the absence of hypertension, an increase in blood glucose is associated with a larger increase in ventricular systolic elastance than arterial elastance. The effect of glucose on ventricular elastance was not entirely explained by altered contractility and it is possible that the effect is mediated by structural changes that increase stiffness.

Potential consequences of an elevated arterial and ventricular elastance

The potential haemodynamic consequences of 'coupled' increases in arterial and ventricular elastance are suggested in the simplified pressure-volume diagrams in Figure 4-6. The diagrams illustrate the hypothetical effects of an isolated increase in ventricular preload (end-diastolic volume) which has the effect of shifting the arterial elastance curve to the right without altering its slope (E_a). Changes from the basal state are depicted in red. Diagram [A] reflects a closely coupled and compliant (low elastances) ventricular-arterial system typical of the lean subjects in this study. The increase in end-diastolic volume leads to a 10ml augmentation of stroke volume which is associated with a 10mmHg change in end-systolic blood pressure and an increase in the area within the box, which represents stroke work. This can be contrasted with [B], which reflects a closely-coupled but stiffer system typical of the obese women in my study. The same basal volumes ejected into a stiffer arterial system, result in a higher basal end-systolic pressure. In addition, the increase in end-diastolic volume results in a greater change in systolic blood pressure and stroke work for the same augmentation in stroke volume. Since the ratio of arterial to ventricular elastance is unchanged, the higher elastances may not adversely affect the augmentation of stroke volume at rest.

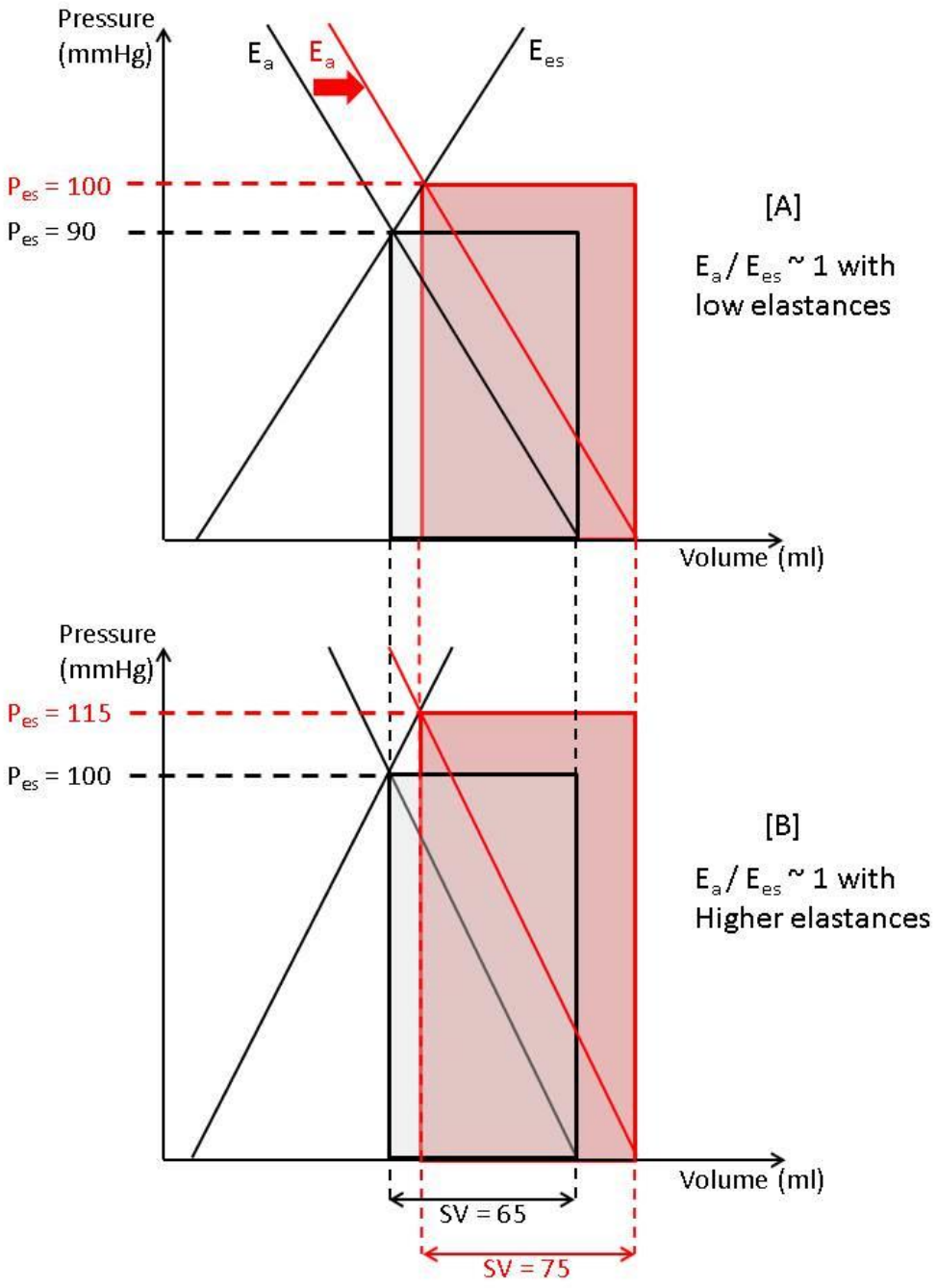

Figure 4-6. Suggested consequence of the same increase in preload on haemodynamic variables in lean [A] and obese [B] subjects. In both subjects an isolated increase in preload causes the arterial elastance curve to shift to the right but the slope (E_a) remains the same. In [A] the elastances are low and the ratio is ~1. Stroke volume (SV) is augmented by 10ml (for example) and this is associated with a 10mmHg (for example) increase in end-systolic pressure (P_{es}) and an increase in stroke work (area shaded within box). In [B] the elastances are higher but the ratio remains ~1. The higher basal elastances result in a higher basal P_{es}. The same augmentation of SV is associated with a larger increase in P_{es} and stroke work.

The effect of PCOS on scaled arterial and ventricular systolic elastance

To the best of my knowledge this is the first study of arterial and ventricular end-systolic elastance in women with PCOS. The data suggest that increased arterial stiffness in PCOS is mediated by the increase in obesity and not by other hormonal changes associated with the syndrome.

Women with PCOS tended to have a higher ventricular elastance than controls and this was weakly associated with testosterone. As a result the coupling ratio tended to be lower in those with PCOS. The results were not statistically significant and this may be a result of the small sample size. Since there was a weak direct association of the coupling ratio with mass (Table 4-5) it is tempting to speculate that the lower coupling ratio may help to explain the finding of a lower rate of LV hypertrophy in obese women with PCOS in my study.

A key finding of this study is that young women with PCOS who are free from comorbidities have similar arterial elastance to age- and BMI-matched controls. The trend towards higher ventricular elastance and lower coupling ratio needs to be confirmed in other studies.

The prognostic value of elastances and their ratio

Ejection fraction is a widely used measure of systolic function with proven prognostic importance at values less than 45% in a wide range of cardiovascular disease.[215] Its strength as a prognostic marker may be underpinned by the fact that it is a *composite* measure which is affected by loading as well as by ventricular contractility. This means it is actually a measure of ventricular-arterial coupling, rather than a pure measure of contractile function, but this factor also hampers its usefulness in unpicking the pathophysiology associated with disease. Elastance measures are determinants of ejection fraction (the relationship is shown below), and may help to explain which elements of pathophysiology are best targeted by therapeutic strategies.

$$Ejection\ fraction\ = \frac{1}{1\ +\ Ea/Ees}$$

There are few studies which consider whether elastance measures have prognostic significance. In the Penn heart failure study, LV end-diastolic volume, increased arterial elastance and a high coupling ratio were associated with increased risk of death, cardiac transplantation or requirement for a mechanical assist device, and increased rates of hospitalization.[126] Surprisingly, LV end-systolic elastance was not an independent predictor of outcomes in the same study. This seems counterintuitive given that all patients had heart failure with reduced ejection fraction (HFrEF) but the authors emphasise that ventricular elastance is the slope of the end-systolic pressure-volume relationship and only fully describes contractile function when considered alongside its volume intercept (V_0) which was related to poorer outcomes. These data help to explain why aggressive afterload reduction is important in patients with HFrEF and suggest that the coupling ratio may be more important in predicting outcomes that individual elastance values.

Similar results have been described in a small study of women with systemic lupus erythematosus (SLE) who were free from overt cardiovascular disease and had normal ejection fraction.[216] Women with SLE (n=48) had higher arterial elastance and higher coupling ratio than controls (n=20) and these variables were significantly elevated in those with SLE who had a cardiovascular event in a three year follow-up period.

Exercise capacity is an accepted prognostic measure of cardiovascular mortality even in low-risk populations,[217] and women who are obese often have exertional dyspnea and reduced exercise capacity.[218] The mechanisms linking these findings with obesity are likely to be multifactorial but one study of U.S. patients with exertional dyspnea and a high burden of obesity (BMI 30.9 ± 6.8kg/m^2) found that arterial elastance independently explained reduced exercise capacity (workload less than eight metabolic equivalents) after adjustment for age, hypertension, LV strain and ejection fraction.[125] None of the measures of ventricular function, including ventricular elastance, were independent predictors of exercise capacity in this population but that might be expected given that they had normal ventricular function. There were no significant between-group differences in resting indices of diastolic function (with the exception of left atrial volume) suggesting that arterial elastance may be better than these measures at predicting exercise tolerance in an

obese population. A strength of this study is that those with reduced ejection fraction and exercise-induced ischaemia were excluded from the study. However, the obesity was complicated by hypertension in 58%, diabetes in 23% and obstructive lung disease in 19% of patients so it is unclear whether the results can be generalised to a population with uncomplicated obesity.

Intervention studies and elastance

A sub-study of the Systolic Heart Failure Treatment with the I_f Inhibitor Ivabradine Trial (SHIFT) has shown the value of elastance measures in understanding the effects of therapy on cardiovascular physiology. Ivabradine is an I_f channel inhibitor which selectively affects the spontaneous activity of the sinoatrial node, leading to a reduction in heart rate without a negative inotropic effect. Patients with HFrEF frequently have increased resting heart rate which is related to worse cardiovascular outcomes. The SHIFT study found that Ivabradine improved outcomes in patients with HFrEF with resting heart rate over 70 bpm as well as improving quality of life. Patients were already optimally medicated at the start of the trial but the improvements were directly related to the magnitude of heart rate reduction associated with Ivabradine. The sub-study (n=275) showed that, after 8 months of treatment with Ivabradine, a significant reduction in heart rate was accompanied by a decrease in arterial elastance and ventricular-arterial coupling ratio compared with the placebo group (p-values <.0001, <.0001 and .002 respectively). In addition, systemic vascular resistance was unchanged but arterial compliance, a surrogate measure of pulsatile load was improved (p=.004). There was no change in ventricular elastance but the decrease in ventricular-arterial coupling ratio led to an increase in stroke volume. The authors suggest that an isolated reduction in heart rate appears to result in systolic unloading of the LV which improves stroke volume in those with systolic heart failure.[219]

Women with obesity and insulin resistance are more likely to develop HFpEF than HFrEF, but I have been unable to find a similar study of elastance in this population. There is evidence from one small, randomised trial (n=61) that short-term treatment (7 days) with Ivabradine resulted in increased exercise capacity and a slower baseline heart rate as well as a slower increase in exercise heart rate compared with those in the placebo group.[220] The patients were predominantly

female, overweight, middle-aged (67±8years) and hypertensive with no significant between-group differences in clinical characteristics. The authors report improved diastolic filling in the Ivabradine group, reflected in higher e′, but the effects of altered afterload are not considered in this study so it is unclear whether the results can be solely attributed to increased diastolic filling time because of slower heart rate or whether reduced afterload was also a contributing factor to improved exercise capacity.

Together these data suggest that the increase in arterial elastance in women with uncomplicated obesity may result in reduced exercise capacity. It would be interesting to conduct a study to confirm this and to examine the relative contributions of heart rate, static load and pulsatile load to reduced exercise capacity. Whatever the haemodynamic mechanisms involved, prognosis may not be affected until the arterial and ventricular elastances become uncoupled but there are no longitudinal studies which confirm this.

Limitations

Cardiovascular risk factors such as cholesterol were not added to multivariate models for elastance because the sample size limited the number of independent variables which could be included and because the close correlation of these risk factors with obesity may have produced problems with multicollinearity. Despite this, the regression models explained a significant proportion of the variability in elastance.

I considered whether the associations of elastance with obesity and with insulin resistance were a function of using body surface area in the scaling method, itself a surrogate measure of obesity. However, the associations remained significant when analyses were adjusted for body surface area (data not presented) so this is unlikely. Scaling elastance to lean mass may be preferable in future studies, since this would provide greater confidence in the results relating to the effects of obesity.

The lack of difference in arterial elastance between women with PCOS and matched controls may be affected by the sample size since the initial power calculation for the study did not include measures of elastance. Nevertheless, this is

one of the largest studies of its type in women with PCOS and the data suggest that any between-group differences are likely to be subtle.

