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Leptin and left ventricular mass in a South African population of African descent

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Chapter 1

Introduction

Obesity-Induced Left Ventricular Hypertrophy: A Potential Role for Leptin.

1.1 Introduction

Cardiovascular diseases (CVD) broadly incorporate disorders of the cerebral vasculature (strokes or cerebrovascular accidents and transient ischaemic attacks); of the coronary arteries (coronary artery disease [CHD] which presents clinically as angina pectoris or myocardial infarction [MI]); of the endocardium, myocardium or pericardium resulting in congestive heart failure (CHF) or cardiac arrhythmias; of the kidneys resulting in renal failure, and of the peripheral vasculature resulting in peripheral vascular disease. Cardiovascular diseases are the leading cause of morbidity and mortality in developed countries (Mancia et al 2007, Williams et al 2004, Chobanian et al 2003, Whitworth 2003) and are now considered a major cause of death and disability in developing countries (Kahn et al 1999).

Once CVD is established, a considerable decline in the quality of life and a high risk of mortality may be predicted. Consequently, an important clinical approach to CVD is primary prevention. In this regard there is an ever increasing emphasis on the early identification of patients at risk of developing CVD and instituting intervention programs at a primary care level to prevent CVD. In this regard, traditionally over the years the major risk factors that have been targeted are an increased blood pressure, diabetes mellitus, dyslipidaemia, and smoking. However, with the greater availability of technology in developed countries, there is an increasing reliance on employing more sensitive approaches to detecting early target organ changes. In this regard, as will be highlighted in the present chapter there is clear evidence that an increased left ventricular mass (LVM) is a risk factor for CVD independent of conventional cardiovascular risk factors including an increased blood pressure, diabetes mellitus, dyslipidaemia and an excess in adiposity.

At a clinical level, the electrocardiogram, which has a low sensitivity for detecting an enlarged heart, is the only tool that may be routinely used at a primary care level for detecting the presence of cardiac hypertrophy. Indeed, the chances of nation-wide screening programs using sensitive techniques for detecting an enlarged heart, such as echocardiography or magnetic resonance imaging, are small. Thus, it is important to better understand the factors that determine an increased LVM in the hopes of identifying those patients whom are most likely to have cardiac hypertrophy.

As will be further underscored in the present chapter, the major modifiable risk factors for an increased LVM are an increased blood pressure (systemic hypertension) and an excess adiposity (overweight or obesity). Presumably, one reason why LVM predicts CVD beyond measures of blood pressure and body size is that blood pressure and body size effects on LVM represent a temporal accrual of the adverse effects of blood pressure and body size on the heart. Alternatively, the impact of blood pressure and body size on LVM and thus CVD may vary from patient to patient depending on genetic and phenotypic variations between patients. Indeed, as shall be highlighted in the present chapter, although blood pressure and adiposity indices are the most consistent factors associated with an increased LVM, these two risk factors may only account for a proportion of the variability in LVM in any population-based study. In essence, blood pressure measurements and measurements of adiposity may only provide crude estimates of the real impact of these two factors on LVM. Indeed there are many potential mechanisms that could contribute toward blood pressure and adiposity-induced effects on LVM, effects that may not be accounted for by simple measures of brachial artery blood pressure or indices of adiposity.

With respect to adiposity effects on LVM, recently proposed hypotheses are that obesity mediates changes in LVM partly through either insulin-resistance (Paolisso et al 1999), obesity-induced leptin release (Paolisso et al 1999, Perego et al 2005), or leptin

resistance (Barouch 2003), changes which themselves occur to a variable extent in overweight or obese population samples. Consequently, it may be possible to more accurately predict target organ changes, such as an increased LVM, in obesity, if measures of insulin resistance or leptin concentrations were determined. However, as will be outlined in the present chapter evidence in favor of these hypotheses is controversial. Consequently, in the present dissertation I further explored the possibility that measures of circulating leptin concentrations may refine our ability to predict an increased LVM in a population sample with a high prevalence of obesity.

The present chapter serves to lead the reader through a series of arguments in favor of conducting the study outlined in the present dissertation. In this regard, in this chapter I will first review the evidence that supports an association between LVM and CVD beyond conventional risk factors. Second, I will discuss the potential determinants of LVM with a focus on the evidence of the impact of excess adiposity on LVM and the mechanisms by which an excess adiposity may produce LV hypertrophy (LVH). Last, I will critically review the evidence to implicate leptin and insulin resistance as factors which in-part explain obesity-induced effects on LVM and suggest approaches that may help resolve some of the controversies, including the value of my own study.

1.2 Left ventricular hypertrophy as an independent predictor of CVD.

Many studies now support the notion that LVH is a predictor of CV risk independent of conventional cardiovascular risk factors, including blood pressure, dyslipidaemia, diabetes mellitus and smoking (Drazner et al 2004, Okin et al 2004, Devereux et al 2004, Gardin et al 2001, Verdecchia et al 2001, Koren et al 1999, Ghali et al 1998, Verdecchia et al 1996, Levy et al 1994, Koren et al 1991, Levy et al 1990, Casale et al 1986). This notion was recognized as early as 1986 when in a study

conducted in 140 men with uncomplicated hypertension followed for a mean duration of 4.8 years, 24% of men with echocardiographic evidence of LVH as compared to 6% of men without echocardiographic evidence of LVH had morbid cardiovascular events (Casale et al 1986). These findings were subsequently supported by evidence obtained in the Framingham Heart Study (Levy et al 1990). In this study, where of the 4850 men and women recruited, 4073 were free of cardiovascular disease and 3220 had high quality echocardiograms, echocardiographic LVH was noted in 218 men (15.5%) and 382 women (21%) (Levy et al 1990). During a 4-year follow-up period, there were 208 cardiovascular events, 37 deaths from cardiovascular disease, and 124 deaths from all causes. In this study, echocardographic LVM was associated with all outcome events, a finding that persisted after adjustment for age, diastolic blood pressure, pulse pressure, treatment for hypertension, cigarette smoking, diabetes mellitus, obesity and electrocardiographic evidence of LVH (Levy et al 1990).

These observations indicating a strong prognostic power of LVH, were subsequently extended by Koren et al (1991) in a much longer follow-up study (10 years) conducted in 253 uncomplicated hypertensives in whom 31% of the men and 20% of the women had LVH according to the criteria of that period (LVM≥125 g/m^2). In this study (Koren et al 1991) cardiovascular mortality was strongly associated with LVH, and all-cause mortality was lowest in patients with normal ventricular geometry, intermediate in those with concentric remodeling or eccentric hypertrophy and highest in those with concentric hypertrophy. The notion that the type of LV remodelling in patients with LVH determines the impact of LVH on cardiovascular events was supported by the findings of Verdecchia et al (1996), whom in 274 hypertensives followed for up to 8.7 years, showed that the risk associated with concentric remodelling in the presence of normal LVM, or with concentric hypertrophy in the presence of an increase in LVM, is

higher by 1 to 1.5 major events per 100 patient-years than the risk associated with normal LV geometry or eccentric hypertrophy, respectively.

More recent prospective studies, conducted with lower blood pressure targets and in an era of more modern antihypertensive agents, have also confirmed the notion that LVH is a strong prognostic predictor. Indeed, in the Italian Multicenter and Prospective Study (Massa Ventricolare sinistra nell' Ipertensione arteriosa) conducted in patients with essential hypertension, irrespective of baseline conventional blood pressure, treatment for hypertension, age, diabetes mellitus and smoking, the presence of LVH doubled the risk for CV events such as cardiac death, MI, stroke, transient ischemic attack, heart failure, unstable angina, arterial occlusive disease and renal failure (Verdecchia et al 2001).

As originally suggested by the Framingham Heart Study (Levy et al 1990), even in the absence of systemic hypertension, LVM is an independent risk factor for CV events. Indeed, in 5888 men and women with a mean age of 73 years who were free of incident cardiovascular disease and who were followed for 6 to 7 years, LVM was related to incident CHD, CHF and stroke after adjustment for anthropometric and traditional CVD risk factors (Gardin et al 2001).

Many of the previous studies assessing the ability of LVM to predict CVD, have not been sufficiently statistically powered to assess the ability of LVM to predict CHF or the development of cardiac dysfunction. However, in a cohort of patients from the Cardiovascular Health Study, where participants were followed for 5 years, LVH was also noted to be associated with a decrease in left ventricular ejection fraction or diastolic function independent of traditional risk factors including conventional blood pressure (Drazner et al 2004). Thus, LVH predicts the transition to cardiac dysfunction and could therefore also predict the development of CHF.

Clearly if LVH predicts the risk of future CVD, then regression of LVH should be associated with a reduced risk of CVD. One method of reducing LVM and decreasing the prevalence of LVH is through antihypertensive treatment (Wachtell et al 2002). It is now well recognized that regression of LVH with antihypertensive therapy is associated with a reduced risk of CVD (Levy et al 1994, Heyden et al 1985). Importantly however, there is now evidence to indicate that the impact of therapeutic agents on either electrocardiographically-determined LVH or echocardiographically-determined LVM and the associated CVD risk reduction is partly independent of blood pressure changes and other conventional cardiovascular risk factors (Okin et al 2004, Devereux et al 2004, Mathew et al 2001). Indeed, the strongest evidence in this regard is probably from the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) randomized clinical trial, conducted from 1995 to 2001, where 941 patients with primary hypertension and electrocardiographic evidence of LVH were followed for a mean (SD) duration of 4.8 (10) years (Devereux et al 2004). In this study (Devereux et al 2004) primary cardiovascular events occurred in 104 patients (11%), including 84 with events after the first year of treatment. After adjusting for baseline LVM index (LVMI), study treatment, and degree of blood pressure lowering, reduction in LVM was strongly associated with reduced risk for the composite end point, CV mortality, stroke, and the secondary end point of all-cause mortality (Devereux et al 2004). These reductions largely persisted after further adjusting for age, smoking, diabetes, prior stroke, prior MI, and heart failure (Devereux et al 2004).

Thus, there is considerable data indicating the clinical value of measuring LVM in cardiovascular risk prediction. Based on these findings, there is no question as to the clinical importance of LVH. However, the factors that determine LVH are nevertheless more obscure.

1.3 Blood pressure and body size as major determinants of left ventricular mass.

There are many factors that are associated with LVM. As indicated in the introduction to the present chapter, of the modifiable factors that are associated with LVM, the two most consistently related to LVM have been blood pressure and body size. The following summarizes the evidence and highlights some revealing controversies in this regard.

1.3.1 Blood pressure as a determinant of left ventricular mass

Systemic hypertension is a primary determinant of LVH. However, the correlation between conventional blood pressure and LVM is not as consistent as one would expect (Devereux et al 1987). This relationship may be almost undetectable in some groups. Indeed, in previous studies conducted in Caucasian hypertensives and normotensives (Polonia et al 1992, Bauwens et al 1991, Cerasola et al 1991) and in a recent study conducted in Congolese hypertensive patients (Lepira et al 2006), the relationship between conventional blood pressure and LVM or LVH was almost absent or indeed was absent.

A potential explanation for the poor relationship between conventional blood pressure and LVM in some studies is that the haemodynamic load on the LV cannot be predicted from office or clinic (conventional) blood pressure measurements, measurements obtained at a single point in time (Devereux et al 1987). Thus, 24-hour ambulatory blood pressure was considered as potentially a better predictor of LVM than conventional blood pressure. Although many cross-sectional studies have reported on the superiority of ambulatory blood pressure over conventional blood pressure when

predicting LVM (reviewed by Fagard et al 2002), an analysis of the relationship between the correlation coefficients of the office blood pressure-LVM and ambulatory blood pressure-LVM relations of many of these studies has been particularly revealing (Fargard et al 1995). This analysis supported a conclusion that some clinic blood pressures may predict LVM as well as ambulatory blood pressure, rather than a conclusion that ambulatory blood pressure is indeed superior to clinic BP in predicting LVM (Fagard et al 1995).

With respect to the method of determining conventional blood pressure so that the values obtained achieve close correlations with LVM, multiple measurements (Fagard et al 1995) or visits (Prisant and Carr 1990) may be required to obtain a better assessment of the relationship between conventional blood pressure and LVM. Moreover, the assessment of conventional blood pressure in a quiet room may produce just as close a correlation with LVM as ambulatory blood pressure (Ganau et al 1990). The use of the same observer and multiple conventional blood pressure measurements (5 at each visit) at two visits in a quiet room at a similar time of day is also associated with similar correlation coefficients between conventional and ambulatory blood pressure and LVM (Fagard et al 1992). The use of high quality measurements by trained nurse observers may also achieve close correlations with LVM. Indeed, a recent study conducted by members of our group has demonstrated in ~400 participants from a population sample, that standardized, nurse-recorded conventional blood pressure is as closely associated with LVM as is 24-hour ambulatory blood pressure (Woodiwiss et al 2008). These data are consistent with the finding that nurse-recorded blood pressures are as closely associated with change in LVM with antihypertensive therapy as is 24-hour ambulatory blood pressure (Skudicky et al 2001). Thus, there is still no real consensus as to whether ambulatory blood pressure is superior to conventional blood pressure in predicting LVM.

Arterial stiffness mediates effects on blood pressure in-part through an impact of early reflective waves on central aortic pressures, a pressure that is not accurately predicted by brachial artery blood pressure measurements (Williams et al 2006, Karamanoglu et al 1993). Thus, an alternative explanation for the limited correlation between conventional blood pressure and LVM is that conventional blood pressure measured at the brachial artery does not reflect the impact of arterial stiffness on central blood pressures. However, although many studies have shown that indices of arterial stiffness are associated with LVM (Leoncini et al 2006, Gates et al 2003, Deague et al 2001, Roman et al 2000, Baguet et al 2000, Iketani et al 2000, Chen et al 1998, Roman et al 1996, , Kobayashi et al 1996, Boutouyrie et al 1995, Tatchum et al 1995, Bouthier et al 1985), much of the evidence indicates that measures of arterial stiffness do not provide information beyond that afforded by measures of brachial artery (peripheral) systolic blood pressure. However, these studies were limited by the use of study groups with a high prevalence of treated hypertension or a low prevalence of risk factors for large artery stiffness. In this regard, in a recent study conducted by our group in randomly recruited untreated participants with a high prevalence of risk factors for large artery stiffness, a strong independent association between arterial stiffness and LVM was noted beyond measures of brachial artery blood pressure, including ambulatory blood pressure (Libhaber et al 2008). Thus, poor correlations between brachial artery blood pressure and LVM may not necessarily reflect a low impact of blood pressure on LVM, but rather the inability to measure blood pressure in central arteries.

1.3.2 Excess adiposity as a determinant of left ventricular mass

With respect to obesity as a modifiable risk factor for an increased LVM, the prevalence of obesity has achieved epidemic proportions in both developed and in developing countries (Ogden et al 2006, Bourne et al 2002, Pouane et al 2002, Flegal et al 2000). In the United States of America, the prevalence of obesity in 2004 was ~32% and the prevalence of extreme obesity (a body mass index [BMI] > 40 kg/m^2) was as high as 6.9% in women (Ogden et al 2006). In South Africa, the prevalence of obesity prior to 2002 was estimated to be 29% in men and 57% in women (Pouane et al 2002) and this prevalence is likely to be increasing. The high prevalence of obesity worldwide is therefore likely to make obesity the major modifiable risk factor for an increased LVM. What is the evidence that obesity is a major determinant of LVM?

The strong relationship between body size and LVM is supported by a number of studies with large sample sizes (Lauer et al 1991, Lauer et al 1992, de Simone et al 1992, Gottdiener et al 1994, Urbina et al 1995, Gardin et al 1995, Sherif et al 2000; Lorber et al, 2003; Fox et al, 2004). Importantly, in large cohorts of mild-to-moderate hypertensive patients, BMI is the strongest predictor of LVM (Lauer et al 1992, Gottdiener et al 1994). The effects of adiposity are thought to be through the impact of fat-free mass (Bella et al 1998). However, the relationship between body size and LVM may not be restricted to lean body mass only. Indeed, in healthy young Caucasian and African-American adults of the Coronary Artery Risk Development in Young Adults cohort study, a strong correlation was found between LVM and subscapular skin-fold thickness in both genders and ethnic groups (Gardin et al 1995). Similarly, in pre-menopausal American women, LVM index correlated with indices of central adiposity (Sherif et al 2000). Thus, there is no question that excess adiposity is a cause of an increased LVM. However, what remains uncertain are the mechanisms responsible for

adiposity effects on LVM. In this regard there are a number of factors that could contribute to the impact of an excess adiposity on LVM. In the following discussion I will first outline the evidence that indicates that haemodynamic factors and the renin-angiotensin-aldosterone system are likely to play a major role in obesity-induced LVH. In the subsequent sections I will then argue in favor of novel factors that may contribute toward obesity-induced LVH.

1.3.2.1 Haemodynamic factors may mediate obesity-induced effects on left ventricular hypertrophy.

Obesity may promote a number of haemodynamic changes many of which may result in an increase in LVM. Because of the high metabolic activity of excessive fat, obesity may result in a decrease in total peripheral resistance and an increase in venous return, stroke volume and cardiac output (Messerli et al 1983, Carabello and Gittens 1987, Paulson and Tahiliani 1992). Further, as sympathetic nervous system activity may be enhanced in obesity (Haynes et al 1997, Haynes et al 1998, Casto et al 1998, Wofford and Hall 2004, Tentolouris et al 2006, Yang and Barouch 2007), heart rate and cardiac contractility may also contribute toward an increased cardiac output. Increases in stroke volume and cardiac output are well known to promote increases in systolic blood pressure, a blood pressure change that is more closely associated with LVM than is diastolic blood pressure. Moreover, a number of studies have demonstrated a relationship between excess adiposity and increases in arterial stiffness (Resnick et al 1997, Sutton-Tyrrell et al 2001, Mackey et al 2002, Wildman et al 2003, Ferreira et al 2004, Snijder et al 2004, Majane et al 2008) and increases in arterial stiffness through the impact of early reflective waves also increases systolic blood pressure. Thus, through increases in cardiac output and arterial stiffness, excess adiposity may promote

increases in LVM through changes in systolic blood pressure. Hence, one argument is that measuring systolic blood pressure should be sufficient to account for the hemodynamic effects of excess adiposity on LVM. However, this may not be the case.

In multivariate regression analysis conducted in a large population sample, stroke volume, cardiac output or filling volumes predicted LVM well beyond even systolic blood pressure (Chen et al 1998). Further, in a recent study conducted by our group, an index of large artery stiffness was shown to be strong predictor of LVM in women beyond conventional, central or 24-hour systolic blood pressure (Libhaber et al 2008). Consequently, measures of systolic blood pressure determined in the periphery (brachial artery) may not entirely account for adipose tissue-induced haemodynamic effects on LVM.

1.3.2.2 The renin-angiotensin II system may mediate obesity-induced effects on left ventricular hypertrophy.

The renin-angiotensin-aldosterone system (RAAS) is activated in obesity. Indeed, angiotensinogen, a protein synthesized principally in the liver and transformed by renin to produce angiotensin I which is converted to angiotensin II and hence promotes the release of aldosterone from the adrenal gland, is also synthesized by adipocytes, particularly visceral adipocytes (Rahmouni et al 2005). Indeed, circulating angiotensinogen concentrations increase in obesity (Umemura et al 1997). Moreover, angiotensin-converting enzyme, an enzyme largely derived from endothelial cells in the lung parenchyma, and which is responsible for the conversion of angiotensin I to angiotensin II, is also synthesized by adipocytes (Barton et al, 2000) and circulating angiotensin-converting enzyme activity increases in obesity (Barton et al, 2000).

Furthermore, adipocytes produce a lipid substance that is converted to aldosterone by adrenal cortical cells (Ehrhart-Bornstein, 2003).

The importance of the RAAS in promoting cardiac hypertrophy independent of effects on blood pressure is now well recognized. These data are largely derived from studies demonstrating that antihypertensive agents that activate the RAAS, such as hydralazine, have no beneficial effects on cardiac hypertrophy (e.g. Tsotetsi et al 2001), whilst RAAS blockers are very effective at reducing LVM. This notion was further extended in a number of human studies that were subsequently formally reviewed in a series of systematic reviews and meta-analyses of these smaller studies. In a meta-analysis by Dahlof including 109 treatment studies, angiotensin-converting enzyme inhibitors were noted to produce a more pronounced reduction of LVH than other drug classes (Dahlof et al 1992). Moreover, in a meta-analysis conducted by Schmieder et al (1998) with 89 randomized double-blind studies included in the analysis, angiotensin-converting enzyme inhibitors were noted to produce a greater decrease in LVH than beta-blockers or diuretics.

Based on the notion that some classes of antihypertensive agents may be superior to others in their ability to reduce LVM in hypertension, further large studies were conducted with direct comparisons made between specific classes of agents. In the LIFE study, the angiotensin II receptor blocker, losartan, produced a greater regression of LVM than did a beta-adrenergic receptor blocker, atenolol, after adjusting for conventional blood pressure effects (Devereux et al 2004). The conventional blood pressure-independent benefits of losartan on LVM in the LIFE study were also associated with a class-specific effect on CV risk reduction. The data from the LIFE study were further supported by a study conducted with a similar design, where the angiotensin II receptor blocker, losartan, was also found to be superior to the beta-

blocker atenolol at regressing LVH despite comparable effects on conventional blood pressure (Dahloff et al 2002).

Although it may be argued that RAAS activation in excess adiposity mediates obesity-induced increases in LVM, studies evaluating the impact of RAAS blockers on obesity-induced increases in LVM have been limited. In this regard, pre-clinical studies have demonstrated an ability of RAAS blockers to prevent obesity-induced LVH (du Toit et al 2005), whether this effect was mediated by haemodynamic or direct myocardial effects was not determined.

1.3.2.3 Insulin resistance may mediate obesity-induced effects on left ventricular hypertrophy.

Insulin resistance may occur as a consequence of excess adiposity. This change is thought not only to lead to diabetes mellitus, but also to adverse cardiovascular changes independent of diabetes mellitus. In obesity, insulin-stimulated protein kinase activity of the insulin receptor, which mediates tyrosine autophosphorylation, is reduced and it is further decreased in patients with obesity-induced type II diabetes mellitus (Caro et al 1989). However, euglycaemia is maintained in the initial stages of insulin-resistance through compensatory hyperinsulinaemia. Indeed, there is considerable evidence to suggest that a large proportion of obese individuals are insulin-resistant, but that compensatory hyperinsulinaemia maintains fasting blood glucose concentrations (Reaven 2003, Keller 2006). Thus, insulin resistance goes undetected in these individuals.

Insulin resistance will undoubtedly promote adverse cardiovascular effects once type II diabetes mellitus develops and the effects of hyperglycaemia are noted (Groop 2000, Goldstein 2002). However, insulin resistance may also promote adverse

cardiovascular changes prior to the development of diabetes mellitus and poor blood glucose control through a number of mechanisms. Insulin resistance has more recently been considered as a potential determinant of adiposity-induced increases in LVM. In this regard, insulin resistance may increase LVM through activation of the sympathetic nervous system. Indeed, there is considerable evidence to suggest that the sympathetic nervous system is activated in obesity (Haynes et al 1997, Haynes et al 1998, Casto et al 1998, Wofford and Hall 2004, Tentolouris et al 2006, Yang and Barouch 2007, Mark et al 1999) a change that may in-part be attributed to the compensatory hyperinsulinaemia that occurs in response to insulin resistance (Hall 2002). Because insulin-mediated glucose uptake in central hypothalamic neurons regulates sympathetic activity in response to dietary intake, hyperinsulinaemia is thought to provoke sympathetic nervous system stimulation (Landsberg 1999).