Finally, the study population comprised young women with uncomplicated obesity and the results may not be generalizable to males, an older population or those with co-existing hypertension or diabetes.

Conclusions

The data from this study confirm that young women with uncomplicated obesity have evidence of increased scaled arterial and ventricular elastance compared with lean controls, but these increases are matched so that the cardiovascular system remains closely-coupled and stroke volume at rest is not significantly altered. However, obese women have higher peripheral and central blood pressure as a result of increased arterial elastance, and this is more sensitive to changes in preload. These data add to the current literature by extending the findings to a younger population who were free from confounding factors such as hypertension and diabetes.

To the best of my knowledge this study represents the most comprehensive assessment of body composition, glucose handling and elastance measures in a young female population with obesity. A novel finding of this study is that increases in arterial elastance were not particularly affected by fat location since visceral fat and insulin resistance were not stronger associates of elastance than BMI and waist circumference. In addition, the increase in ventricular elastance associated with obesity appears to be driven by the increase in arterial elastance but visceral fat may have a small direct effect on ventricular function.

I have been unable to find other published studies of elastance and ventricular-arterial coupling in women with PCOS. A novel finding of this thesis is that women with PCOS have similar arterial elastance as age- and BMI-matched controls. This confirms that increased afterload in women with PCOS is attributable to the obesity associated with the syndrome. There was a trend towards a higher ventricular elastance and lower ventricular arterial coupling ratio in women with PCOS and this might be related to testosterone levels. It is unclear whether this finding is relevant to

the lower rates of LV hypertrophy in obese women with PCOS and this is worthy of further study.

The principle determinant of arterial elastance in this study was waist circumference which independently explained a significant proportion of arterial elastance after adjustment for systemic vascular resistance, heart rate and systemic arterial compliance (a measure of pulsatile load). The principle determinant of ventricular end-systolic elastance was arterial elastance but after adjustment for this, contractile function, late diastolic function and waist circumference remained independent contributors to the model. Interestingly, a direct univariate association between ventricular elastance and waist circumference became an inverse association after adjustment for arterial elastance. It is unclear whether this reflects an early negative effect of obesity on ventricular contractility but other measures of contractile function were not significantly altered. Together these findings suggest that both haemodynamic and non-haemodynamic factors are important contributors to ventricular-arterial coupling in this population.

It is unclear whether arterial elastance fully describes pulsatile afterload. I found that it was significantly affected by heart rate and static load but that arterial compliance was also a significant contributor. Others have found that arterial elastance is a poor measure of pulsatile load and this is a key factor in the development of LV hypertrophy. For this reason a comparison of elastance and WI measures of loading is included in Chapter 5.

5. Obesity, polycystic ovary syndrome and arterial waves

The study of arterial waves provides an additional opportunity to quantify the ventricular-arterial interaction. One non-invasive method of studying these waves involves using ultrasound to detect simultaneous changes of diameter-derived blood pressure and velocity in the common carotid artery. A detailed description of the development of this method is given in the general introduction. Briefly, the product of the change in blood pressure and velocity is called WI and this reflects the energy or power carried by a longitudinal wave as it moves through blood. A commercially available system generates net WI signals but it is possible to separate these into forward and backward components of the wave using mathematical software. The separated waves are considered to provide more detailed information about the time-course of the ventricular-arterial interaction.

Waves travelling forward from the heart into the circulation have positive values of WI while backward waves reflected from the circulation have negative values. In addition, waves that increase pressure are considered to be 'compression' in nature and those that decrease pressure are 'expansion' in nature. In summary, a forward compression wave (FCW) generated by the heart causes an increase in arterial blood pressure and velocity i.e. it may be considered as having a 'pushing' effect. This is reflected back from the circulation as a backward compression wave (BCW) which increases backward pressure but opposes the velocity of blood. In contrast, the forward expansion wave (FEW) generated by the LV at the end of systole has a 'pulling' effect, causing a reduction of arterial blood pressure and decelerating blood velocity. This is reflected from the circulation as a backward expansion wave (BEW) which also reduces blood pressure but pulls the flow towards the circulation.

Whereas elastance measures represent end-systolic stiffness of the ventricular-arterial system, WI measures may provide more detailed information about the time-course of ventricular-arterial coupling. This may have relevance to the timing and quantification of pulsatile loading which may be particularly relevant to the development of LV hypertrophy.

It is unclear whether WI analysis is feasible in an obese, female population, or whether these methods are sufficiently sensitive to detect subclinical dysfunction associated with uncomplicated obesity or PCOS.

Aims of this chapter

The primary aim of this chapter is to establish the effect of uncomplicated obesity and PCOS on net WI and separated waves. Wave intensity signals are said to provide a more comprehensive means of assessing pulsatile load so the data should complement the results detailed in Chapter 3 which characterised ventricular-arterial coupling using elastance methods.

The secondary aim is to establish whether central obesity and insulin resistance independently contribute more to the variation in WI than general obesity.

The final aim is to establish whether detailed quantitative measures of ventricular-arterial coupling can independently explain the variation in LV mass and diastolic function in this study population.

Hypotheses

In young women who are free from diabetes, hypertension and cardiovascular disease

1. those who are obese will have wave intensity signals that are altered in magnitude compared with lean controls but a similar reflection co-efficient,
2. those with PCOS will have similar wave intensity signals to age- and BMI-matched controls,
3. central obesity will independently explain more of the variation in key measures of wave intensity than BMI, and
4. quantitative measures of ventricular-arterial coupling will help to explain the finding of increased left ventricular mass and worse diastolic function in obese subjects.

Specific methods

Women with PCOS and control volunteers were recruited from local clinics and the general community. Full details of recruitment, exclusion criteria and general methods are described in Chapter 2.

Net wave intensity

Subjects were studied in the supine position after resting for at least 10 minutes. WI signals were derived using a commercially available ultrasound system (Aloka Prosound SSD-5500, Tokyo, Japan).

A perpendicular scan line was applied to a longitudinal view of the common carotid artery ~1cm from the bulb, to acquire changes in diameter through the cardiac cycle using an echo-tracking subsystem (Figure 5-1) which has been described elsewhere.[146] Arterial pressure waveforms were derived from diameter waveforms and calibrated by automated sphygmomanometer measurement of brachial systolic and diastolic blood pressure from the ipsilateral arm as previously validated.[155] The design of the ultrasound system allows for a second, steerable pulsed-wave Doppler beam to be aligned to the vessel walls in order to simultaneously acquire velocity data and diameter-derived pressure. Colour-flow Doppler over the vessel lumen allowed the angle of incidence to be more closely aligned with blood flow and the sample volume was altered to encompass ~70-80% of the lumen diameter. Arterial diameter and flow velocity were continuously recorded for 20 seconds.

During off-line analysis, individual beats affected by artefact or ectopics were rejected. The aim was to include at least 10 consecutive beats which were signal-averaged to derive a single waveform of diameter and velocity (Figure 5-2). Wave intensity was calculated as the product of the time-derivatives of pressure and flow velocity [(dP/dt) x (d*U*/dt)]. A simultaneous electrocardiogram (ECG) was used as a time reference for the cardiac cycle.

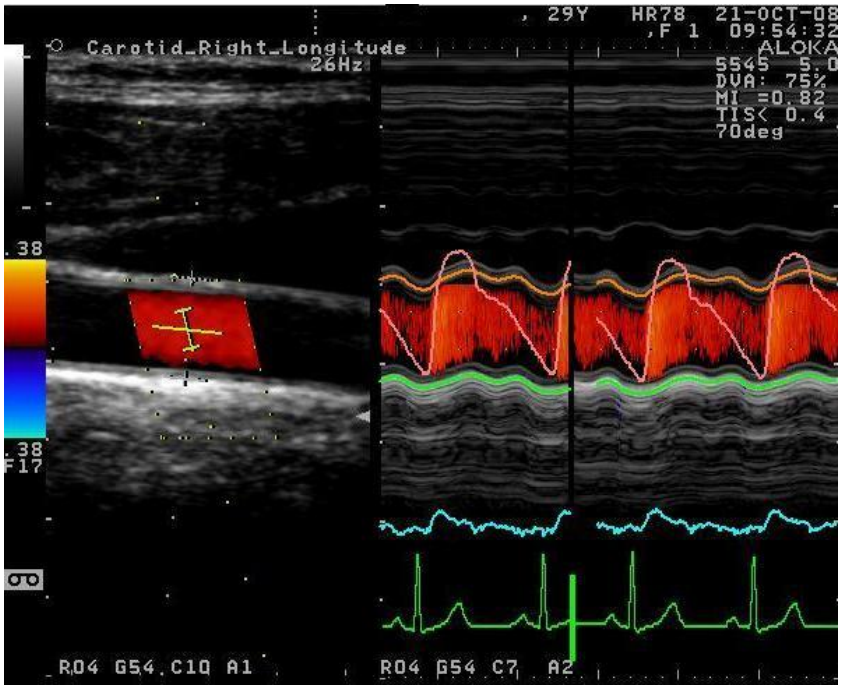

Figure 5-1. Simultaneous recordings of velocity and diameter-derived pressure used to derive wave intensity signals. The vertical cursor on the longitudinal image shows the m-mode recording position (displayed on the right). Echo-tracking software follows diameter changes of the near and far carotid adventitia (orange and green lines). From this a diameter waveform is constructed (shown in pink) which can be calibrated with brachial systolic and diastolic blood pressure. The flow velocity waveform derived from Doppler information is shown in blue.

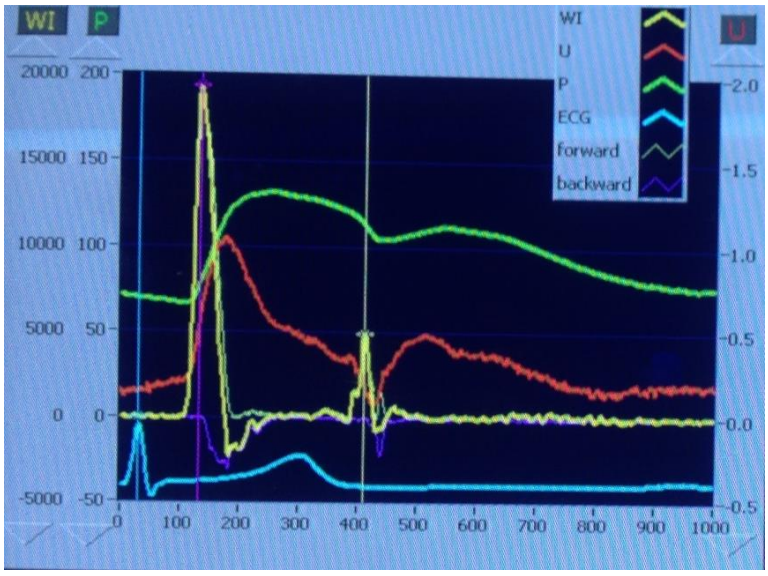

Figure 5-2. Screenshot from Aloka showing diameter-derived pressure (green), Doppler derived flow velocity (orange) and net wave intensity (yellow).

Separated wave analysis

A customised programme (MATLAB, MathWorks Inc., Natick, Massachusetts) was used to calculate the local wave speed and the intensity of separated forward and backward travelling waves as previously described by our lab.[149] Briefly, the time-course of simultaneous changes in pressure and velocity across the cardiac cycle was displayed as a loop as shown in Figure 5-3; the onset of systole is in the bottom left corner. In early systole there is a linear relationship between pressure and velocity. Local wave speed was calculated by

$$c = \frac{dP}{dU} \times \frac{1}{\rho}$$

where c is the local wave speed, dP is the change in pressure in early systole, dU is the change in velocity in early systole and ρ is the density of blood (1040kg/m^3).

The programme uses formulae described by Jones *et al.* [139] to separate forward and backward waves using net WI signals and the local wave speed in early systole as follows

$$Pf = \frac{(pressure - Po) + (\rho.c.velocity)}{2}$$

$$Pb = \frac{(pressure - Po) - (\rho.c.velocity)}{2}$$

where Pf is the part of artery pressure from a forward travelling wave, Pb is the part of artery pressure from a backward travelling wave, pressure is the net pressure, velocity is the net velocity, Po is the diastolic blood pressure, ρ is the density of blood and c is the local wave speed in early systole. For the forward and backward components of velocity:

$$Uf = \frac{Pf}{\rho.c}$$

$$Ub = -\frac{Pb}{\rho.c}$$

where Uf is velocity of a forward travelling wave and Ub is the velocity of a backward travelling wave. Finally,

$$WIf = dPf.dUf$$

$$WIb = dPb.dUb$$

where WIf is the wave intensity of the forward travelling wave and WIb is the wave intensity of the backward travelling wave.

Figure 5-4 shows separated forward and backward components of pressure and velocity obtained using this method and the final separated forward and backward wave intensity signals are shown in Figure 5-5.