What evidence is there that sympathetic nervous system activation may mediate deleterious effects on the heart? In this regard, LVH is associated with excessive myocardial sympathetic effects (Schlaich et al 2003) and blockade of β-adrenoreceptors, the receptors in the heart through which the sympathetic nervous system mediates its effects, may reduce LVM through effects that cannot be attributed solely to an impact on blood pressure (Varagic et al 2001). Moreover, activation of the sympathetic nervous system can promote the transition from compensated LVH to heart failure (Badenhorst et al 2003, Veliotes et al 2005), a change that may further promote increases in LVM. Thus, insulin-induced increases in sympathetic nervous system activation in obesity may be one mechanism that explains the relationship between obesity and LVH. Although alternative hypotheses indeed exist, the proposed relationship between hyperinsulinaemia and sympathetic nervous system activation was one hypothesis that prompted the question as to whether insulin resistance in obesity was not in-part responsible for obesity-induced LVH. However, this has been a difficult question to

answer because of the strong relationship between insulin resistance and obesity, a relationship that reduces the ability to segregate body size-induced increases in LVM and insulin resistance-induced effects on LVM. Moreover, insulin resistance is associated with hypertension, thus again making it difficult to segregate an afterload-induced effect on LVM from insulin resistance-induced effects on LVM (Young et al 2002). Further, the exact mechanism by which insulin resistance mediates LVM is obscure. In the following section I will review the controversy regarding the hypothesis that insulin resistance may in-part mediate obesity-induced increases in LVM.

1.3.2.3.1 Relationship between Insulin resistance and left ventricular mass.

Data showing an independent relationship between insulin resistance and LVM in persons without type II diabetes mellitus are largely derived from studies with small sample sizes or where the effects of blood pressure or body size may not have been completely accounted for. In this regard, in 29 non-obese participants with a high-normal BP, insulin sensitivity (minimal model of glucose kinetics) was related to LVM independent of blood pressure (Phillips 1998). Further, in 40 normotensive, non-diabetic obese participants, insulin resistance (intravenous glucose tolerance test) and the associated hyperinsulinaemia was strongly associated with LVM independent of BMI and blood pressure (Sasson et al 1993). In 101 never-treated, non-diabetic, hypertensive subjects, insulin and insulin growth factor-1 were independent determinants of LVM and geometry (Verdecchia et al 1999). Further, post-load glucose and insulin concentrations, measured two hours after glucose administration, were noted to be more important than fasting insulin as a potential determinant of LVM (Verdecchia 1999). The relatively small sample sizes (n=29-101) of these studies obviously raises the question of false positive associations. Nevertheless, glucose intolerance without

diabetes mellitus was associated with LVM indexed to height$^{2.7}$ in 1345 non-diabetic patients (457 of whom had glucose intolerance) (Ilercil et al 2001). In that study (Ilercil et al 2001) glucose intolerance in non-diabetic participants is presumably attributed to insulin resistance. However, whether the relationships between indices of insulin resistance and LVM are independent of ambulatory blood pressure in any of these studies is uncertain. As indicated in the aforementioned discussion, it is possible that adjustments for conventional blood pressure measurements may not entirely account for BP effects, as 24-hour blood pressure values may be more closely associated with LVM than conventional blood pressure.

Evidence suggesting that insulin resistance without diabetes mellitus is not associated with LVM independent of confounding factors comes from studies with both small and large sample sizes. In 50 subjects without diabetes mellitus, insulin concentration, secretion or action (euglycaemic clamp) was not independently associated with LVM when accounting for body mass or blood pressure (Galvan 2000). In 153 healthy subjects, neither insulin resistance (homeostasis model of insulin resistance which mirrors the outcomes of the more sensitive glucose clamp technique (Bonara et al 2000), nor fasting insulin concentrations were associated with LVH, independent of obesity (Ebnic 2006). Furthermore, in a large community-based sample of 2623 Framingham Study subjects, although LVM and wall thickness increased with worsening glucose intolerance and insulin resistance (as determined by the homeostasis model of insulin resistance) in non-diabetic participants, an effect that was more striking in women compared with men, this relation was largely accounted for by the presence of obesity (Rutter et al 2003).

1.3.2.4 Leptin may modulate obesity-induced left ventricular hypertrophy.

A more recent hypothesis for the effect of excess adiposity on LVM is through leptin, through direct myocardial effects or its associated effects on blood pressure, sympathetic nervous system activation or through concurrent leptin resistance which frequently accompanies obesity.

Leptin is a 16-kDa adipocytokine, synthesized and secreted by adipose tissue in proportion to current body size. The principle role of leptin is to mediate body weight homeostasis (Considine et al 1996) through effects on food intake and energy expenditure (Leyva et al 1998). Circulating concentrations of leptin are increased in obesity (Livshits et al 2005, Hall et al 2001) and under physiological conditions the amount of leptin produced by fat tissue is directly related to the mass of adipose tissue (Campfield et al 1996). Upon release by adipocytes, leptin reaches hypothalamic leptin receptors in the brain via facilitated passage across the blood-brain barrier (Halaas et al 1995). In addition to reducing appetite and thus controlling weight gain, leptin activates the sympathetic nervous system thus increasing metabolic rate and promoting further weight loss. The mechanisms of sympathetic nervous system activation in obesity are still unclear but this may be via stimulation of neurons within the ventromedial hypothalamus (Satoh et al 1999). Although the biological actions of leptin are mediated largely through interactions with its cognate receptor expressed in the hypothalamus (Tartaglia et al 1996), subsequent studies have demonstrated that leptin receptors have a widespread tissue distribution including the liver, kidney, lungs, pancreas and heart (Chen et al 1999, Hoggard et al 1997, Lee et al 1996, Van Heek et al 1996, Kieffer et al 1996). Thus leptin may have a wide ranging influence on a variety of tissue including the cardiovascular system.

The most common leptin abnormality in patients is “receptor insensitivity” or peripheral leptin resistance, leading to a compensatory or secondary excess in circulating leptin concentrations (Frederich et al 1995). In the clinical setting of leptin resistance or leptin receptor insensitivity, there is concurrent insulin resistance or insulin receptor insensitivity. Under these circumstances, leptin concentrations may be associated with insulin resistance. Indeed, in a factor analysis, inter-individual variations in plasma leptin concentrations were strongly related to the principal components of the insulin resistance syndrome (Leyva et al 1998). Thus, leptin concentrations may be employed as an appropriate surrogate for insulin resistance in epidemiological studies where difficulties are encountered in assessing insulin-resistance in non-fasted individuals.

A complete explanation for the relationship between leptin resistance and insulin resistance has not been forthcoming. However, this relationship may be explained in-part by the fact that leptin directly affects insulin sensitivity by regulating the efficiency of insulin-mediated glucose metabolism by skeletal muscle (Cohen et al 1996, Liu et al 1997).This insulin-resistant state is well recognized in obesity, hypertension, heart failure and after myocardial infarction (Levya et al 1998, Agatha et al 1997, Reaven et al 1995) and in these settings, circulating leptin concentrations are elevated, and serum leptin concentrations are an independent predictor for cardiovascular morbidity and mortality (Wallace et al 2001, Soderberg et al 1999a and 1999b). Importantly, as leptin influences insulin sensitivity, it is possible that leptin concentrations may reflect a relationship between insulin resistance and sympathetic nervous system activation.

Leptin may be intricately involved in mediating obesity-induced arterial hypertension (Mark et al 1999a, 1999b, Haynes et al 1998, Shek et al 1998) and thus through afterload-mediated effects on the left ventricle, promote LVH. Although when acutely administered, leptin has no effect on blood pressure, probably because it

concomitantly stimulates the sympathetic nervous system and produces counteracting depressor changes such as a natriuresis and nitric oxide (NO)-dependent vasorelaxation, by contrast, chronic hyperleptinaemia increases blood pressure (Shek et al 1998). The chronic effects of excessive leptin on blood pressure are probably because the acute depressor effects are impaired and/or additional sympathetic nervous system-independent pressor effects appear, such as an increased oxidative stress, NO deficiency, enhanced renal Na^+ reabsorption and overproduction of endothelin (Shek et al 1998). Although a cause-effect relationship between leptin and high blood pressure in humans has not been directly demonstrated, many clinical studies have shown elevated plasma leptin concentrations in patients with essential hypertension and a significant positive correlation between leptin and blood pressure independent of body adiposity both in normotensive and in hypertensive individuals (Abbas et al 2004).

In addition to a potential impact on LVM through blood pressure effects, excessive leptin secretion in obesity, or leptin resistance in obesity have been implicated in moderating LVM independent of blood pressure effects. As leptin promotes sympathetic nervous system activation directly (Hall et al 2001, Satoh et al 1999), or through concurrent insulin resistance, excess leptin secretion could promote LVH in obesity through direct sympathetic effects. Alternatively, as leptin promotes cardiac hypertrophy in isolated cardiac myocytes (Rajapurohitam et al 2003), obesity may promote cardiac hypertrophy through the direct myocardial growth-inducing actions of leptin. In contrast, by promoting leptin resistance, obesity may moderate a potential protective effect of leptin on cardiac growth. Indeed, the leptin deficiency in the ob/ob mouse is associated with LVH where the degree of hypertrophy is independent of body mass (Barouch et al 2003). A protective effect of leptin on cardiac growth in the ob/ob model of obesity is supported by the effects of exogenous administration of leptin, which

reduces LVH (Barouch et al 2003), thus indicating that leptin may mediate anti and not necessarily pro-hypertrophic effects on the heart.

Importantly, irrespective of whether leptin promotes LVH in obesity through direct myocardial effects; through blood pressure effects and sympathetic nervous system activation; or whether leptin protects the heart against LVH, plasma leptin concentrations could more closely predict the impact of excess adiposity on LVM. If leptin promotes LVH through direct myocardial effects, blood pressure effects and sympathetic nervous system activation, then plasma concentrations of leptin may be more closely related to LVM than indices of excess adiposity *per se*. Similarly, if leptin were protective against LVH, plasma leptin concentrations may reflect the degree of leptin resistance in obesity and similarly be more closely related to LVM than indices of excess adiposity *per se*. Thus, in either instance it is reasonable to hypothesize that plasma leptin concentrations may be more closely associated with LVM than indices of adiposity. What is the current evidence regarding the notion that plasma leptin concentrations are more closely associated with LVM than indices of adiposity?

1.3.2.4.1 Leptin and left ventricular mass or geometry.

Studies assessing the relationship between plasma leptin concentrations and LVM have provided inconsistent outcomes. In this regard, a positive correlation between plasma leptin and left ventricular wall thickness was noted in 40 hypertensive men and 15 apparently healthy men independent of indices of adiposity or blood pressure (Paolisso et al 1999). Furthermore, in 31 morbidly obese normotensive participants and 30 lean participants, leptin correlated with LVM, independent of sex, age, BMI, insulin sensitivity and blood pressure and the decrease in LVM noted to occur one year after gastric bypass surgery in these patients, correlated with a decrease in plasma leptin

concentrations (Perego et al 2005). Although an independent relationship between plasma leptin concentrations and LV wall thickness or LVM in 55 (Paolisso et al 1999) and in 31 (Perego et al 2005) participants could represent false positive outcomes, the independent relationship between changes in plasma leptin concentrations and LVM following weight loss (Paolisso et al 1999, Perego et al 2005) provides prospective data to support the hypothesis. In these previous studies however (Paolisso et al 1999, Perego et al 2005), only hypertensive participants with insulin resistance (Paolisso et al 1999), or morbidly obese (Perego et al 2005) participants were studied. Hence, these data could reflect unique characteristics of the samples studied rather than effect of excess adiposity in general.