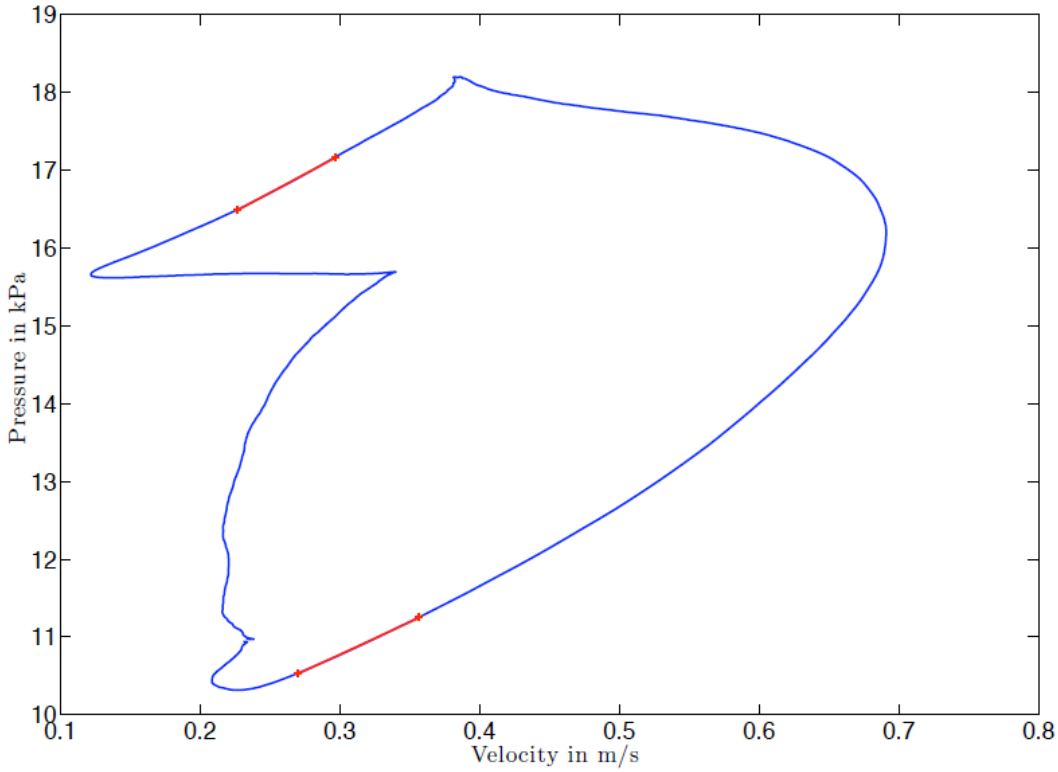

Figure 5-3. Example of a pressure-velocity loop across the cardiac cycle generated by customised software. The onset of systole occurs in the bottom left corner. The first red section marked by asterisks represents the linear portion in early systole used to calculate local wave speed.

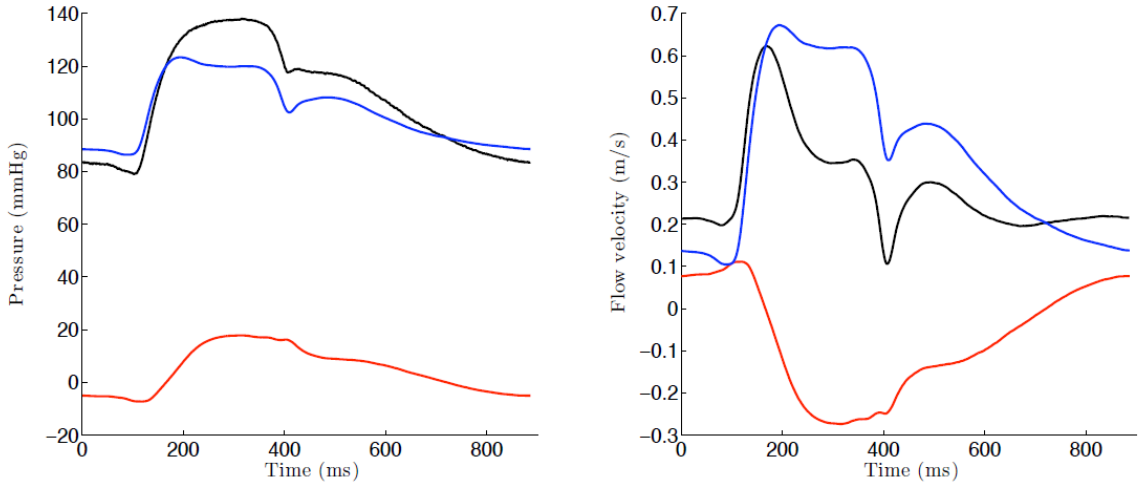

Figure 5-4. Example of the total (black), forward (blue) and backward (red) components of pressure and velocity which were separated using the method described. Note that the net pressure represents the addition of the forward and backward pressure (it is augmented by the backward pressure). Whereas the net flow velocity represents the subtraction of the backward from the forward flow velocity.

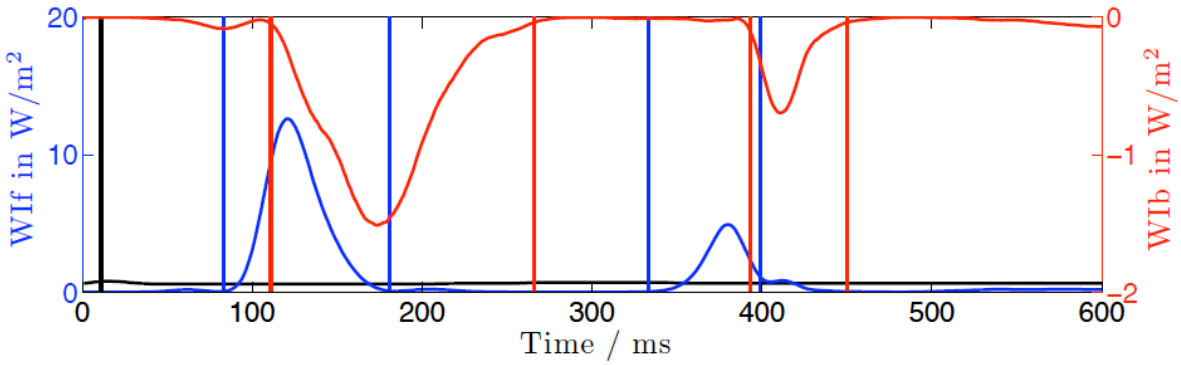

Figure 5-5. Final separated forward (blue) and backward (red) wave intensity signals.

Local artery stiffness was calculated using established formulae and the changes in common carotid diameter and diameter-derived pressure.

$$\beta \text{ stiffness index} = \ln [(Ps/Pd) / ((Ds-Dd)/Ds)]$$

$$\text{Peterson's elastic modulus, } \varepsilon = [(Ps - Pd) / (Ds - Dd)] \cdot Dd$$

Reproducibility of wave intensity measures

The intra-observer inter-session repeatability of WI measures using an Aloka prosound SSD-5500 and these methods of wave separation has previously been established and published by a member of our group.[149] The mean (s.d.) differences of two measurements taken from the right common carotid artery, separated by at least one week were as follows; epsilon 13(10)%, beta stiffness index 12(9)%, wave speed 8(8.3)%, augmentation index 14(7)%, forward compression wave 12(6)%, backward compression wave 21(11)%.

Data analysis

I analysed the effects of obesity and PCOS on the following measurements (the average of left and right common carotid artery measures was used for net and separated WI analysis):

- net wave intensity
    - W_1 amplitude,
    - W_2 amplitude,
    - negative area,
- separated wave intensity
    - local wave speed,
    - forward compression wave amplitude (FCW) and integral,
    - backward compression wave (BCW) amplitude and integral,
    - time from the R wave of the ECG to BCW,
    - forward expansion wave (FEW) amplitude and integral,
    - reflection coefficient (BCWintegral/FCWintegral x 100),
    - local beta stiffness index, and
    - local Peterson's elastic modulus.

Results

Feasibility

Net WI analysis was feasible in 90% of subjects and was limited by the ability to obtain high quality and stable images of *both* the left and right common carotid artery since I elected to use the average of these measurements in analyses. The feasibility was not worse in obese subjects.

Wave intensity measures were not related to body size

In the lean control subjects there were no associations of body size with net WI measures but this was a small group (n=56). I assessed the relationship of height with WI measures in the pooled sample (n=127) but there were no significant associations.

Association of net wave intensity measures with cardiovascular risk factors

Table 5-1 shows univariate correlations of net WI measures with cardiovascular risk factors. There were few significant associations and none of the measures was related to BMI.

There was a weak direct association of age with the amplitude of W_1 (originating from the LV) and with the negative area (representing reflections). In addition, there was a weak but direct association of the negative area with visceral fat and an inverse association with HDL cholesterol.

Table 5-1. Univariate correlates [Spearman's rho (p-value)] of net wave intensity measures with cardiovascular risk factors.

	W_1		W_2		NA	
Age	**.18**	**(.048)**	.11	(.218)	**.21**	**(.020)**
BMI	−.03	(.733)	−.06	(.531)	.10	(.290)
Waist	.02	(.805)	−.04	(.690)	.09	(.312)
Hip	.05	(.545)	.01	(.906)	**.18**	**(.039)**
Waist/hip	−.09	(.321)	−.14	(.125)	−.09	(.328)
TFA	−.04	(.696)	−.02	(.862)	.16	(.093)
SFA	−.04	(.690)	−.03	(.716)	.15	(.116)
VFA	.01	(.941)	.01	(.943)	**.19**	**(.043)**
Glucose	.10	(.271)	−.02	(.867)	.06	(.509)
Glucose AUC	−.04	(.627)	<.01	(.987)	−.02	(.844)
Insulin	−.13	(.157)	−.04	(.637)	−.01	(.956)
Insulin AUC	−.03	(.753)	−.06	(.516)	.04	(.700)
HOMA-IR	−.11	(.227)	−.07	(.456)	<.01	(.981)
hs-CRP	.07	(.439)	-.05	(.588)	.09	(.302)
Testosterone	−.01	(.931)	.02	(.830)	.06	(.530)
Adiponectin	.08	(361)	.10	(.266)	−.07	(.417)
Cholesterol	.02	(.844)	−.08	(.375)	−.01	(.938)
HDL	−.04	(.671)	.01	(.922)	**−.18**	**(.045)**
LDL	.05	(.605)	−.06	(.532)	−.01	(.960)
Triglycerides	−.04	(.685)	−.14	(.126)	.05	(.595)

TFA = total abdominal fat area, SFA = subcutaneous fat area, VFA = visceral fat area, AUC = area under the curve.

Local artery stiffness and cardiovascular risk factors

Table 5-2 shows univariate correlations of cardiovascular risk factors with measures of local carotid artery stiffness. There was a direct association of age with the local beta stiffness index, epsilon and the augmentation index, but not with local wave speed which is an important determinant of wave intensity. There were no associations of obesity or insulin resistance with the beta stiffness index in this young female population. In contrast, both general and central obesity were directly associated with local wave speed, epsilon and the local augmentation index. The local augmentation index was directly associated with the post-prandial glucose levels and triglycerides. There was an unexpected direct association of wave speed with HDL cholesterol.

Separated wave intensity measures and cardiovascular risk factors

Univariate correlations of cardiovascular risk factors with separated WI measures are shown in Table 5-3. The first two columns show the relationships with waves generated by the LV. There was a direct association of visceral fat area and HOMA-IR with the forward compression wave. The correlations of this wave with other measures of obesity were weaker and, in most cases, not significant. Age tended to be inversely associated with the energy in the FCW but this did not reach statistical significance. The forward expansion wave was directly associated with all measures of obesity and with HOMA-IR; the strongest association was with visceral fat area.

The next column shows the relationships with the reflected wave from the cerebrovascular circulation. Age was *inversely* associated with the reflected compression wave. Central obesity was directly associated with the reflected wave. When the reflected wave was normalised for the energy in the preceding forward travelling wave (to produce a reflection coefficient), only the inverse association with age remained.

The final column shows the data for the timing of the reflected wave. This was most strongly associated with age, visceral fat and fasting glucose levels (all inverse).

Table 5-2. Univariate correlates [Spearman's Rho (p-value)] of carotid artery stiffness measures with cardiovascular risk factors.