In contrast to the positive relations between plasma leptin concentrations and LVM noted in two previous studies conducted in small study samples (Paolisso et al 1999, Perego et al 2005) in 191 participants with a BMI range of 24.8 and 27.1 kg/m^2, plasma leptin concentrations were not independently associated with LV wall thickness or LVM after adjustments for body size (Malmqvist et al 2002). However, it could be argued that a lack of independent relationship between plasma leptin concentrations and LVM or wall thickness in participants with a BMI range of 24.8 and 27.1 kg/m^2 (Malmqvist et al 2002) may represent a low sensitivity to detect an independent relationship in a population with a low prevalence of excess adiposity. Indeed, plasma leptin concentrations correlated with only relative wall thickness and not with LVMI before adjustments for adiposity indices in this prior study (Malmqvist et al 2002).

In contrast to the positive independent relations noted between plasma leptin concentrations and LVM in two previous studies (Paolisso et al 1999, Perego et al 2005) and the lack of independent relationship noted by Malmqvist et al (2002), in a cross-sectional study of 478 participants, plasma leptin concentrations were inversely associated with LVMI and wall thickness (Pladevall et al 2003). This inverse relationship

may reflect a protective effect of leptin on the heart as described in one preclinical study (Barouch et al 2003). The apparent discrepancy in the outcome of this study (Pladevall et al 2003) as compared to other studies (Paolisso et al 1999, Perego et al 2005, Malmqvist et al 2002), may nevertheless, also reflect differences in the populations sampled. For example, in this previous study (Pladevall et al 2003), the population sampled had a mean BMI of ~26.3 kg/m^2. Thus the precise role of leptin in mediating LVH in obese humans independent of indices of adiposity, if any, remains unclear.

1.4 Aims of the present study

As the majority of studies assessing the relationship between plasma leptin concentrations and LVM or geometry have been conducted in small study samples in severely obese participants, or in population samples with a relatively low mean BMI, these studies may not reflect the relationship between plasma leptin concentrations and LVM in the population at large of a group recognized as having a high prevalence of excess adiposity. Consequently, in the present study I aimed to assess the relationship between plasma leptin concentrations and LVM or geometry in a relatively large population sample with high prevalence of excess adiposity and a wide range of BMI values (Norton et al 2008, Woodiwiss et al 2008a 2008b, Majane et al 2008, Libhaber et al 2008 , Majane et al 2007, Maseko et al 2007, Shiburi et al 2006).

Chapter 2

Methods

2.1 Study participants

The study protocol was approved by the University of the Witwatersrand Committee for Research in Human Subjects (approval number: M02-04-72). All participants gave written, informed consent. Participants of African ancestry were recruited from the South Western Township (SOWETO) of Gauteng (a metropolitan area of Johannesburg). Random recruitment of spouses and siblings living in households (nuclear families) from formal dwellings represented in the last census conducted (2001) was performed. Nuclear families were recruited if at least one or two offspring of at least 16 years of age and one or both parents were available for examination. Households were allocated numbers and numbers were selected from a random number generator. People residing in informal dwellings or institutions/homes were not recruited. Although the minimum age for participation in the study was 16 years, there was no upper age limit. A lower age limit was included to avoid the impact of rapid growth effects on cardiovascular structure and function. The participants were from the Nguni (Zulu, Xhosa, Ndebele, Swati), Sotho (South Sotho, North Sotho and Tswana) and Venda chiefdoms. The groups largely consisted of Nguni and Sotho chiefdoms. The lack of representation from the Venda chiefdom reflects a lack of individuals of this chiefdom residing in these areas of Johannesburg. No participants of mixed, Asian, or European ancestry were recruited and no Khoi-San persons were recruited.

Recruitment for the present study was initiated in October 2003 and data obtained up until November 2007 were used for the present dissertation. Of the 1058 participants that were invited to be part of the study, 811 (~77%) agreed to participate. Of the 811 participants enrolled in the study, 418 (~52%) agreed to echocardiograph assessments. I discarded 15 participants from the analysis because echocardiograms were of poor quality and a further 8 who did not have conventional blood pressure

measurements obtained on three separate occasions as part of this study. Thus, in total 399 participants were assessed in this study. Of these participants 378 had complete blood results available.

2.2 Questionnaire

Participants completed a standard questionnaire. In order to avoid translational errors, the questionnaire was not translated into an African language, but study assistants familiar with all languages spoken in these townships and who either previously lived in SOWETO or currently reside in SOWETO assisted with the completion of each questionnaire. Only same sex assistants were used to assist each family member with the completion of the questionnaire. Assistance was only provided when requested. The majority of participants were reasonably proficient in English. Study assistants first visited homes of families that agreed to participate in the study in order to develop a trusting relationship. The questionnaire was only completed at a subsequent clinic visit and then ambiguities checked by performing a follow-up home visit. If family members were absent at follow-up home visits, data was checked with them personally via telephonic conversations whenever possible. Ambiguities in answers to the questionnaire were detected by an independent observer prior to the second home visit. A pilot study was conducted in 20 participants to ensure that data obtained in the questionnaires were reproducible when obtained with the assistance of two separate study assistants.

The questionnaire requested specific answers to date of birth, gender, previous medical history, the presence of hypertension, diabetes mellitus and kidney disease, prior and current drug therapy (analgesic use included), prior and current occupation, level of education, smoking status (including the number of cigarettes smoked in the

past and at the present time), daily alcohol consumption (beer, traditional beer or other forms of alcohol and the daily quantity), caffeine consumption (number of cups of tea or coffee and whether they are decaffeinated and the number of cola's a day), exercise frequency and family history of hypertension and cardiovascular events. For females, menstrual history, history of pregnancies and oral contraceptive use was evaluated. Most of the questions simply required a "yes"-"no" answer, but understanding was assessed by requesting some short answers. If participants were unable to provide the name of medication taken these were obtained on the second home visit. Although a crude assessment of socioeconomic status was calculated from the combined levels of education, present occupations and annual income, these data have not been analysed at this point as an appropriate score has not been derived.

2.3 Conventional blood pressure measurements

Trained nurse-technician observers measured blood pressure using a standard mercury sphygmomanometer at the clinic visit. Doctors were not utilized to measure blood pressure as they are perceived by the community as being in positions of authority and hence may elicit "white-coat" effects. After being trained in the procedure, including being shown the pitfalls of blood pressure measurement (positioning of the cuff, positioning of the arm, first estimating systolic blood pressure using a radial pulse measure in order to avoid increasing cuff pressures too high, detecting auscultatory gaps, releasing valve pressure at the correct speed, using the correct cuff size, etc.), assistants had to demonstrate an ability to perform the procedure on 20 participants. The study assistants were then tested on their ability to measure blood pressure in two ways. First they were asked to measure blood pressure on a separate group of 20 participants including patients with hypertension and their readings had to be within 4

mm Hg of an experienced investigator's readings obtained with a stethoscope with two ear pieces. Second, study assistants were asked to watch a video showing a simulated mercury column with Korotkoff sounds where observers were tested on their ability to detect phase I and V sounds under different circumstances including in the presence of a wide auscultatory gap and where phase V Korotkoff was taken as a "muffling" rather than a "disappearance" of sounds (Blood Pressure Measurement, British Medical Journal, BMA House London). To qualify as observers all readings (n = 20) had to be within 4 mm Hg of the reference standard. Assistants who failed on the assessment the first time were given more time to practise on participants and then asked to repeat the tests.

To determine blood pressure, participants were seated and asked to rest for 5 minutes. A standard cuff with a 12 $\times$ 24 cm inflatable bladder was used for blood pressure measurement, but if upper arm circumference exceeded 31 cm, larger cuffs with a 15 $\times$ 35 cm inflatable bladder were used. After 5 minutes of rest in the seating position, five consecutive blood pressure readings were taken 30 to 60 seconds apart with the participants in a sitting position, followed by a pulse rate count. The cuff was deflated at approximately 2 mm Hg per second and phase I (systolic) and phase V (diastolic) blood pressure recorded to the nearest 2 mm Hg according to the recommendations of the European Society of Hypertension (O'Brien et al 2003). The average of the five readings was taken as the conventional blood pressure.

In the present study, quality control of conventional blood pressure assessments was assessed as previously described (Kuznetsova et al 2002). In participants with echocardiographic and blood assessments, only 2.4% of visits had fewer than the planned blood pressure recordings. The frequency of identical consecutive recordings was 0.54% for systolic blood pressure and 0.81% for diastolic blood pressure. The

occurrence of blood pressure values recorded as an odd number was 0.03 %. Of the 3702 systolic and diastolic blood pressure readings, 29.0% ended on a zero (expected =20%). A diagnosis of hypertension was made if participants were receiving antihypertensive therapy and/or if the average of the conventional blood pressure readings was ≥140/90 mm Hg (O'Brien et al 2003).

2.4 Anthropometric Measurements

Body height, weight, waist and hip circumference and triceps and subscapular skin-fold thickness (Harpenden Skinfold Calliper, Bedfordshire, UK) were measured during the clinic visit by a trained observer. Height and weight were measured with the participants standing and wearing indoor clothes with no shoes. Waist circumference and hip circumference were measured using conventional approaches. Body mass index was calculated as weight in kilograms divided by the square of height in meters and waist-to-hip ratio calculated as an index of central obesity. Skin-fold thickness was reported as the mean of triceps and sub scapular values. Participants were identified as being overweight if their BMI was ≥25 kg/m² and obese if their BMI was 30 kg/m².

2.5 Blood measurements

Blood samples were obtained on the day of the clinic visit and sent to the South African National Health Laboratory Services (NHLS) to perform a full blood count and differential count, to measure urea, creatinine and electrolyte (sodium, potassium, chloride) concentrations, to assess liver function (from alanine transaminase, aspartate transaminase, gamma gluteryl transaminase, alkaline phosphatase, albumin, total protein and plasma albumin, total bilirubin, and conjugated and unconjugated bilirubin

concentrations) and plasma urate concentrations, to obtain a lipid profile (total cholesterol, low density lipoprotein cholesterol concentrations, high density lipoprotein cholesterol concentrations and triglyceride concentrations), a blood glucose measurement, percentage glycated haemoglobin (HbA1c) and a follicle stimulating hormone concentration (Bayer, Leverkusen, Germany) in females. These data were used to identify medical conditions, syndromes that may affect blood pressure or LVM and to confirm menopausal status in females. A “spot” urine analysis was also performed to screen for major clinical conditions, such as diabetes mellitus and renal pathology prior to obtaining laboratory results. The NHSL was utilized for blood measurements to ensure reproducibility and reliability as these laboratories have been accredited as fulfilling all criteria of “good laboratory practise”. As a number of participants did not have an 8 hour fast (as determined from the questionnaire) prior to attending the clinic, in those participants without a prior history of diabetes mellitus, a percentage glycated haemoglobin (HbA1c) rather than a fasting blood glucose concentration was utilized in the study to assess blood glucose control. Diabetes mellitus or inappropriate blood glucose control was defined as the use of insulin or oral hypoglycaemic agents, HbA_{1c} (Roche Diagnostics, Mannheim, Germany) >6.1% (Bennett et al 2007)

2.6 Plasma leptin concentrations

All blood samples were centrifuged, the plasma was then aliquoted and stored at -40℃ for later analysis. Plasma leptin concentrations wer e measured using a human leptin ELISA, (BioVendor; Human leptin ELISA, Clinical Range, Modrice, Czech Republic, RD191001100R), which is a sandwich enzyme immunoassay for the

quantative measurement of human leptin in serum and plasma. The assay kits were stored at 2-8℃. The total assay time was less than 4 ho urs.

Each vial of quality control was reconstituted with 0.35 ml of distilled water at least 30 minutes prior to use. The reconstituted quality controls were diluted 1:3 with dilution buffer prior to use i.e. 100 µl standards + 200µl dilution buffer for duplicates. Diluted standard solutions were not stored for future use, but rather discarded. One hundred ml of wash solution concentrate was diluted with 900 ml de-ionized (distilled) water. The remaining wash solution after the assay had been completed was stored at 2-8 ℃ for future use in subsequent assays. Samples wer e diluted 1:3 with dilution buffer i.e. 100 µl sample + 200 µl dilution buffer for duplicates.

One hundred µl of diluted standards, quality controls and samples were placed in duplicate in appropriate wells, incubated at room temperature (25 ℃) and shaken for 1 hour at 300 rpm on an orbital microplate shaker. After an hour the wells were washed 3-times with wash solution (0.35 ml per well). One hundred µl of conjugate solution was added to each well and the plate was incubated at room temperature (25 ℃) for a further 1 hour, shaking at 300 rpm on an orbital microplate shaker. The wells were washed again 3-times with wash solution (0.35 ml per well). One hundred µl of substrate solution was added to each well and the plate was incubated for 10 minutes at room temperature with the plate covered with aluminium foil, without shaking. Colour development was stopped by the addition of 100 µl of stop solution to each well. Absorbance was read within 5 minutes on a microtiter plate reader at 450 nm. The plate reader performed automatic calculations of analyte concentration. The calibration curve was constructed by plotting the absorbance (Y) of standards versus *log* of the known concentration (X) of the standards. The results were reported as concentration of leptin (ng/ml) in samples. Results exceeding 50 ng/ml were repeated using a dilution of 1:5. Dilution factors were taken into consideration when the concentration of leptin was

calculated. Representative data obtained from standards and quality controls are shown in the Table 2.1.

2.7 Echocardiography

Echocardiographic measurements were obtained using two-dimensional guided M-mode echocardiography performed using a pulse colour Doppler Hewlett Packard model 5500 recorder coupled to a 2.5 MHz transducer. Data obtained in the short axis view were analyzed according to the American Society of Echocardiography convention (Sahn et al 1978) (Figure 2.1). During recordings the transducer was placed perpendicular to the chest wall or pointed slightly inferiorly and laterally at the end of the long axis (Sahn et al 1978). Measurements were obtained at the left ventricular minor axis at the level of the *chordae tendinae* just beyond the mitral leaflet tips. All measurements were recorded on videotape and analyzed off-line by a single experienced investigator (Carlos Libhaber), who was unaware of the clinical condition of the subjects. I assisted in approximately 50% of the measurements. The interventricular septal wall thickness (IVS) at end diastole and end systole, the posterior wall thickness (PWT) at end diastole and end systole and the end diastolic and end systolic internal dimensions of the left ventricle were measured only when appropriate visualization of both the right and the left septal surfaces was obtained (Sahn et al 1978) (Figure 2.1). Left ventricular mass was derived according to an anatomically validated formula:

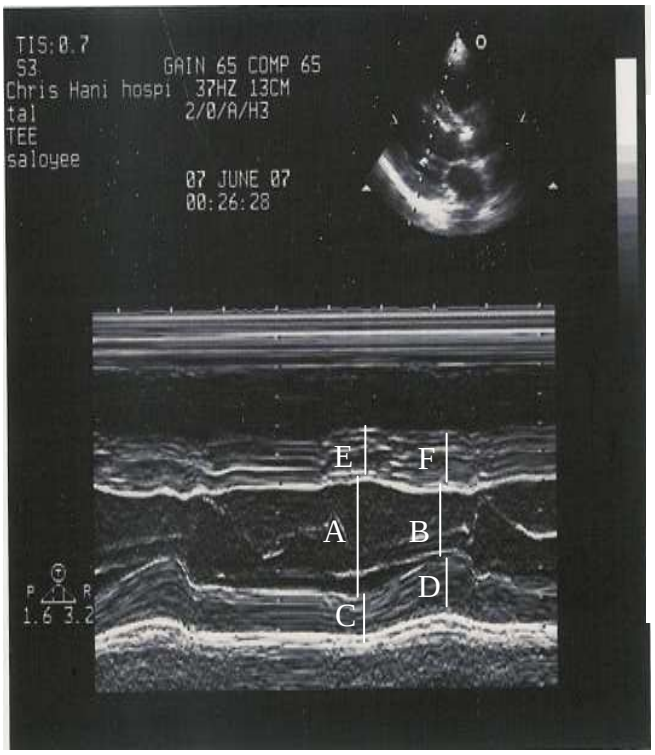
$$LVM = 0.8 \times [1.04 (LVEDD + IVS + PWT)^3 - (LVEDD)^3] + 0.6g,$$

where LVEDD = LV internal diameter, IVS =LV septal thickness, PWT = posterior wall thickness, all measured at diastole in centimeters (Devereux et al 1986).

Table 2.1. Representative data obtained from leptin standards and quality controls.

Standards/ Quality Controls	*Average*	*Concentration Expected (ng/ml)*	*Concentration Obtained (ng/ml)*
Standard 1	3.014	50	49.94
Standard 2	1.719	20	20.13
Standard 3	0.966	10	9.83
Standard 4	0.549	5	5.15
Standard 5	0.241	2	1.91
Standard 6	0.159	1	1.04
Blank	0.038	-	-
QC High	1.631	**19.86 (15.9-23.8)**	18.77
QC Low	0.510	**4.83 (3.9-5.8)**	4.73

QC, quality control.



A-Left ventricular end diastolic (LVED) diameter

B-Left ventricular end systolic (LVES) diameter

C-LVED posterior wall thickness.

D-LVES posterior wall thickness.

E-LVED septal wall thickness.

F-LVES septal wall thickness

Figure 2.1. Upper panel illustrates the Hewlett Packard model 5500 utilized to assess left ventricular dimensions in the study sample. The lower panel shows an M-Mode image (courtesy of Dr. Carlos Libhaber).

Intra-observer variability was assessed on 29 subjects in whom repeat echocardiographic measurements were performed within a two week period of the initial measurements. The Pearson's correlation coefficients for LV end diastolic diameter, septal wall thickness and posterior wall thickness were 0.76, 0.94 and 0.89 (all $p<0.0001$) respectively, and the variances (mean % difference ± SD) were 0.12±5.95%, -0.77±4.47% and 0.67±5.57% respectively. In addition, no significant differences between repeat measurements were evident on paired t-test analysis ($p=0.99$, $p=0.42$ and $p=0.48$ respectively).To adjust for the influence of growth on LVM without eliminating the impact of excess adiposity, LVM was indexed to height$^{2.7}$ (LVMI). Left ventricular mean wall thickness was calculated as the mean of LV posterior and LV septal wall thickness at end diastole. Left ventricular hypertrophy was defined as an LVMI>51g/m$^{2.7}$ in both men and women (Nunez et al 2005).

2.8 Data analysis.

Database management and statistical analyses were performed with SAS software, version 9.1 (The SAS Institute Inc., Cary, North Carolina, USA). Data from individual subjects were averaged and expressed as mean±SD. The X^2-statistic was used to compare proportions. As plasma leptin concentrations were abnormally distributed, analysis was conducted by first transforming plasma leptin concentrations (square root of plasma leptin concentration). In the initial analysis univariate relations (unadjusted correlations) between phenotypic variables (including adiposity indices and plasma leptin concentrations) and LVM, LVM index and mean wall thickness were identified. Thereafter, independent relationships between plasma leptin concentrations and either LVM, LVM index, mean wall thickness or LVH or between indices of excess adiposity and either LVM, LVM index, mean wall thickness or LVH were determined from

multivariate regression analysis with adjustments for age (or age^2), height (for LVM and LV mean wall thickness only), conventional systolic blood pressure, diabetes mellitus or an HbA1c>6.1%, antihypertensive treatment, regular tobacco or alcohol intake and pulse rate, menopausal status and oestrogen contraception. Due to the marked sex differences in plasma leptin concentrations, analysis was performed in sex-specific groups. Probability values were further adjusted for non-independence of family members in non-linear models (Mixed procedure as defined in the SAS package).

Chapter 3

Results

3.1 General demographic and clinical characteristics of the participants.

Table 3.1 compares the demographic and clinical characteristics of the people who agreed to participate in the study and those who had echocardiography and plasma leptin data. No differences were noted between the groups.

Table 3.2 give the demographic and clinical characteristics of the participants with echocardiographic and plasma leptin measurements. More women than men participated. In general the group had a high BMI, with ~26% being overweight and ~41% being obese. Approximately 22% (85/378) of participants had severe obesity as defined by a BMI>35kg/m^2. More women (77%) were overweight or obese than men (54%, $p<0.0001$). Although women had increased values for all indices of adiposity, waist-to-hip ratio was similar between men and women. On average a low proportion of participants either regularly smoked or consumed alcohol, but the percentage was far greater in men than in women. Of women 41.5% were postmenopausal as confirmed with follicle stimulating hormone measurements. Of women 7.5% were receiving oestrogen contraception, but none were receiving hormone replacement therapy. A greater proportion of women than men were hypertensive. On average 21% of participants were receiving antihypertensive therapy, with a greater proportion of women (24%) than men (16%) receiving antihypertensive medication. On average ~24% were either receiving therapy for diabetes mellitus or had an HbA1c >6.1%, with marginally more women than men either receiving glucose lowering agents or with an abnormal blood glucose control. No participants were receiving statin therapy. Plasma leptin concentrations ranged from 0.04 to 192.1 ng/ml and the mean (±SD) leptin concentration was 27.4±28.7 ng/ml with markedly higher concentrations noted in women ($p<0.0001$) as compared to men (Table 3.2). Furthermore, after adjustments for waist circumference, I

Table 3.1. Characteristics of all participants (All) as compared to characteristics of participants with echocardiography and plasma leptin measurements (Study group).

	All (n= 811)	Study group (n= 378)
Age (years)	43.0±18.2	43.4 ±17.9
% Female	63.5	63.8
Body weight (kg)	75.6 ±19.1	76.6±18.5
Body Height (cm)	161.2±8.7	161.9±8.8
Body mass index (kg/m^2)	29.3 ±7.8	29.3±7.4
Mean skin-fold thickness (cm)	2.09±1.11	2.14±1.10
Waist circumference (cm)	90.0±15.9	90.3 ±15.5
Hip circumference (cm)	107.8±15.3	107.7±14.7
Waist-to-hip ratio	0.84±0.10	0.84±0.11
% overweight/obese	23.2/39.7	26.7/41.8
Regular tobacco intake (%)	12.5	11.9
Regular alcohol intake (%)	21.0	22.2
% Postmenopausal	41.2	41.5
% Oestrogen contraception	4.6	7.5
% with DM or an HbA1c>6.1%	23.3	24.3
Hypertension (%)	39.2	40.5
Conventional SBP (mm Hg)	131±22	130±22
Conventional DBP (mm Hg)	85±13	84±12
Total/HDL cholesterol (mmol/l)	3.51±2.18	3.57±2.81

DM,diabetes mellitus; $HbA_{1c,}$ glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 3.2. Characteristics of the study group by sex.

	Women (n=241)	Men (n=137)
Age (years)	42.9±17.4	44.2±18.8
Body weight (kg)	78.1±19.4	73.9±16.7
Body Height (cm)	157.9.1±6.8	169.0±7.2
Body mass index (kg/m^2)	31.3±7.6	25.8±5.4
Mean skin-fold thickness (cm)	2.43±1.07	1.62±0.94
Waist circumference (cm)	92.1±15.7	87.2±14.5
Hip circumference (cm)	112.1±14.2	99.9±12.2
Waist-to-hip ratio	0.82±0.10	0.88±0.11
% overweight/obese	22.8/53.9	33.6/20.4
Regular tobacco intake (%)	2.5	28.5
Regular alcohol intake (%)	14.1	36.5
% Postmenopausal	41.5	-
% Oestrogen contraception	7.5	-
% with DM or an HbA1c>6.1%	25.3	22.6
Hypertension (%)	40.8	40.0
Antihypertensive therapy (%)	23.7	16.1
Conventional SBP (mm Hg)	129±23	132±20
Conventional DBP (mm Hg)	84±13	85±11
Total/HDL cholesterol (mmol/l)	3.55±3.39	3.62±1.29
Leptin (ng/ml)	38.0±30.4	8.6±9.7

DM,diabetes mellitus; $HbA_{1c,}$ glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

noted no differences in plasma leptin concentrations (ng/ml) between fasting (28.1±25.6) and non-fasting (24.1±25.2) participants (p=0.36).