	Local wave speed		**Local β stiffness index**		**Local Epsilon**		**Local Augmentation index**	
Age	.02	(.859)	**.47**	**(<.0001)**	**.54**	**(<.0001)**	**.49**	**(<.0001)**
BMI	**−.20**	**(.021)**	−.05	(.599)	.17	(.051)	**.34**	**(<.0001)**
Waist	**−.21**	**(.017)**	−.03	(.757)	**.20**	**(.019)**	**.32**	**(<.0001)**
Hip	**−.22**	**(.011)**	−.05	(.590)	**.18**	**(.041)**	**.33**	**(<.0001)**
Waist/hip	−.04	(.634)	.08	(.358)	**.19**	**(.032)**	.16	(.064)
TFA	−.17	(.060)	−.08	(.392)	.12	(.183)	**.22**	**(.015)**
SFA	−.17	(.059)	−.10	(.269)	.09	(.331)	**.18**	**(.041)**
VFA	−.17	(.055)	.03	(.775)	**.23**	**(.011)**	**.34**	**(<.0001)**
Glucose	−.03	(.699)	.16	(.071)	.17	(.055)	.16	(.063)
Glucose AUC	.05	(.581)	.04	(.693)	.06	(.500)	**.19**	**(.029)**
Insulin	−.02	(.829)	−.08	(.333)	.03	(.748)	.10	(.263)
Insulin AUC	−.04	(.698)	−.10	(.286)	−.02	(.816)	.02	(.798)
HOMA-IR	−.05	(.606)	−.07	(.417)	.05	(.578)	.14	(.112)
hs-CRP	−.10	(.234)	−.06	(.473)	.10	(.268)	.15	(.075)
Testosterone	.09	(.298)	−.02	(.864)	−.03	(.759)	.02	(.809)
Adiponectin	.04	(.652)	.07	(.438)	−.05	(.570)	−.10	(.280)
Cholesterol	.03	(.706)	.12	(.167)	.22	(.011)	.11	(.223)
HDL	**.22**	**(.012)**	.13	(.128)	.08	(.368)	<.01	(.976)
LDL	−.07	(.442)	.05	(.570)	**.17**	**(.049)**	.08	(.383)
Triglycerides	.01	(.937)	<.01	(.979)	.08	(.375)	**.26**	**(.002)**

TFA = total abdominal fat area, SFA = subcutaneous fat area, VFA = visceral fat area, AUC = area under the curve.

Table 5-3. Univariate correlates [Spearman's rho (p-value)] of separated wave intensity measures with cardiovascular risk factors.

	FCWi	FEWi	BCWi	BCWi/FCWi	R-BCW
Age	−.15 (.077)	.02 (.795)	**−.24 (.006)**	**−.19 (.026)**	**−.24 (.004)**
BMI	.10 (.252)	**.18 (.032)**	.09 (.327)	.04 (.627)	−.13 (.143)
Waist	.15 (.089)	**.19 (.028)**	.13 (.129)	.05 (.563)	−.14 (.102)
Hip	.12 (.177)	**.17 (.048)**	.11 (.226)	.05 (.552)	−.13 (.130)
Waist/hip	.11 (.195)	.09 (.327)	.10 (.273)	.01 (.956)	−.11 (.203)
TFA	**.19 (.037)**	**.24 (.006)**	**.17 (.050)**	.07 (.421)	−.10 (.256)
SFA	.17 (.055)	**.24 (.008)**	.17 (.051)	.09 (.304)	−.07 (.448)
VFA	**.22 (.014)**	**.25 (.005)**	**.19 (.033)**	.02 (.800)	**−.25 (.005)**
Glucose	.03 (.718)	.10 (.249)	−.02 (.845)	−.06 (.464)	**−.24 (.006)**
Gluc AUC	.02 (.828)	−.07 (.423)	−.05 (.606)	−.13 (.141)	**−.19 (.034)**
Insulin	.16 (.068)	**.17 (.044)**	.09 (.331)	−.05 (.547)	−.13 (.141)
Ins AUC	.10 (.296)	.05 (.619)	.07 (.464)	.01 (.897)	−.15 (.106)
HOMA-IR	**.20 (.028)**	**.21 (.014)**	.11 (.190)	−.04 (.610)	−.15 (.082)
hs-CRP	.17 (.055)	.16 (.067)	.16 (.072)	.07 (.429)	−.09 (.309)
Testosterone	.01 (.937)	−.02 (.801)	−.02 (.784)	−.06 (.529)	.03 (.744)
Adiponectin	−.07 (.451)	−.04 (.673)	−.05 (.543)	−.03 (.724)	.03 (.765)
Cholesterol	.03 (.751)	.10 (.256)	<.01 (.994)	−.06 (.508)	−.05 (.536)
HDL	−.12 (.179)	−.11 (.227)	−.11 (.231)	−.02 (.782)	.10 (.271)
LDL	.07 (.444)	.15 (.093)	.05 (.547)	−.02 (.854)	−.05 (.599)
Triglycerides	.08 (.337)	.03 (.704)	.05 (547)	−.05 (.569)	−.07 (.424)

FCWi = forward compression wave integral, BCWi = backward compression wave integral, R-BCW = time from R wave to backward compression wave peak, BCWi/FCWi = compression wave reflection coefficient, FEWi = forward expansion wave integral, TFA = total abdominal fat area, SFA = subcutaneous fat area, VFA = visceral fat area, AUC = area under the curve.

These data suggested that WI measures of pulsatile loading were more strongly influenced by central obesity and metabolic health than by general obesity. As a result, I tested the effect of a diagnosis of PCOS and/or insulin resistance on separated waves.

Clinical characteristics of the population when grouped by insulin resistance and PCOS status

The clinical characteristics of the study population when stratified by PCOS and insulin resistance are presented in Table 5-4. Women in the 'control' group were free from PCOS and insulin resistance (PCOS-IR-). There were only 12 subjects with a diagnosis of PCOS but without insulin resistance, this group size was significantly smaller than the others and was not considered in further inferential statistics. Women in the 'insulin resistance' group were obese but free from PCOS (PCOS-IR+). Women in the 'PCOS' group had both PCOS and insulin resistance (PCOS+IR+).

As expected, the insulin resistance group had higher levels of general and central obesity than the metabolically healthy controls, with lower levels of HDL and adiponectin, and higher levels of inflammation.

By design, women with PCOS had higher testosterone levels than the other two groups. Women with PCOS had similar levels of obesity as the group with insulin resistance. Measures of glucose handling were also similar with the exception of insulin area under the curve, which was higher in those with PCOS.

Table 5-4. Clinical characteristics (mean ± s.d.) of the study population when grouped by PCOS status and HOMA-IR >2.5.

	Control (PCOS-IR-) (n=50)	**Insulin Resistance (PCOS-IR+) (n=29)**	**IR vs. Control p-value**	**PCOS (PCOS+IR+) (n=43)**	**PCOS vs. Control p-value**	**PCOS vs. IR p-value**
Age (years)	31 ± 8	32 ± 7	.947	30 ± 6	.614	.374
BMI (kg/m^2)*	25 ± 4	32 ± 6	**<.0001**	32 ± 7	**<.0001**	.977
Waist (cm)*	79 ± 10	94 ± 13	**<.0001**	96 ± 17	**<.0001**	.969
Hip (cm)	100 ± 11	115 ± 15	**<.0001**	115 ± 17	**<.0001**	.996
Waist-hip ratio	0.80 ± 0.05	0.82 ± 0.05	.296	0.83 ± 0.05	0.12	.762
TFA (cm^2)	224 ± 105	380 ± 90	**<.0001**	362 ± 127	**<.0001**	.896
SFA (cm^2)	204 ± 97	339 ± 78	**<.0001**	327 ± 113	**<.0001**	.925
VFA (cm^2)*	19 ± 13	42 ± 21	**<.0001**	38 ± 27	**<.0001**	.640
Glucose (units)	4.5 ± 0.4	4.8 ± 0.3	**.047**	4.7 ± 0.4	**.037**	.995
Gluc AUC (mmol min/l)*	670 ± 120	743 ± 145	.066	775 ± 141	**.001**	.738
Insulin (units)	45 ± 19	107 ± 28	**<.0001**	132 ± 91	**<.0001**	.239
Ins AUC (pmol min/l)*	36460 ±16357	73478 ± 45023	**<.0001**	105583 ±61173	**<.0001**	**.021**
HOMA-IR*	1.4 ± 0.6	3.8 ± 1.1	**<.0001**	4.6 ± 3.3	**<.0001**	.759
hs-CRP (mg/l)*	1.6 ± 2.1	3.8 ± 3.4	**<.0001**	3.8 ± 4.6	**<.0001**	.990
Testosterone (nmol/l)*	0.92 ± 0.28	0.88 ± 0.27	.944	1.52 ± 0.86	**<.0001**	**<.0001**
Adiponectin (µg/ml)*	12 ± 7	7 ± 6	**.002**	11 ± 9	.207	.179
Cholesterol (mmol/l)	4.6 ± 0.8	4.7 ± 0.9	.989	4.8 ± 0.7	.856	.981
HDL (mmol/l)	1.5 ± 0.4	1.2 ± 0.2	**.003**	1.3 ± 0.3	**.023**	.714
LDL (mmol/l)	2.7 ± 0.7	3.0 ± 0.9	.248	2.9 ± 0.7	.375	.969
Triglycerides (mmol/l)	0.9 ± 0.5	1.0 ± 0.4	.764	1.1 ± 0.5	.176	.853

*TFA = total abdominal fat area, SFA = subcutaneous fat area, VFA = visceral fat area, AUC = area under the curve. *Variables were log-transformed prior to use in inferential statistics.*

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Standard measures of cardiovascular function and elastance when grouped by insulin resistance and PCOS status

Women with insulin resistance (but without PCOS) had higher heart rate, brachial and central systolic blood pressure, scaled arterial and ventricular elastance than controls (Table 5-5). The differences that were evident in LV wall thickness, mass and diastolic function when groups were stratified by BMI (Chapter 3) were no longer significant when stratification was based on insulin resistance.

Women with PCOS who were insulin resistant had higher heart rate than controls but similar blood pressure. In addition, those with PCOS had a shorter isovolumic relaxation time than both other groups.

The effects of insulin resistance and PCOS on wave intensity measures

Table 5-6 shows separated WI measures according to PCOS and insulin resistance status. Measures of artery stiffness were comparable across the groups.

Compared with metabolically healthy controls, women with insulin resistance had greater energy forward compression and expansion waves from the LV, as well as greater energy backward wave reflections from the circulation. The reflection coefficient and timing of the backward compression wave were not different between groups.

Women with PCOS had WI signals that were similar to metabolically healthy controls, despite having higher levels of obesity and insulin resistance. In addition, those with PCOS had significantly lower energy forward expansion waves and backward compression waves than those with insulin resistance, despite similar levels of obesity and insulin resistance. These data are also illustrated in Figure 5-6 for clarity.

Table 5-5. Standard measures of cardiovascular function and elastance in the study population when grouped by PCOS status and insulin resistance.

	Control (PCOS-IR-) (n=50)	**Insulin Resistance (PCOS-IR+) (n=29)**	**IR vs. Control**	**PCOS (PCOS+IR+) (n=43)**	**PCOS vs. Control**	**PCOS vs. IR**
Heart rate (bpm)	67 ± 9	75 ± 12	**.004**	75 ± 10	**.002**	.995
bSBP (mmHg)	109 ± 10	117 ± 9	**.004**	113 ± 8	.170	.343
bDBP (mmHg)	68 ± 9	66 ± 7	.331	66 ± 9	.338	.996
cSBP (mmHg)	93 ± 11	99 ± 9	**.048**	96 ± 9	.558	.458
cDBP (mmHg)	63 ± 10	67 ± 8	.265	67 ± 9	.160	1.0
SVR (dynes/s/cm^5)	1512 ± 343	1419 ± 296	.581	1356 ± 280	.074	.817
SAC (ml/mmHg)	1.39 ± 0.31	1.29 ± 0.26	.488	1.40 ± 0.28	.999	.418
ccIMT (mm)*	0.51 ± 0.06	0.51 ± 0.06	.966	0.50 ± 0.07	.914	.725
AIx -75 (%)	2.7 ± 15.4	7.1 ± 14.1	.475	3.2 ± 11.0	.997	.587
PWV (m/s)*	6.15 ± 0.89	6.59 ± 0.88	.109	6.34 ± 0.92	.704	.571
RWT*	0.36 ± 0.06	0.39 ± 0.07	.286	0.36 ± 0.05	1.0	.301
LV mass I (g/m$^{2.7}$)*	32 ± 8	36 ± 8	.127	34 ± 7	.490	.786
FS (%)	34 ± 5	34 ± 6	.997	35 ± 5	.460	.709
s′ (cm/s)*	9.7 ± 1.6	10.2 ± 2.1	.779	9.8 ± 1.6	.999	.868
MAPSE (mm)	1.33 ± 0.13	1.34 ± 0.15	.961	1.36 ± 0.14	.620	.941
e′/a′*	1.9 ± 0.6	1.8 ± 0.6	.629	1.8 ± 0.1	.686	.998
IVRT (ms)	85 ± 9	82 ± 10	.539	78 ± 11	**.012**	.553
FPV (cm/s)	56 ± 10	59 ± 11	.700	58 ± 11	.913	.967
E_aI (mmHg/ml/m$^{2.4}$)*	3.1 ± 0.5	3.8 ± 0.8	**<.0001**	3.6 ± 0.9	**.017**	.494
$E_{es}I$ (mmHg/ml/m$^{2.1}$)*	2.6 ± 0.6	3.1 ± 0.8	**.031**	3.1 ± 0.8	**.008**	1.0
VAC	1.1 ± 0.2	1.1 ± 0.2	.997	1.1 ± 0.2	.273	.533

B: brachial, c: central, SBP: systolic blood pressure, DBP: diastolic blood pressure, SVR: systemic vascular resistance, SAC: systemic arterial compliance, ccIMT: common carotid intima-media thickness, AIx-75bpm: augmentation index for a heart rate of 75bpm, PWV: aortic pulse wave velocity, RWT: LV relative wall thickness, FS: fractional shortening, MAPSE: mitral annular plane systolic excursion, IVRT: isovolumic relaxation time, FPV: flow propagation velocity, E_aI: scaled arterial elastance, $E_{es}I$: scaled ventricular elastance, VAC: ventricular-arterial coupling ratio. Variables were transformed using natural log before use in ANOVA.*

Table 5-6. Selected separated wave measures (mean ± s.d.) of the study population group by PCOS status and/or HOMA-IR > 2.5.

	Control (PCOS-IR-)	**Insulin Resistance (PCOS-IR+)**	**IR vs. Control p-value**	**PCOS (PCOS+IR+)**	**PCOS vs. Control p-value**	**PCOS vs. IR p-value**
Wave speed (m/s)*	3.8 ± 3.7	2.9 ± 0.8	.418	3.4 ± 1.5	1.0	.480
Beta index*	5.5 ± 1.3	5.6 ± 1.3	.996	5.3 ± 1.4	.742	.688
Epsilon (kPa)*	63 ± 16	68 ± 19	.569	61 ± 18	.961	.337
AIx (%)	2.8 ± 9.8	2.3 ± 9.5	.996	4.9 ± 7.8	.657	.615
FCWi (J/m^2)*	526 ± 209	628 ± 170	**.027**	532 ± 144	.929	.122
BCWi (J/m^2)*	219 ± 79	289 ± 123	**.020**	216 ± 87	.996	**.014**
R wave to BCW (ms)*	139 ± 22	133 ± 20	.614	135 ± 13	.746	.989
BCWi/FCWi (%)	42 ± 10	46 ± 17	.582	40 ± 9	.647	.116
FEWi (J/m^2)*	74 ± 39	98 ± 32	**.001**	75 ± 25	.845	**.015**

*AIx: local augmentation index, FCW: forward compression wave, BCW: backward compression wave, FEW: forward expansion wave, i: integral. *Variables were transformed using natural log before use in ANOVA.*

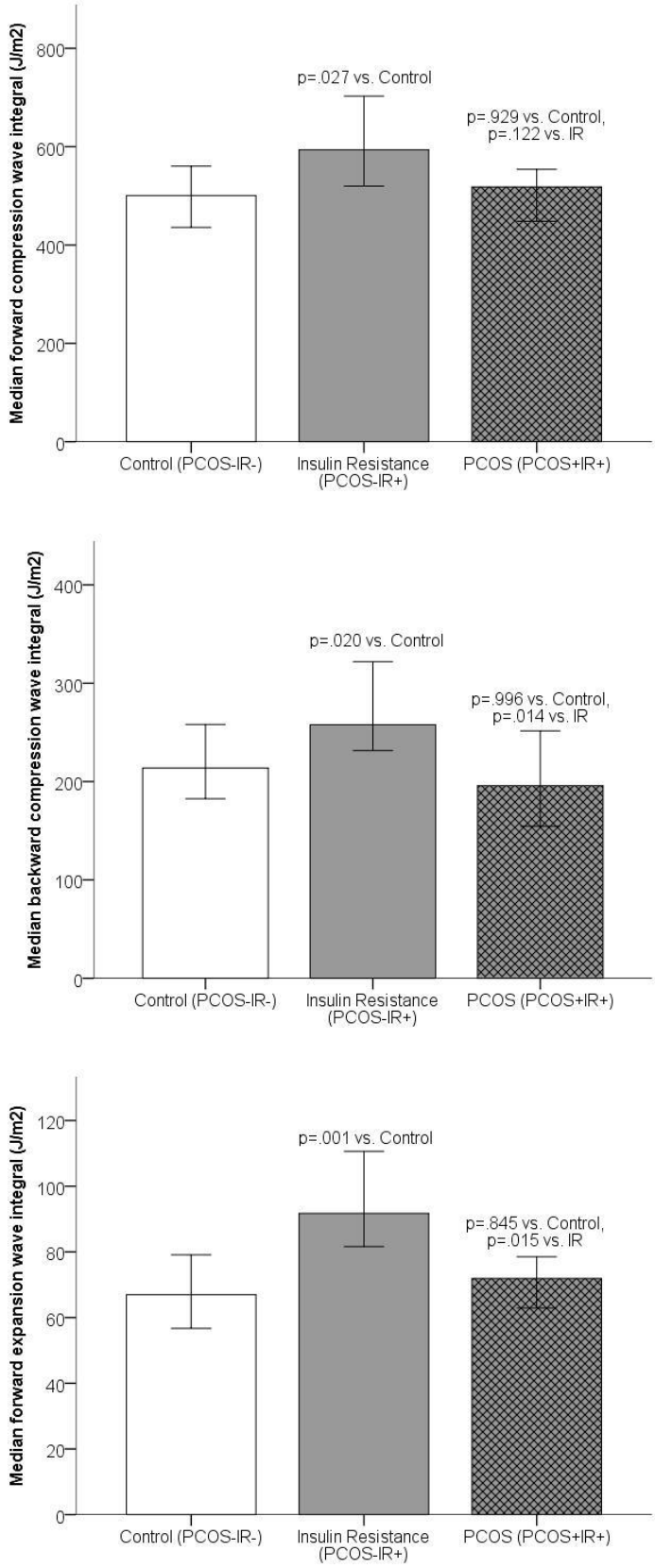

Figure 5-6. Median forward compression, backward compression and forward expansion wave by study group. Error bars give 95% CI.

Associations of separated wave intensity signals with tonometry and echo measures

To improve my understanding of the additional information carried by WI signals, I explored the associations of selected separated signals with other conventional measures of artery structure, regional artery stiffness, ventricular structure, systolic function, and diastolic function, as well as the measures of arterial and ventricular elastance and the coupling ratio calculated in Chapter 4. These data are shown in Tables 5-7 and 5-8.

Waves generated by the left ventricle: The forward compression wave occurs in the carotid artery at the start of ventricular ejection and is an indication of the energy transmitted from the LV into the conduit arteries. It causes an increase in aortic flow velocity as well as an increase in pressure. The timing of the wave means that it has yet to be affected by wave reflections. As such, I expected it to be associated with measures of systolic ventricular function, heart rate, and static vascular resistance. The data confirmed that the most important associations of the separated forward compression wave integral were heart rate, brachial pulse pressure, arterial elastance and longitudinal systolic function (direct associations). The wave was inversely associated with systemic vascular resistance and local wave speed. There was no significant association with fractional shortening or with ventricular elastance.

The forward expansion wave occurs in the carotid artery towards the end of ejection. It is caused by the drop in left ventricular pressure in the protodiastolic period and causes a deceleration of aortic blood flow (as well as a drop in aortic pressure). Since this wave occurs late in systole, I anticipated that it might be associated with measures of pulsatile as well as static loading. I also expected it to be related to early diastolic function. The data confirmed that the separated forward expansion wave integral was directly associated with peripheral and central pulse pressure and mean arterial pressure, arterial end-systolic elastance, regional stiffness (aortic pulse wave velocity), heart rate and epsilon (a measure of local stiffness affected by blood pressure). The wave was inversely associated with local wave speed. I found a direct association of the forward expansion wave integral with left ventricular mass but no significant associations with diastolic function.

Table 5-7. Associations of selected separated wave intensity signals [Spearman's Rho (p-value)] with other measures of artery stiffness and function.

	FCWi	FEWi	BCWi	BCWi/FCWi	R-BCW
Heart rate	**.23 (.009)**	**.20 (.020)**	.13 (.140)	−.09 (.317)	**−.23 (.009)**
bPP	**.36 (<.0001)**	**.30 (<.0001)**	**.30 (<.0001)**	.03 (.776)	.02 (.827)
cPP	.14 (.118)	.16 (.060)	.07 (.435)	−.04 (.659)	−.09 (.291)
SVR	**−.25 (.005)**	−.11 (.207)	**−.24 (.006)**	−.07 (.410)	**−.19 (.031)**
E_aI	**.18 (.034)**	**.27 (.002)**	.13 (.130)	<.01 (.970)	−.05 (.563)
bMAP	.16 (.064)	**.28 (.001)**	.09 (.286)	.02 (.858)	−.06 (.475)
cMAP	.08 (.343)	**.22 (.013)**	.01 (.876)	−.04 (.680)	−.11 (.208)
SAC	−.15 (.085)	−.17 (.052)	−.07 (.393)	.11 (.195)	−.08 (.339)
AIx-75	**−.21 (.018)**	−.02 (.843)	**−.31 (<.0001)**	**−.24 (.005)**	**−.27 (.001)**
ccIMT	.09 (.307)	.05 (.589)	.10 (.246)	.09 (.313)	−.15 (.077)
PWV	.05 (.551)	**.23 (.007)**	−.06 (.517)	−.15 (.092)	**−.21 (.019)**
WS	**−.25 (.003)**	**−.24 (.005)**	**−.45 (<.0001)**	**−.54 (<.0001)**	.04 (.619)
β	−.04 (.611)	.07 (.431)	−.14 (.116)	**−.17 (.046)**	.01 (.905)
ε	.05 (.605)	.17 (.049)	−.04 (.688)	−.10 (.261)	−.04 (.626)

b:brachial, c: central, PP: pulse pressure, SVR: systemic vascular resistance, E_aI: scaled arterial elastance, MAP: mean arterial pressure, SAC: systemic arterial compliance, AIx-75: augmentation index corrected for a heart rate of 75bpm, ccIMT: common carotid intima-media thickness, PWV: pulse wave velocity, WS: wave speed, β: beta stiffness index, ε: epsilon, FCW: forward compression wave, BCW: backward compression wave, FEW: forward expansion wave, and i: integral.

Table 5-8. Univariate correlations of selected separated wave intensity signals [Spearman's Rho (p-value)] with echocardiographic variables, ventricular elastance and coupling ratio.

	FCWi	**FEWi**	**BCWi**	**BCWi/FCWi**	**R-BCW**
LVIDd	.13 (.147)	.17 (.053)	**.23 (.009)**	**.21 (.018)**	**.20 (.023)**
RWT	<.01 (.994)	.05 (.545)	.02 (.822)	**.13** (.137)	−.05 (.580)
LV mass	.10 (.244)	**.24 (.007)**	**.23 (.008)**	**.33 (<.0001)**	.17 (.051)
FS	.07 (.430)	−.05 (.611)	−.01 (.948)	−.13 (.136)	**−.45 (<.0001)**
s′	**.27 (.003)**	.07 (.446)	.16 (.087)	−.10 (.288)	**−.37 (<.0001)**
MAPSE	.18 (.040)	.07 (.429)	.17 (.059)	.05 (.590)	**−.20 (.022)**
$E_{es}I$	.13 (.160)	.11 (.214)	.04 (.694)	−.09 (.299)	**−.38 (<.0001)**
VAC	<.01 (.989)	.13 (.133)	.08 (.386)	.14 (.122)	**.48 (<.0001)**
e′	.16 (.094)	.07 (.478)	.12 (.196)	−.05 (.595)	.06 (.532)
a′	.13 (.176)	.17 (.075)	.02 (.858)	−.12 (.200)	**−.38 (<.0001)**
e′/a′	.03 (.755)	−.05 (.578)	.07 (.458)	.04 (.695)	**.29 (.002)**
IVRT	−.12 (.192)	.03 (.731)	.01 (.923)	**.19 (.028)**	**.20 (.026)**
FPV	.13 (.202)	−.07 (.454)	.08 (.401)	−.05 (.599)	−.04 (.718)

LVIDd: left ventricle internal dimension in diastole, RWT: relative wall thickness, FS: fractional shortening, s': longitudinal systolic tissue velocity, $E_{es}I$: scaled ventricular elastance, VAC: ventricular arterial coupling ratio (E_a/E_{es}), e': early diastolic tissue velocity, a': late diastolic tissue velocity, IVRT: isovolumic relaxation time, FCW: forward compression wave, BCW: backward compression wave, FEW: forward expansion wave, and i: integral.

Wave reflections from the arterial circulation: The backward compression wave occurs in the carotid artery in mid-systole and is an indication of the energy reflected backward to the heart from the circulation. It causes an increase in artery pressure but, due to its backward nature, a decrease in flow velocity. Therefore, it adds to the resistance or loading that the LV has to overcome to continue ejecting blood. Since this wave is a reflection of the preceding forward compression wave I expected it to be associated with systemic vascular resistance and measures of systolic function. In addition, I expected direct relationships with measures of artery stiffness and surrogate measures of pulsatile loading such as mean arterial pressure and augmentation index as well as with left ventricular size and mass.

The data confirmed that the backward compression wave integral was inversely associated with the local wave speed, central augmentation index, and systemic vascular resistance. There was no significant relationship with mean arterial pressure or arterial compliance. The direction of these associations was the same as for the forward compression wave and is likely dominated by that shared factor. I found evidence of a direct association between the backward compression wave integral and left ventricular mass.

When I normalised the backward compression wave with the integral of the forward compression wave, the dominant inverse association with local wave speed remained, as did the direct associations with left ventricular size and mass. A new direct association emerged between this reflection coefficient and the isovolumic relaxation time indicating that higher pulsatile loading was associated with lengthening of this relaxation period. I was surprised that the reflection coefficient was *inversely* associated with the augmentation index since this has been proposed as a measure of the effect of wave reflections by others.