3.2 Echocardiographic characteristics of participants.

Table 3.3 shows echocardiographic data by sex in the study group. Left ventricular (LV) end diastolic diameter, LV posterior wall thickness, LV septal wall thickness and absolute LV mass were greater in men than in women (Table 3.3). However, when indexed to height$^{2.7}$, LVM was similar between the gender groups. Moreover, although absolute LV wall thickness was greater in men, when wall thickness was expressed as a proportion of internal diameter (LV relative wall thickness), no differences were noted between gender groups (Table 3.3). A high proportion of both men and women had LV hypertrophy (Table 3.3).

3.3 Association between indices of obesity.

Table 3.4 shows correlation matrices for the relationships between indices of adiposity in gender-specific groups. All indices of adiposity were strongly associated with each other in both gender groups, with the closest relationships noted between waist circumference and BMI in both women and in men (Tables 3.4). However, the relationship between waist-to-hip ratio and either BMI or skin-fold thickness was markedly lower in women than in men (Table 3.4).

Table 3.3. Echocardiographic characteristics of the study population by sex.

	Women (n=241)	Men (n=137)
LV end diastolic diameter (cm)	4.61±0.47	4.96±0.47*
LV posterior wall thickness (cm)	0.96±0.13	1.02±0.15*
LV septal wall thickness (cm)	1.01±0.16	1.07±0.18*
LV mean wall thickness (cm)	0.98±0.14	1.04±0.16*
LV relative wall thickness (cm)	0.42±0.07	0.41±0.07
LV mass (g)	160±43	195±60*
LV mass index ($g/m^{2.7}$)	47.0±14.2	47.6±15.1
% with LV hypertrophy (%)	26.1	30.7

LV, left ventricle, *p<0.001 vs women

Table 3.4 Correlation matrices between indices of adiposity in gender-specific groups.

	WC (cm)	WHR	Mean skin-fold thickness
	Women (n=241)		
Body mass index (kg/m^2)	0.87**	0.34**	0.57**
Waist circumference (WC) (cm)	-	0.67**	0.53**
Waist-to-hip ratio (WHR)	-	-	0.21*
	Men (n=137)		
Body mass index (kg/m^2)	0.86**	0.50**	0.71**
Waist circumference (WC) (cm)	-	0.50**	0.60**
Waist-to-hip ratio (WHR)	-	-	0.38**

Numbers are correlation coefficients (r). WC, waist circumference; WHR, waist-to-hip ratio. * $p<0.001$, ** $p<0.0001$ for the relationships.

3.4 Relationships between adiposity indices and plasma leptin concentrations.

Figures 3.1 and 3.2 show the univariate correlations between adiposity indices and plasma leptin concentrations in women (Figure 3.1) or in men (Figure 3.2). In women, BMI, waist circumference and mean skin-fold thickness were strongly associated with plasma leptin concentrations (Figure 3.1). Moreover, hip circumference was also strongly associated with plasma leptin concentrations ($r=0.58$, $p<0.0001$). However, waist-to-hip ratio was only weakly associated with plasma leptin concentrations in women (Figure 3.1) and the correlation coefficient was significantly lower than that for the relationships between other adiposity indices and plasma leptin concentrations ($p<0.003$). In men, all adiposity indices were strongly associated with plasma leptin concentrations (Figure 3.2) including hip circumference ($r=0.58$, $p<0.0001$).

3.5 Relationships between plasma leptin concentrations and other demographic or clinical characteristics of the study sample.

On univariate analysis, age and systolic blood pressure were also strongly associated with plasma leptin concentrations in both women (age, $r=0.28$, $p<0.0001$, systolic blood pressure, $r=0.25$, $p=0.0001$) and in men (age, $r=0.31$, $p=0.0002$, systolic blood pressure, $r=0.33$, $p<0.0001$). However, other demographic and clinical characteristics (other than sex) were not associated with plasma leptin concentrations on univariate analysis.

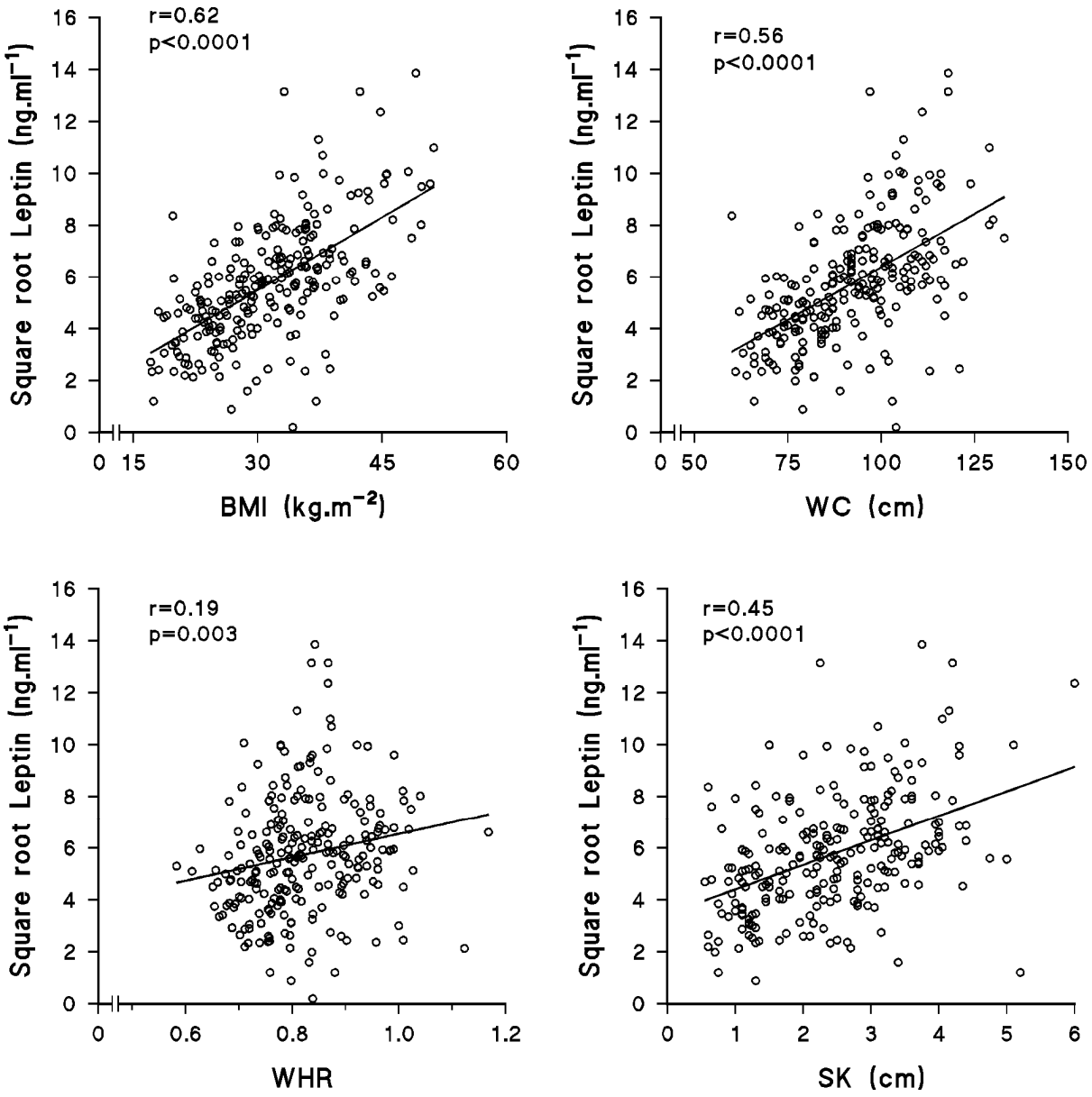


Figure 3.1. Correlations between indices of adiposity and plasma leptin concentrations in women from the study sample. BMI, body mass index; WHR, waist-to-hip ratio; WC, waist circumference; SK, mean skin-fold thickness.

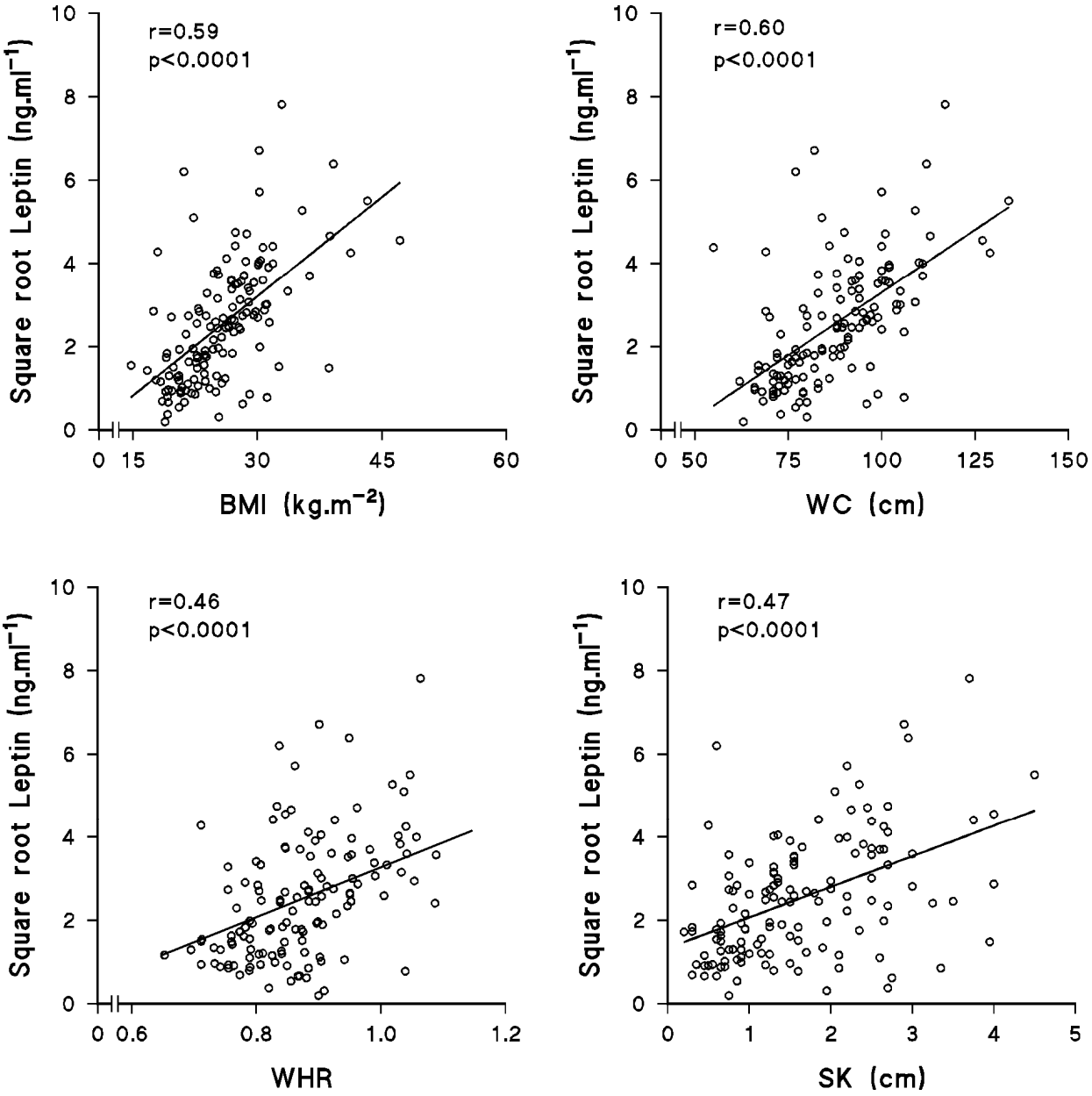


Figure 3.2. Correlations between indices of adiposity and plasma leptin concentrations in men from the study sample. BMI, body mass index; WHR, waist-to-hip ratio; WC, waist circumference; SK, mean skin-fold thickness.

3.6 Relationship between adiposity indices and left ventricular mass index and geometry.

Figures 3.3 and 3.4 show the univariate relationships between either BMI or waist circumference and LVM in women (Figure 3.3) and in men (Figure 3.4). Figures 3.5 and 3.6 show the univariate relationships between either BMI or waist circumference and LVM indexed to height$^{2.7}$ (LVMI) in women (Figure 3.5) and in men (Figure 3.6). In both women and in men a strong relationship between BMI and either LVM or LVMI and between waist circumference and LVM or LVMI was noted. Although skin-fold thickness and waist-to-hip ratio were also correlated with LVM in both women (skin-fold thickness, $r=0.28$, $p< 0.0001$, waist-to-hip ratio, $r=0.27$, $p<0.0001$) and in men (skin-fold thickness, $r=0.30$, $p=0.0005$, waist-to-hip ratio, $r=0.36$, $p<0.0001$) and with LVMI in both women (skin-fold thickness, $r=0.23$, $p=0.0003$, waist-to-hip ratio, $r=0.32$, $p<0.0001$) and in men (skin-fold thickness, $r=0.32$, $p=0.0002$, waist-to-hip ratio, $r=0.32$, $p=0.0001$), the correlation coefficients were not as striking as those for BMI and waist circumference (Figures 3.3 to 3.6).

Figures 3.7 and Figure 3.8 show the univariate relationships between either BMI or waist circumference and LV mean wall thickness in women (Figure 3.7) and in men (Figure 3.8). In both women and in men a strong relationship between BMI and LV mean wall thickness and between waist circumference and LV mean wall thickness was noted. Although skin-fold thickness and waist-to-hip ratio were also correlated with LV mean wall thickness in both women (skin-fold thickness, $r=0.24$, $p=0.0002$, waist-to-hip ratio; $r=0.35$, $p<0.0001$) and in men (skin-fold thickness, $r=0.27$, $p=0.0016$, waist-to-hip ratio, $r=0.37$, $p<0.0001$), again the correlation coefficients were not as striking as those for BMI and waist circumference (Figures 3.7 and 3.8).

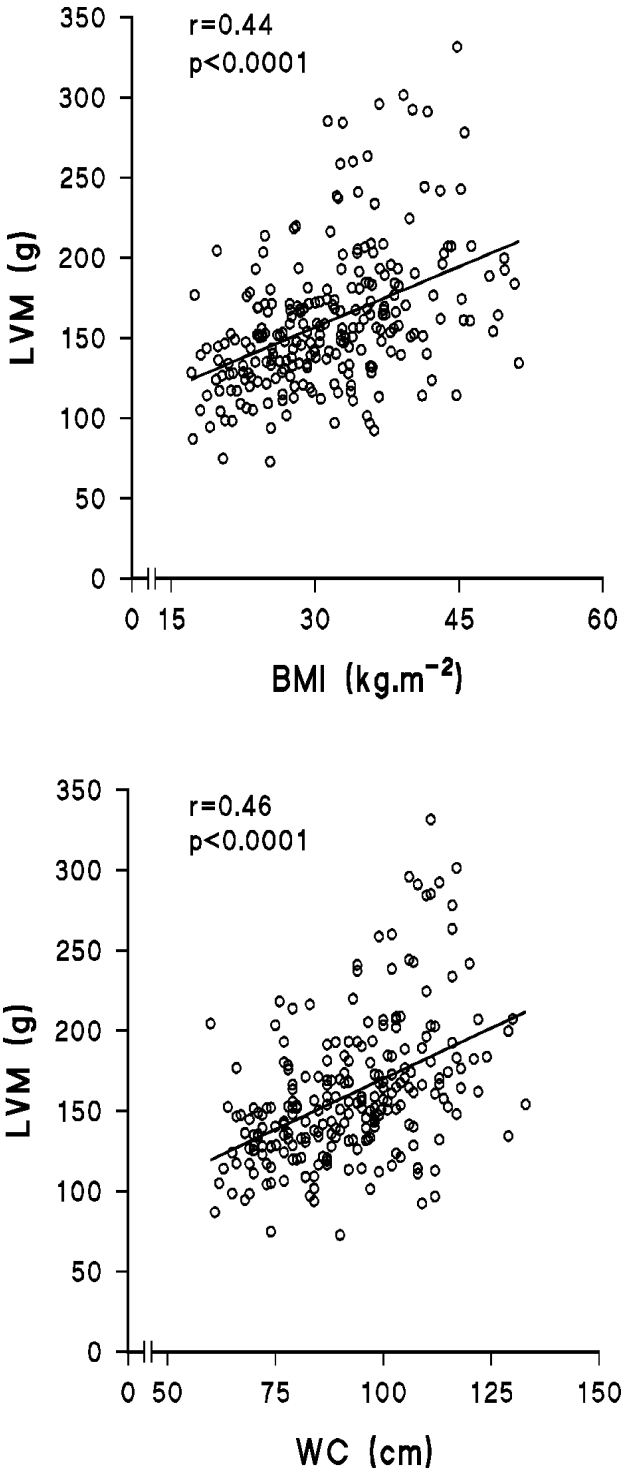


Figure 3.3. Correlations between indices of adiposity and left ventricular mass (LVM) in women from the study sample. BMI, body mass index; WC, waist circumference.

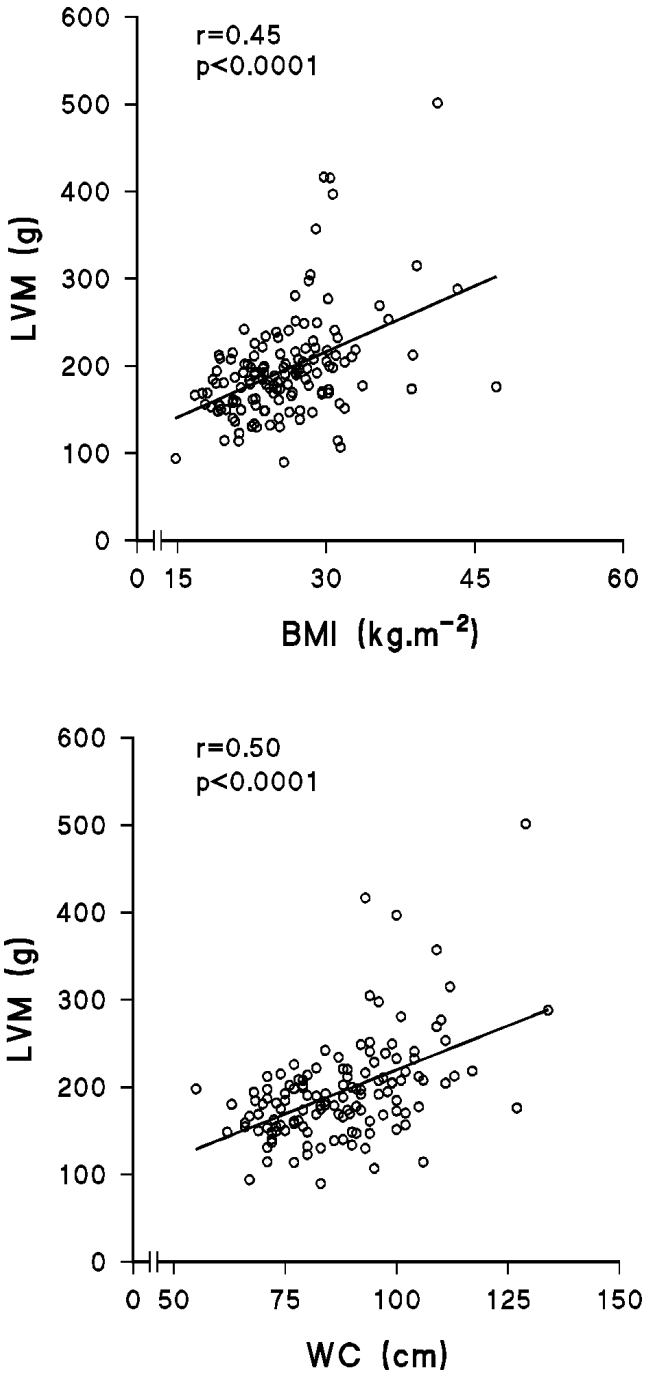


Figure 3.4. Correlations between indices of adiposity and left ventricular mass (LVM) in men from the study sample. BMI, body mass index; WC, waist circumference.

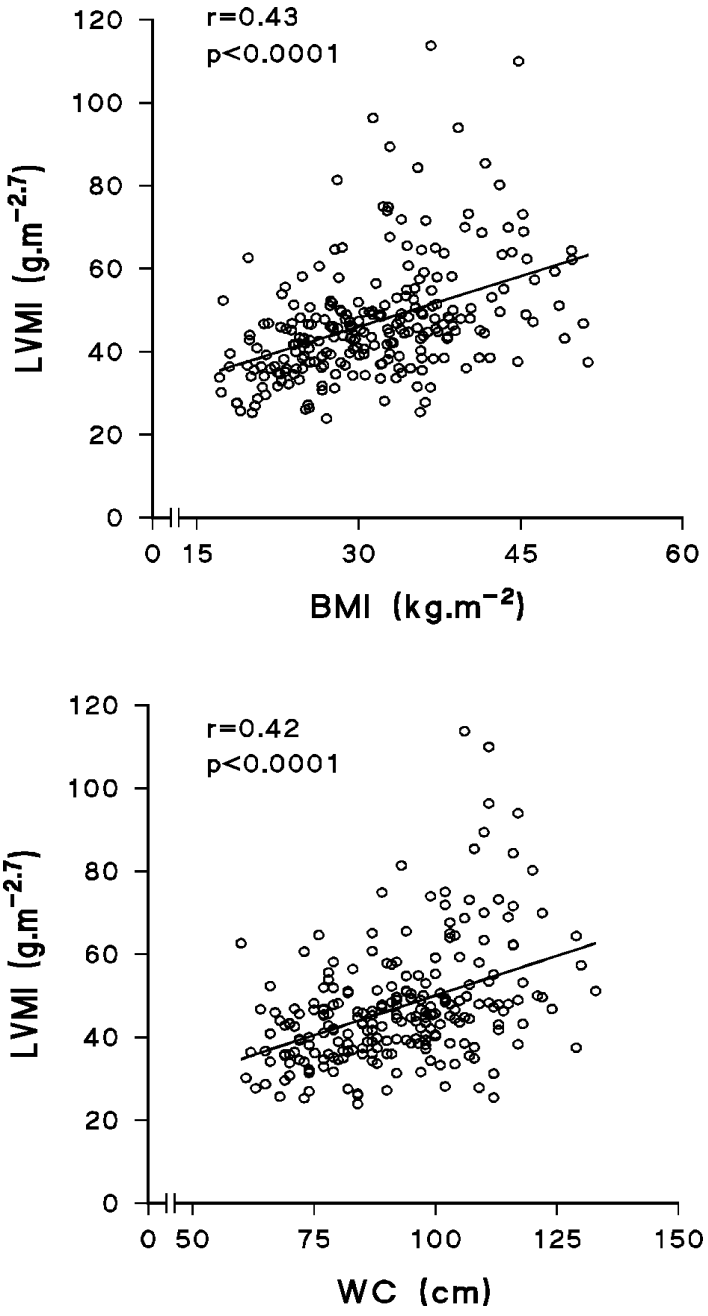


Figure 3.5. Correlations between indices of adiposity and left ventricular mass indexed to height$^{2.7}$ (LVMI) in women from the study sample. BMI, body mass index; WC, waist circumference.