Timing of wave reflections: The timing of reflections back towards the heart was assessed by measuring the time from the R wave of the ECG to the peak of the backward compression wave. The strongest correlates of the reflected compression wave timing were the ventricular-arterial coupling ratio (direct) and fractional shortening (inverse). There were also inverse associations with other measures of systolic function, ventricular elastance, the late diastolic tissue velocity, heart rate, augmentation index and pulse wave velocity.

Independent contributions of wave intensity measures to left ventricular mass

In Chapter 3, the variation in LV mass was predicted by a model that included weight, systolic blood pressure and common carotid intima-media thickness (r^2 = .503, p<.0001). I tested whether adding measures of elastance and WI improved the model and independently explained some of the variation in LV mass. These variables were added to the second block of a hierarchical model using a stepwise approach.

The compression wave reflection coefficient, timing of the backward compression wave, intrinsic local artery stiffness and the scaled arterial elastance to the model explained a further 15% of the variation in left ventricular mass (r^2 = .651, p<.0001). The final model is shown in Table 5-9. Weight explained most of the variation in LV mass but similar independent contributions were made by the other variables. Arterial elastance had an unexpected inverse association with LV mass in the final model after adjustment for other factors.

No independent contribution of wave intensity measures to diastolic function

In Chapter 3, the variation in diastolic function (e′/a′) was explained reasonably well by a basic model comprising age, visceral fat area, insulin area under the curve and systolic blood pressure (r^2 = .585, p<.0001). I tested whether adding measures of arterial elastance, ventricular arterial coupling or the timing of the backward compression wave improved the model and independently explained some of the variation in diastolic function (e′/a′). These variables were added to the second block of a hierarchical model using a stepwise approach. This approach did not result in an improved model. When visceral fat and insulin resistance were removed from the model, the measures of elastance and ventricular arterial coupling were independent predictors but the model explained less of the variation in diastolic function (r^2= .445, p<.0001).

Therefore, the components of the model remain as shown in Chapter 3 although the relevant contributions are slightly altered because this iteration only included those in whom WI measures were feasible (Table 5-10). Age explained most of the variation in diastolic function, followed by insulin area under the curve, visceral fat and central systolic blood pressure.

Table 5-9. Final multivariate regression model for left ventricular mass.

*Dependent variable: LV mass**	**Standardised β**	**P-value**	**Part correlations**
Weight*	.671	<.0001	.488
E_aI*	-.255	.001	-.184
BCWi/FCWi*	.237	<.0001	.226
ccIMT*	.186	.004	.159
Local β stiffness index*	.203	.003	.164
R-BCW*	.224	<.0001	.214
Central systolic BP	.186	.009	.144
Model r^2 = .650, p<.0001			

*E_aI: scaled arterial elastance, BCWi: backward compression wave integral, FCWi: forward compression wave integral, ccIMT: common carotid intima-media thickness, R-BCW: time from R wave of ECG to peak of backward compression wave. *Variables were transformed by taking the natural log prior to use in multivariate regression.*

Table 5-10. Final multivariate regression model for e′/a′.

Dependent variable: e'/a'	**Standardised β**	**P-value**	**Part correlation**
Age*	-.492	<.0001	-.420
Insulin AUC*	-.262	.001	-.241
Visceral fat area*	-.193	.020	-.165
Central systolic BP	-.161	.049	-.139
Model r^2 = .555, p<.0001			

*AUC: area under curve. *Variables were transformed by taking the natural log prior to use in multivariate regression*

Discussion

Feasibility and reproducibility

Carotid ultrasound images were obtained by an operator who was experienced in performing cardiac ultrasound but a novice to vascular ultrasound. Despite this, WI measures were feasible in the majority of subjects regardless of the presence of obesity. None of the published studies explicitly document the feasibility of the technique but the implication in each paper is that feasibility was 100%. However, the majority of studies elected to use data from either the left or right common carotid artery. The feasibility in my study may by lower (90%) because I elected to take an average of the two sides in an effort to understand the general effects of obesity and PCOS on ventricular-arterial coupling in conduit arteries. In addition, I believed that averaging the measures would reduce error associated with noisy data.

The repeatability and reproducibility of WI measures in our lab is comparable with figures in the published literature.[141, 144, 146, 148, 151] There is consistently less variation in the larger amplitude forward compression wave compared with the smaller forward expansion wave and wave reflections. Others have reported that the variation is likely related to physiological variability of pressure and flow velocity signals rather than systematic bias.[146] This variability in data may affect the sensitivity of WI measures to detect subtle but significant between-group differences in the sub-clinical phases of disease. Nevertheless, I was able to detect between-group differences using this technique.

Net wave intensity indices

There is evidence that the forward compression wave which dominates the first net WI signal, W_1, is directly associated with local augmentation index [156] and that central augmentation index is associated with height.[221] Therefore, I anticipated that net WI measures would be associated with height or body surface area in lean control subjects. This was not the case so I used unscaled measures in between-group analyses. My findings are in agreement with Hughes *et al.* who used different methods to derive a wave reflection coefficient but also reported no association with

height in a small study of healthy, normotensive individuals (n=65).[144] This should be confirmed in a larger study of the healthy, community-dwelling population.

The mean values of net WI in the lean control group were higher than the normal values reported by others who used similar methods but subject characteristics are not reported in their paper and there is no information about how the 'healthy status' of volunteers was assured.[146] As a result, it was difficult to suggest reasons for the discrepancy. It is unlikely that the differences are due to the all-female population in my study since women tend to have lower WI values than men. The subjects in my study were comprehensively characterised in terms of metabolic, anthropometric and cardiovascular health so I consider that the data accurately reflect normal values in a young and healthy female population.

PCOS and general obesity did not affect net wave intensity measures

Neither PCOS nor general obesity was associated with altered net WI signals. This may suggest that net WI signals, which sum forward and backward components at any given point in the cardiac cycle, are not sufficiently precise or sensitive to detect sub-clinical differences attributable to increased body size. I considered whether the lack of between-group difference may be attributable to the study design; the initial power calculation suggested a sample size sufficient to detect differences in standard tonometric and echocardiographic variables. Wave intensity signals were not among the primary outcome measures and there is typically greater physiological variation in the signals. It may be that a greater sample size is needed to reach sufficient power for these measures.

Insulin resistance due to obesity did not affect local artery stiffness

In Chapter 3 I found that regional arterial stiffness (aortic pulse wave velocity) was increased in obese subjects without PCOS compared with controls. However, aortic pulse wave velocity is not a true measure of the intrinsic stiffness of the artery since it is affected by blood pressure (which tends to be increased in obesity), ejection time and the early systolic rise in left ventricular pressure (dP/dT).[222]

A key finding of this study is that women with insulin resistance due to obesity had similar intrinsic carotid artery stiffness (β stiffness index) to controls.

In the pooled data, there were no associations of the beta stiffness index with measures of obesity or insulin resistance. In contrast, epsilon was associated with central obesity but not insulin resistance. I found a trend towards higher epsilon measures in those with insulin resistance compared with controls but this was not significant.

These findings differ from Malshi *et al*. who report an increase in carotid artery stiffness measures in overweight (n=23) and obese (n=57) subjects compared with controls (n=25).[158] However, details of the mean stiffness measures and the statistical significance of these are not reported in the brief conference proceedings. In addition, the subjects were drawn from a wider age range (18-68 years) than in my study and it is unclear whether their groups were matched for age. The same group also found that in a small, predominantly female population (n=33, 9 males), obesity tended to cause a non-significant increase in beta and epsilon.[159] It seems intuitive that obese subjects are likely to have elevated epsilon and local augmentation index since these are affected by blood pressure [148] which tends to be higher in obesity.

In my study the standard deviation of local augmentation index, another measure of local artery stiffness, was wide in comparison with the mean (e.g. in controls it was 2.75 ± 9.80 %). This is likely to affect the ability to detect small between-group differences.

Insulin resistance due to obesity was associated with increased wave intensity signals

A key finding of this study is that obese women with insulin resistance had increased amplitude separated wave intensity signals compared with age-matched controls. These changes suggest increased energy transmitted from the LV into the circulation, and increased reflections from the circulation which augment pulsatile loading of the LV.

The forward compression wave is initiated by ventricular ejection and arrives in the common carotid artery shortly after the aortic valve opens. It correlates strongly with the rise in left ventricular pressure during early systole (dP/dT) [147] which creates the momentum to move blood (stroke volume) into the aorta. This rise in ventricular pressure generates a wave that propagates into the circulation at a rate

faster than the blood travels. Therefore, the forward compression wave can be thought of as a wave that 'pushes' blood forward into the circulation – it is associated with an increase in both pressure and flow at the measurement site. The increase in arterial pressure must be overcome by the LV as it continues to eject so the forward compression wave contributes to early systolic loading of the LV.

Wave intensity is the flux of energy per unit area carried by such waves and is defined by the product of instantaneous changes of pressure and flow velocity (dP.dU). This gives information about the amplitude of the wave in units of W/m^2 but others have suggested that the duration of the wave may be as important as the amplitude, therefore I chose to focus my analysis on the *integral* of the forward compression wave (the area under the wave) which has units of J/m^2.[135]

In my study obese women with insulin resistance had higher forward compression wave integral than metabolically healthy controls. The mechanisms underlying this increase are likely to include sympathetic nervous system overactivity because of hyperinsulinaemia, as well as decreased systemic vascular resistance which is a feature of general obesity in the absence of hypertension.[223] Indeed, I confirmed significant associations with heart rate (direct), systolic longitudinal tissue velocity (direct), and systemic vascular resistance (inverse). A similar effect of increased adrenergic activity and vasodilation on the forward compression wave has been described in a canine experimental study using dobutamine and nitroglycerin,[139] and in a clinical study where caffeine was found to have a positive inotropic effect.[153]

My data add to those of Tian *et al.* who describe a significant increase in W_1 of patients with type 2 diabetes compared with controls and higher values still in those with diabetes and hypertension.[224] In addition, Avgeropoulou *et al.* describe an increase in W_1 in subjects with type 2 diabetes and hypertension (n=65 with type 2 diabetes compared with 57 controls), some of whom were taking cardiovascular medication.[148] While I found that net WI signals were insensitive to sub-clinical cardiovascular changes, I demonstrated an increased separated forward compression wave integral in obese women with pre-clinical levels of dysglycaemia who were normotensive.

I was rather surprised that the forward compression wave was not associated with other echocardiographic measures of systolic function (beyond s'), or ventricular end-systolic elastance and cannot explain this. To the best of my knowledge I am the first to explore relationships between echocardiographic measures of ventricular function and separated WI signals so there are no other studies from which to draw comparisons.

The forward compression wave is reflected back toward the heart from sites of impedance mismatch in the arterial system. Impedance mismatches tend to occur at bifurcations where there is a change in vessel diameter or at the resistance vessels (arterioles).[135] The reflected wave is of the backward compression type which has the effect of pushing back towards the heart in mid-systole, causing an increase in pressure and opposing ejection. Therefore, this wave increases the pulsatile loading on the LV and is affected by the amplitude of the forward compression wave, as well as by artery stiffness.[156]

In my study obese women with insulin resistance had higher backward compression wave integral than metabolically healthy controls. This appeared to be largely explained by the increase in the preceding forward compression wave since the two were highly correlated ($r = .853$, $p<.0001$), and there were no between-group differences in the reflection coefficient which normalizes the backward compression wave by the integral of the forward compression wave. Furthermore, measures of obesity and insulin resistance were not related to the reflection coefficient in my study population. While this suggests that obesity and insulin resistance do not affect the degree of impedance mismatch at reflection sites, the very fact that the percentage reflection does not increase, suggests that a greater energy may be transmitted to the distal circulation. It is tempting to speculate that this might have implications for the microvasculature and this is worthy of further study. In addition, a larger forward compression wave with an unchanged reflection coefficient will result in an increase in the absolute energy of the backward compression wave so that pulsatile loading is increased.

Roberts *et al*. describe an increased separated backward compression wave in 18 older patients (mean age 67 years) with newly diagnosed type 2 diabetes who were naïve to treatment compared with age comparable controls.[225] My data add to these

findings because I found an increased backward compression wave integral in young obese women with pre-clinical levels of abnormal glucose handling.

My data showed that that the reflection coefficient was *inversely* associated with the central augmentation index. This appears counterintuitive since the augmentation index has been proposed as a surrogate marker of wave reflections, with increased augmentation reflecting increased wave reflections. Others have also shown a weak but negative association of augmentation index with the ratio of forward to backward pressure (r=-.200, p=0.1) in a group of healthy volunteers (n=65, age 21-78 years, 43 male, r=).[144] The authors suggest that the poor relationship between the two may be affected by the shape of the aortic pressure waveform and the presence of re-reflected waves in mid systole which were evident in some subjects. Together, these data suggest that the augmentation index cannot be used as a measure of wave reflection in all subjects.

The aortic pressure waveform is affected by the *timing* of reflected compression waves as well as their amplitude,[138] which suggests that both contribute to ventricular pulsatile loading. The earlier a reflected wave arrives back at the heart, the greater the maximum systolic pressure in the artery. In pooled data I found significant inverse associations between the timing of the backward compression wave and age, visceral fat and fasting glucose. In between-group analyses those with insulin resistance tended to have earlier compression wave reflections than controls but the differences were not significant. This may be because subjects were young and free from impaired fasting glucose.

The forward expansion wave occurs toward the end of systole in the protodiastolic period. At this time, the ventricular pressure begins to decrease and this decelerates the blood that is moving forward (stroke volume), eventually leading to closure of the aortic valve. This generates a wave that propagates forward into the circulation but has the effect of pulling back towards the heart, decreasing arterial pressure and flow velocity. Therefore, the forward expansion wave plays a key role in the rapid unloading of the LV at the end of ejection.

In an experimental canine study, Wang *et al*. [226] showed that the expansion wave generated by the ventricle continued through the isovolumic relaxation period until

the mitral valve opened, suggesting that the time-course of the wave was similar to that of the untwisting of the LV. In addition, the energy of the forward expansion wave was related to LV end-systolic volume as well as the rate at which the ventricle relaxed. Finally, the authors found that while early diastolic filling of the LV was largely dependent on passive decompression of the left atrium, it was also affected by the early diastolic suction of the LV.

In my study, young women with insulin resistance and obesity had increased forward expansion wave integral compared with metabolically healthy controls. This increase is intuitive given increase in the forward compression wave. The rate of decline in left ventricular pressure (and therefore the deceleration in flow) is likely affected to some degree by the preceding rate of increase in left ventricular pressure. Therefore, an increase in forward compression wave should be followed by an increased forward expansion wave and it might be expected that both are affected by adrenergic state. Indeed, I found direct associations of both forward waves with heart rate and arterial elastance which is affected by vasomotor tone. Furthermore, the associations of forward waves with visceral fat and insulin resistance were no longer significant when heart rate was controlled in the analysis (data not presented).

In Chapter 3 I found that obese women had worse diastolic function than controls. Therefore, I was surprised that the forward expansion wave integral was not associated with measures of diastolic function in the pooled data but my results are comparable with other limited reports in the published literature.[227] I found that when the forward expansion wave integral was divided by the forward compression wave integral, an inverse association was revealed with the flow propagation velocity, a measure of early diastolic function which reflects LV suction (r= -.238, p=.014). This may suggest that a *relative* increase in the forward expansion wave is associated with poorer early diastolic function of the LV.

One study has shown that the net W_2 signal (which is dominated by the forward expansion wave) tended to be increased in patients with type 2 diabetes but the differences were not statistically significant.[148] These subjects also had an increase in W_1. However, over half of the patients with diabetes had abnormal diastolic function according to age-corrected cut off velocities and it may be that the forward expansion wave is increased early in the course of disease then declines with the

advent of clinically relevant diastolic dysfunction. If this was true then including subjects with subclinical and more advanced disease would unwittingly negate significant differences in the WI signal.

Arterial elastance and wave intensity signals independently explain some of the variation in left ventricular mass

A key finding of this study is that arterial elastance, the normalised backward compression wave and the timing of the backward compression wave independently explained some of the variation in left ventricular mass in a model that included weight and systolic blood pressure.

There is a general consensus that obese subjects are more likely to develop LV hypertrophy than lean counterparts. A systematic review of echo studies used to identify left ventricular hypertrophy in obese subjects confirmed an odds ratio of 4.2 in obese compared with non-obese counterparts.[45] In addition, body size (BMI) was directly associated with LV hypertrophy and the eccentric pattern of hypertrophy was more prevalent than a concentric pattern (66 vs. 34%, $p<.01$). However, of the 15 studies and ~5,000 obese subjects included in the review, 70% were hypertensive, 50% were taking cardiovascular medication and 10% had type 2 diabetes. These confounding factors are likely to influence the development of hypertrophy so it is unclear whether the results are applicable to those who are at an earlier stage in the disease process.

There is recent evidence from the Multi-Ethnic Study of Atherosclerosis (MESA) that both pulsatile and static loading independently contribute to LV mass, with the former explaining a greater proportion of the variation.[161] The methods included cardiac magnetic resonance imaging to determine LV mass and arterial tonometry to derive forward and backward pressure waves. The backward pressure wave, which occurred in mid-late systole, was the main correlate of LV mass, emphasizing the importance of the time-course of loading in the development of hypertrophy. By design, subjects in the study were older (mean age 61.3±10.1 years) and included those with hypertension (42%) and diabetes (12%).

My data add to the findings of these studies by confirming that body weight was the main contributor to the variation in LV mass in a young female population

without hypertension or diabetes, and who were not taking cardiovascular medication. I also found that the eccentric pattern of hypertrophy was more prevalent. In addition, I demonstrated that WI measures of local artery stiffness and mid-late systolic pulsatile loading independently explained some of the variation in mass when weight and systolic blood pressure were included in the model. These aspects of pulsatile loading were related to central obesity and insulin resistance and it is tempting to speculate that these are more likely to result in the development of a concentric pattern of hypertrophy.

Central obesity independently contributes to the variation in diastolic function but wave intensity signals do not improve the model

In Chapter 3 I found that a significant proportion of the variation in diastolic function (e′/a′) could be explained by age, regional artery stiffness (aortic pulse wave velocity) and visceral fat.

A key finding of this study is that when subjects are grouped by BMI, those who are obese have poorer diastolic function than controls, but when subjects are grouped by insulin resistance the differences did not reach significance. In addition, measures of arterial elastance and wave intensity did not independently explain any of the variation in diastolic function when added to the model.

These findings may seem incongruent with those of Chapter 3, but an explanation may lie in the possibility that diastolic function was impaired because of increased epicardial fat rather than alterations in ventricular afterload. Epicardial fat is the visceral fat around the heart and there is evidence from magnetic resonance imaging that epicardial and abdominal visceral fat are strongly associated (r=.864, p=.01).[66]

There is some evidence in the literature that epicardial fat can be reliably measured from echocardiographic images.[66] I reviewed the stored images from my study but could not perform this measurement because images had not been optimised to provide maximum resolution in the near-field.

The mechanisms linking epicardial fat and diastolic function are yet to be elucidated. It is possible that epicardial fat may affect the left ventricular transmural

pressure in early diastole, impeding passive ventricular filling with the consequence of increased left atrial pressure. This, in turn, would generate a larger contribution to diastolic ventricular filling during the subsequent atrial contraction (as noted in my data). In addition, epicardial fat is anatomically contiguous with the myocardium (there is no dividing muscle fascia) and functions as an endocrine and paracrine organ, secreting bioactive molecules such as inflammatory cytokines. Therefore, epicardial fat may also affect left ventricular function through biochemical pathways.[64]

Women with PCOS have normal wave intensity signals despite obesity and insulin resistance

I expected to find similar energy WI signals in women with PCOS as in the insulin resistance group. Both groups had comparable levels of central obesity and women with PCOS had worse insulin resistance; features which were directly associated with WI signals in the pooled data.

A key finding of this study is that women with PCOS had wave intensity signals that were lower than counterparts in the insulin resistance group who were free from PCOS. In fact, wave intensity signals in the PCOS group were similar to metabolically healthy controls, despite the presence of obesity and insulin resistance.

I believe this adds further evidence to my hypothesis that women with PCOS are somehow protected from the deleterious effects of obesity on cardiovascular haemodynamics. In particular, it appears that pulsatile loading is mitigated by some feature of PCOS since arterial and ventricular elastance were elevated in this group.

An alternative explanation would be that the intensities were reduced because of poorer ventricular systolic function but I performed comprehensive echocardiograms and could find no evidence of this. Others have similarly shown that left ventricular function is similar in obese women with PCOS and insulin resistance compared with obese controls with insulin resistance.[98]

Given the lower prevalence of left ventricular hypertrophy in women with PCOS in my study, it is tempting to speculate that the lower pulsatile loading of the

ventricle in PCOS is, at least in part, delaying the development of left ventricular hypertrophy.

Since measures of pulsatile loading were not independent contributors to diastolic function, the effects of central obesity on diastolic function should be comparable in those with PCOS and in controls. This was evident in my data and it is worth noting that increased ventricular mass was not an independent predictor of diastolic function in the subclinical phase of disease of these young women.

Limitations

Carotid ultrasound images and WI analysis were performed by a novice to vascular ultrasound (although experienced in echoes) after a short training period. This may have reduced feasibility and led to greater variability in data. In addition, WI measures were not among the primary outcome measures. Nevertheless, I was able to detect between-group differences in WI associated with subclinical levels of disease.

The large physiological variability in WI signals means that this technique may not be suitable for the clinical environment but I am not proposing that it should be used in this way. My aim was to use the technique to better understand the effects of obesity and PCOS on cardiovascular haemodynamics and in that respect I believe the technique has led to new insights, particularly in relation to women with PCOS.

I would have liked to explore whether separated WI measures could explain the variation in hypertrophy patterns but this was not possible because of the small numbers of women with hypertrophy in the study. This could easily be addressed in a larger study of those who are further along the disease pathway although the confounding influence of hypertension may impact the results.

The case-control nature of this study means that I cannot infer causality between central obesity, pulsatile loading and left ventricular hypertrophy. However, my data suggest that this could be explored in a longitudinal study with an intervention that results in improved insulin sensitivity, lower central obesity or reduced sympathetic activity.

Conclusions

My data suggest that young women with uncomplicated obesity have evidence of altered pulsatile loading of the LV.

In the rapid ejection phase the forward compression wave was increased, and this is likely to be attributable to sympathetic overactivity. This increase in early systolic loading was not independently associated with ventricular mass. The backward compression wave in mid-ejection was increased, despite an absence of between-group differences in intrinsic artery stiffness, and represents increased mid-systolic loading of the LV. The timing and amplitude of the backward compression wave were independent contributors to the variation in left ventricular mass confirming that mid-late systolic loading is important in the development of hypertrophy.

The reflection coefficient and timing of the backward compression wave were not significantly different in obese women in my study. This supports the finding that ventricular-arterial coupling is not altered in obesity when there are no confounding influences of hypertension or impaired fasting glucose.

My hypothesis that women with obesity would have a decreased forward expansion wave associated with diastolic function was not supported by the data. I found that the forward expansion wave was increased in obesity and that it was not associated with diastolic function. However, when the wave was normalised for the forward compression wave there was an inverse association with the flow propagation velocity through the valve (a measure which represents diastolic suction of the LV). It may be that the normalised forward expansion wave provides information about early diastolic suction of the LV and this is worthy of further study.

An important finding of this study is that women with PCOS had lower WI signals than age and BMI-matched controls with similar levels of obesity and insulin resistance. Furthermore, the WI signals were similar to those of metabolically healthy control. These data suggest that women with PCOS who have yet to develop diabetes are protected from the increased pulsatile loading by an as yet unappreciated factor.

Analysis of separated WI signals has provided new insights to the mechanisms underpinning increased left ventricular mass in obese women. While the most important contributor to ventricular mass was weight (body size), a significant proportion of the variation was explained by WI measures of pulsatile loading, even in the absence of hypertension or increased intrinsic artery stiffness.

6. General discussion

This thesis was designed to investigate the effects of obesity and pre-diabetic states on ventricular-arterial coupling in young women. The motivation for the study came from a lack of evidence about the natural history of cardiovascular dysfunction in obese women despite epidemiological evidence of an increased risk of diabetes,[2] HFpEF [228] and cardiovascular mortality [229] in those who are older. The relative risk of cardiovascular disease is higher in those with diabetes [8] and it appears that women with diabetes have a higher relative risk of cardiovascular mortality than men.[10] Finally, there is evidence that early diagnosis and intervention are necessary because once diabetes has developed it may be too late to prevent adverse cardiovascular outcomes.[12]

LV hypertrophy [45] and diastolic dysfunction [48] are well-established consequences of obesity and potent markers of cardiovascular risk,[49] but the precise mechanisms linking uncomplicated obesity and pre-diabetic states with these features are not well defined. Early diagnosis of sub-clinical dysfunction and its precise nature may be useful in identifying those at highest risk of altered cardiovascular outcomes and may inform interventional studies. Since the relative contributions of central obesity and metabolic disturbances on ventricular loading were particularly unclear it seemed appropriate to develop a study to address this question.

The hypothesis was that quantitative measures of ventricular-arterial coupling would complement standard measures of cardiovascular function and lead to an improved understanding of sub-clinical pathophysiology.

Two non-invasive methods of quantifying the ventricular-arterial interaction were used in this work (i) arterial and ventricular end-systolic elastance and their ratio (ii) measures of carotid artery WI. These methods were chosen because they have been validated against gold-standard measurements in experimental and clinical studies and because they could be obtained from ultrasound techniques which are ideal to study cardiovascular function in a relatively healthy population.

There were three specific aims of the work. The first was to examine whether the non-invasive methods of quantifying the ventricular-arterial interaction were

sufficiently sensitive to detect sub-clinical changes in cardiovascular function associated with uncomplicated obesity and insulin resistance. The second was to determine whether measures of ventricular-arterial coupling were more strongly associated with central obesity and insulin resistance than with general obesity. The third was to determine whether measures of ventricular-arterial coupling independently contributed to left ventricular mass and diastolic function.

In the planning stages of this project an opportunity arose to collaborate with an endocrinologist who was interested in studying the effects of body composition on cardiovascular function in women with PCOS, a common condition which is associated with a high prevalence of obesity [82] and which is considered a non-modifiable risk factor for diabetes. As a result it seemed sensible to combine our planned studies to maximize the data yield from subject participation and, as a result, we obtained a very rich data-set which comprehensively characterised lean and obese women with and without PCOS.

Novel findings of this thesis

The novel findings of this thesis are summarised below.

1. Both elastance and wave intensity measures of ventricular-arterial coupling were sufficiently sensitive to detect sub-clinical differences in physiology associated with obesity and pre-diabetic states in women (Table 4-2, page 135 and Table 5-5, page 183 respectively).
2. These methods of quantifying ventricular-arterial coupling helped to explain the haemodynamic effects of general obesity, central obesity and insulin resistance on the cardiovasculature. Both general and central obesity were important contributors to the finding of increased arterial and ventricular elastance (Table 4-3, page 137). In contrast, visceral fat and insulin resistance were more important contributors to the finding of altered wave reflections which served to increase mid- to late-systolic pulsatile loading of the left ventricle (Table 5-3, page 179).
3. Measures of ventricular-arterial coupling helped to explain the finding of increased LV mass in obese young women. General obesity (weight) was the most important independent contributor to LV mass but arterial

elastance, local artery stiffness and the amplitude and timing of wave reflections also made independent contributions even after adjustment for systolic blood pressure (Table 5-9, page 192).

4. In contrast, measures of ventricular-arterial coupling did *not* help to explain the finding of worse diastolic function in this population. Neither was this explained by the finding of increased LV mass. The most important independent contributors to diastolic function after adjustment for pulse pressure and regional aortic stiffness were age, insulin levels and visceral fat (Table 5-10, page 192).
5. Obese women with PCOS had a lower odds ratio of having LV hypertrophy than obese controls despite similar body composition and worse insulin resistance (Table 3-4, page 99). This may be explained by lower amplitude wave reflections (Figure 5-6, page 185) perhaps as a result of better arterial compliance and lower intrinsic carotid artery stiffness which tended to be closer to normal values.

Themes arising from the findings

No single test fully described cardiovascular function: No single test within the protocol fully described the sub-clinical cardiovascular dysfunction associated with obesity in young women. Between-group differences in LV mass and diastolic function were evident from echocardiography but even sensitive measures of systolic function such as myocardial velocity and strain were unchanged. Non-invasive measures of systolic elastance allowed a neat and intuitive visualization of the ventricular-arterial interaction in the pressure-volume plane. The diagrams derived from these results are familiar to those working in cardiology and helped to describe the effects of obesity on blood pressure sensitivity to volume changes and stroke work. However, these diagrams are an oversimplification of the intact circulation and arterial elastance did not fully capture the effects of pulsatile loading.

Wave intensity signals added information about the amplitude and timing of pulsatile loading but these measures had considerable physiological variation and were more challenging to interpret since they reflect complex interactions and are subject to phenomena such as re-reflections and wave-trapping. While composite measures of arterial and ventricular function such as PWV and EF have established

prognostic significance and are clinically useful measures, they do not provide sufficiently detailed information to inform research studies whose aim is to define subclinical pathophysiology.

There was increased loading of the left ventricle in the absence of altered ventricular-arterial coupling *per se*: The ratio of elastances and the reflection coefficient were unaltered in obesity and, as such, the ventricular-arterial system remained 'closely-coupled' but this did not mean that ventricular loading was unaffected. The separate components of ventricular arterial coupling (arterial and ventricular systolic elastance, forward and backward compression wave) provided greater insight to the components of static and pulsatile load on the LV.

When the system is closely coupled, ejection fraction and stroke volume remain normal but at the expense of higher systolic blood pressure. In addition, a higher forward compression wave with an unchanged reflection coefficient (%) necessarily means that there is a larger backward compression wave which will augment blood pressure and oppose late systolic ejection of the LV. Furthermore, there will be a larger amplitude residual wave transmitted to the distal circulation. It is unclear whether this can be interpreted as increased pulsatile flow transmitted to the more distal circulation which could have relevance to the finding of microvascular dysfunction which is evident in more advanced diabetic states.

The late-systolic pulsatile loading appeared to be driven by increased sympathetic nervous system activation because of hyperinsulinaemia and not body size: Wave intensity measures of pulsatile loading appeared to be specifically elevated in those who were obese *and* had insulin resistance. The main determinant of the reflected compression wave was the preceding forward compression wave driven by the LV. The forward compression wave was determined by heart rate, systemic vascular resistance and LV contractile function, but only heart rate was different between IR and control groups. This suggests that heart rate and perhaps the rate of ejection are important determinants of pulsatile loading in the sub-clinical phase of disease associated with obesity. It may be worth confirming this in a larger number of subjects since pulsatile loading contributes to LV hypertrophy and there is a drug (Ivabradine) which can selectively reduce heart rate without having a negative impact on ventricular contractile function.

It is tempting to speculate that the mixed pattern of eccentric and concentric hypertrophy in obese subjects is related to whether there is significant insulin resistance and increased heart rate. This could be determined by classifying subjects by obesity and metabolic health which I did not attempt because the sample sizes would have been too small to be meaningful.

In uncomplicated obesity, diastolic dysfunction may be predominantly related to the mechanical and paracrine effects of epicardial fat or the endocrine effects of visceral fat: In this study the impaired diastolic function in obese subjects was not determined by pulsatile loading or LV mass and was more strongly associated with visceral fat than with BMI. This suggests that in the earliest stages, diastolic dysfunction may be more closely related to the mechanical and paracrine effects of epicardial fat or the endocrine effects of visceral fat, rather than to the haemodynamic effects of obesity which lead to left ventricular hypertrophy. My findings add to those of Kozakova *et al*. who also reported that diastolic function was most strongly associated with abdominal obesity and fasting insulin in obese children,[61] but they did not characterise body composition and pulsatile loading as I have.

PCOS is not a good model for obesity and insulin resistance in the general population: I had envisaged that PCOS may be a good model to study the effects of obesity and pre-diabetic conditions on cardiovascular function in women. However, my data suggest that the results from women with PCOS should not be generalised to a non-PCOS population. There appeared to be some element of PCOS which mitigates the effects of central obesity and insulin resistance on pulsatile loading and the subsequent increased LV mass. This finding may have relevance for the lack of conclusive evidence of altered cardiovascular mortality in women with PCOS.[86]

There may be a PCOS phenotype that is associated with hyperadiponectinaemia: I found that lean women with PCOS tended to have higher levels of HMW-adiponectin than lean controls despite similar body composition and worse insulin resistance. However, this group was small (n=20) and there was considerable between subject variation. The difference was not statistically significant when the parametric test ANOVA was used with post-hoc corrections for

multiple group comparisons, but the equivalent non-parametric test was significant (Kruskal-Wallis p=.006 for lean PCOS vs. lean controls).

It is unclear whether there is a specific reproductive phenotype of PCOS that is associated with hyperadiponectinaemia. Since HMW-adiponectin has insulin-sensitising, anti-inflammatory and anti-atherogenic effects,[21] it may be that those with higher values are less likely to develop significant insulin resistance and are therefore protected against the deleterious effects on cardiovascular function. In support of this I found that adiponectin was inversely associated with ccIMT, aortic PWV, LV mass, as well as scaled elastances and their ratio. In addition, it was among the independent predictors of ccIMT after adjustment for age. However, it is possible that a situation of adiponectin resistance exists (in the same way as cells can develop insulin resistance) so that higher than normal circulating levels actually indicate an already altered state. This may be an as yet unappreciated feature of milder forms of PCOS. Furthermore, others have described a paradoxical finding of *increased* cardiovascular mortality in those with higher adiponectin levels after adjustment for other risk factors,[34] This suggests that there may be circumstances where the higher adiponectin has a toxic effect or is a marker of an as yet unappreciated risk factor for disease.

This debate may be addressed by including a measure of HMW-adiponectin in a large longitudinal study of women with PCOS who are classified by reproductive phenotype. Such a study should also include comprehensive assessment of the ventricular-arterial interaction and body composition as well as having HFpEF among the key outcome measures.

Limitations

There are several limitations of this work. I chose to study women with uncomplicated obesity i.e. they were free from hypertension and diabetes. The majority of women in the study were Caucasian and all were younger than 45 years. As a result the findings may not be applicable to other populations. Women with PCOS were largely drawn from a hospital database and may not be representative of those with PCOS in the general population. The initial power calculation for the study was based on detecting clinically relevant differences in established measures

of cardiovascular function (aortic pulse wave velocity and diastolic function). Quantitative measures of ventricular-arterial coupling were not considered and there was insufficient published information in uncomplicated obesity and in PCOS to perform the calculation. Despite this, I found significant differences in obese versus lean subjects across several key measures.

It was not possible to establish whether increased arterial elastance and wave reflections were among the *causes* of increased LV mass in this case-control study and it would be useful to establish this in an interventional study.

Wave reflections in the section of aorta adjacent to the heart are likely to be most relevant to ventricular loading but I am not aware of a non-invasive method for measuring these. I analysed reflections in the common carotid artery but this has different geometry, branching morphology and wall structure from the proximal aorta. In addition, backward wave reflections in the carotid artery are from the cerebrovascular system which may have different vasomotor tone from the systemic circulation. These differences are likely to affect both the conduit function of the artery (and static resistance) as well as the cushioning or Windkessel effect of the artery (and pulsatile resistance). However, I found that the amplitude and timing of wave reflections in the carotid artery were independently associated with LV mass so they are likely to have pathological significance regardless of the mechanisms driving reflections.

I recruited a separate sample of six individuals to test reproducibility of the elastance and WI measures at two separate visits prior to much of the analysis for this thesis. These individuals were drawn from a wide range of age and BMI but they were not necessarily representative of final sample of young, female women with obesity and/or PCOS. In addition, the Aloka equipment used to measure WI developed a technical fault in the period between data collection and thesis writing. This meant that it was not possible to access the repeated WI measures of this small sample group so I have included the measures previously reported by our lab (using the same environment, equipment and software). Nevertheless, the standard deviation of measurements within the study population was sufficiently narrow to detect subtle between-group differences associated with sub-clinical disease.

I did not study the interaction effects of testosterone and adiponectin (or other metabolic factors) on cardiovascular function and this may be important in some women with PCOS.

Conclusion

This is the first study to comprehensively study the effects of uncomplicated obesity, insulin resistance and PCOS on ventricular-arterial coupling. Quantification of the ventricular-arterial interaction using ultrasound is feasible in an obese, female population and sufficiently sensitive to detect changes in the sub-clinical phase of disease. In addition, the measures independently contribute to the finding of increased LV mass in the study population who are at risk of developing HFpEF and of having a higher cardiovascular mortality later in life. Thus, the data provide new information about the early natural history of cardiovascular dysfunction which may be useful in counseling patients about life-style modifications and may inform intervention studies which seek to reverse altered static and pulsatile loading before irreversible changes in cardiovascular structure and function occur.

Publications related to this thesis

Peer-reviewed publication

Rees E, Coulson R, Dunstan F, Evans WD, Blundell HL, Luzio SD, Dunseath G, Halcox JP, Fraser AG and Rees DA. Central arterial stiffness and diastolic dysfunction are associated with insulin resistance and abdominal obesity in young women but polycystic ovary syndrome does not confer additional risk. *Human Reproduction*. 2014; 9: 2041-9. http://dx.doi.org/10.1093/humrep/deu180

Presentations to learned societies

Rees E, Rakebrandt F, Rees DA, Halcox JP and Fraser AG. Altered ventricular-arterial coupling in obese young women with insulin resistance: insights from separated wave analysis. *European Heart Journal of Cardiovascular Imaging*. 2013; 14 (Supp 2): ii85, P506. http://dx.doi.org/10.1093/ehjci/jet203

Rees E, Hocking R, Dunstan F, Lewis M, Tunstall K, Halcox JP, Fraser AG and Rees DA. Does adiponectin protect against the cardiovascular dysfunction associated with central adiposity in young women with polycystic ovary syndrome? *European Heart Journal.* 2013; 34 (Supp 1): 143, P712. http://dx.doi.org/10.1093/eurheartj/eht307.P712

Rees E, Rakebrandt F, Halcox JP, Rees DA and Fraser AG. The effect of insulin resistance on ventricular-arterial coupling: Insights from separated wave analysis in young women with and without PCOS. *Artery Research*. 2013; 7 (3-4): 151, P5.28. http://dx.doi.org/10.1016/j.artres.2013.10.176

Rees E, Hocking R, Dunstan F, Lewis M, Tunstall K, Rees DA, Halcox JP and Fraser AG. Relationship of insulin with cardiovascular dysfunction in young women with polycystic ovary syndrome. *European Heart Journal of Cardiovascular Imaging*. 2012; 13 (Supp 1): i84, P512. http://dx.doi.org/10.1093/ehjci/jes257

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