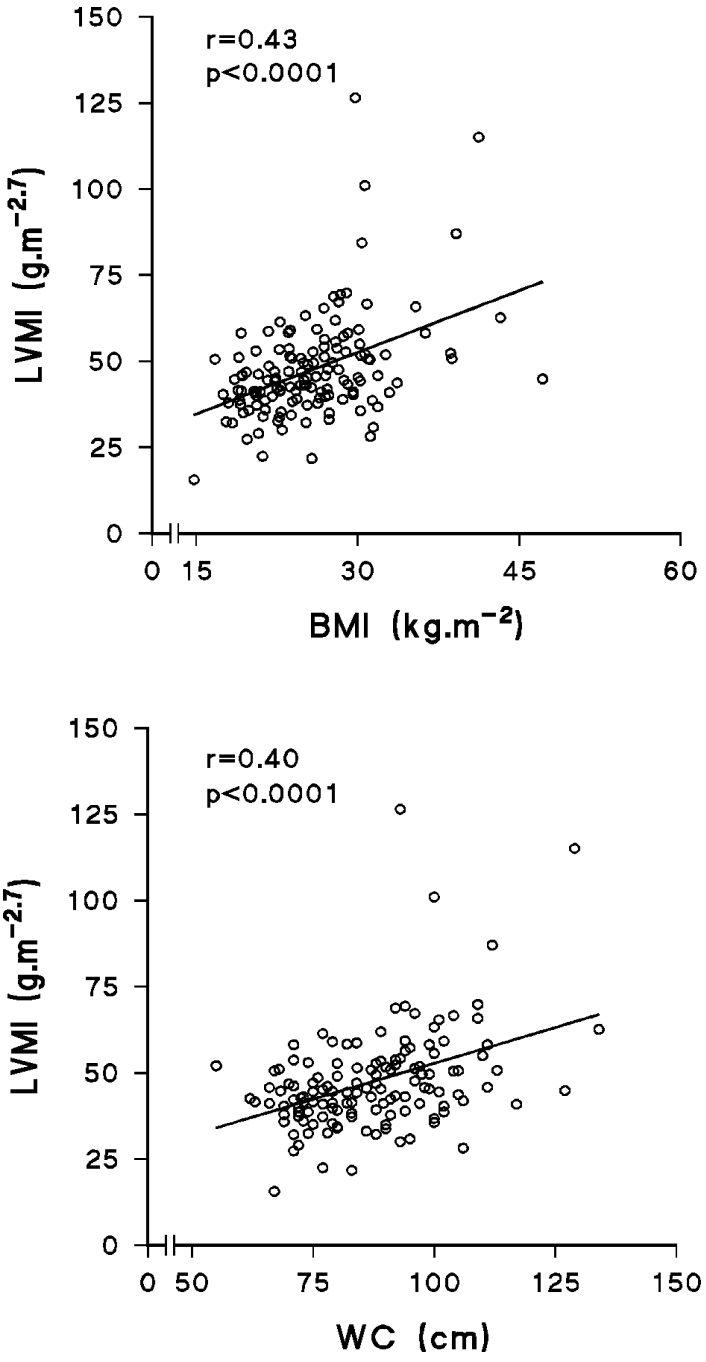


Figure 3.6. Correlations between indices of adiposity and left ventricular mass indexed to height$^{2.7}$ (LVMI) in men from the study sample. BMI, body mass index; WC, waist circumference.

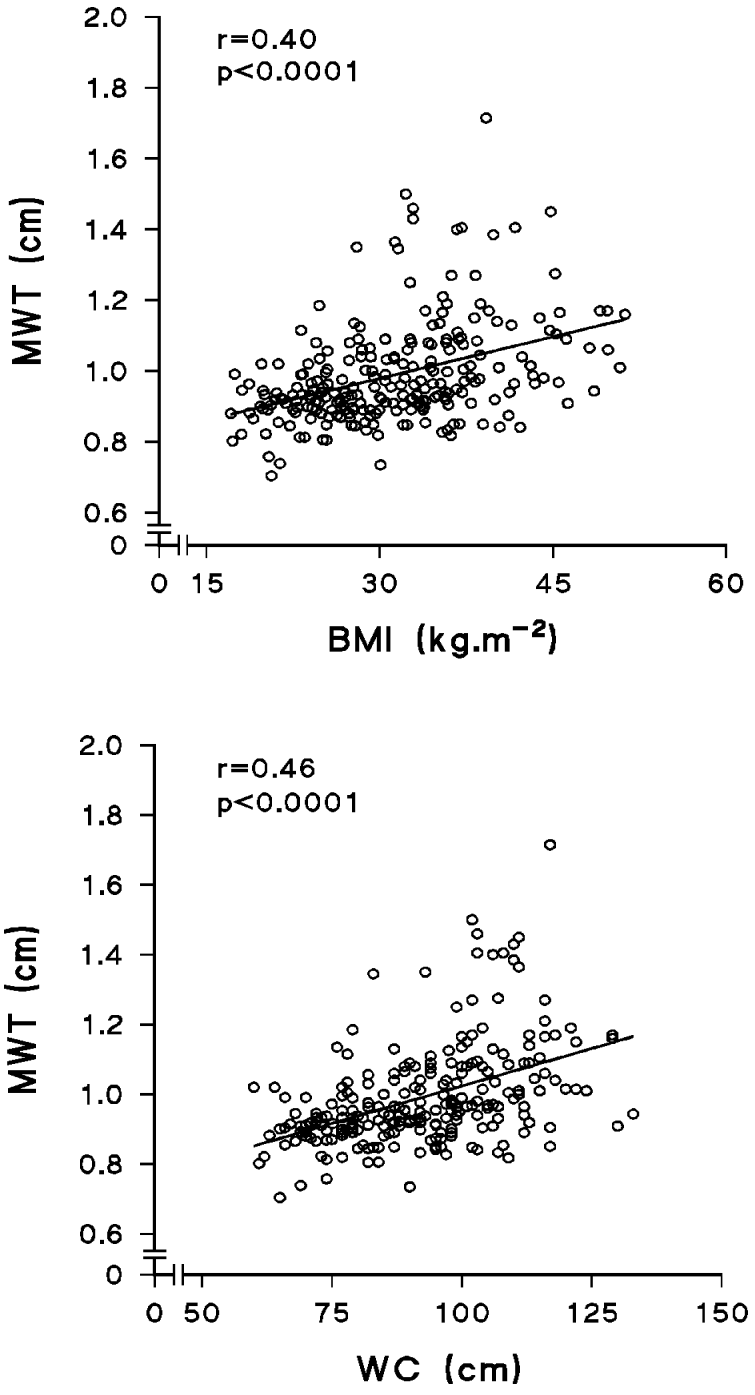


Figure 3.7. Correlations between indices of adiposity and left ventricular mean wall thickness (MWT) in women from the study sample. BMI, body mass index; WC, waist circumference.

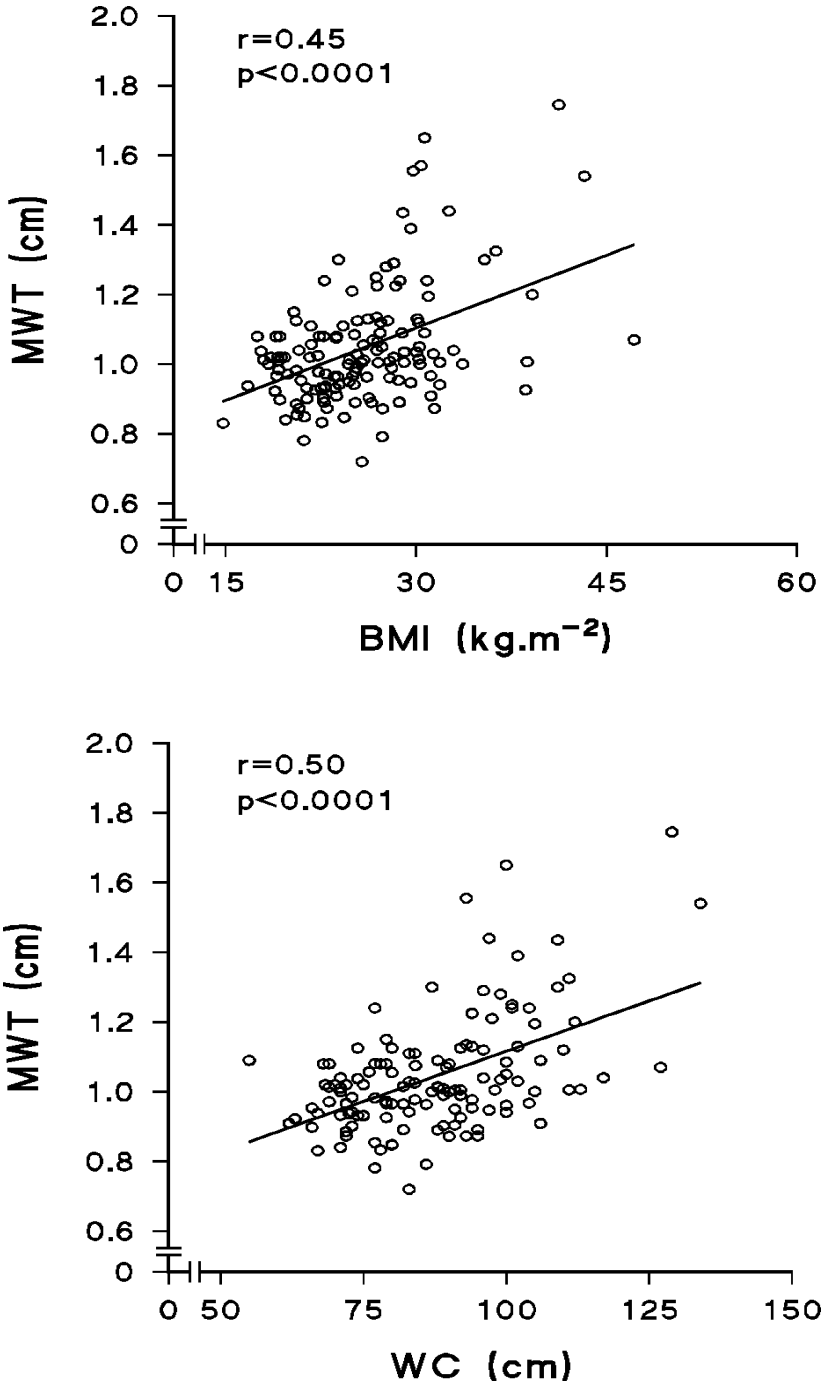


Figure 3.8. Correlations between indices of adiposity and left ventricular mean wall thickness (MWT) in men from the study sample. BMI, body mass index; WC, waist circumference.

3.7 Relationship between plasma leptin concentrations and left ventricular mass index and geometry.

Figures 3.9 and 3.10 show the univariate correlations between plasma leptin concentrations and either LVM (Figure 3.9), LVMI (Figure 3.9) or LV mean wall thickness (Figure 3.10) in women. Figure 3.11 and 3.12 show the univariate correlations between plasma leptin concentrations and either LVM (Figure 3.11), LVMI (Figure 3.11) or LV mean wall thickness (Figure 3.12) in men. In both women and in men plasma leptin concentrations were strongly correlated with LVM, LVMI and LV mean wall thickness. Moreover, plasma leptin concentrations were markedly higher in participants with as compared to those without LVH (Figure 3.13).

3.8 Other factors associated with left ventricular mass index and geometry.

In women, other factors associated with LVMI on univariate analysis included age ($r=0.26$, $p<0.0001$), and systolic blood pressure ($r=0.35$, $p<0.0001$). In women, age ($p<0.0001$) and systolic blood pressure ($p<0.0001$) were also associated with LV mean wall thickness. In men, age ($r=0.36$, $p<0.0001$) and systolic blood pressure ($r=0.27$, $p=0.0012$) were also correlated with LVMI. However, in men, no other factors were associated with LV mean wall thickness.

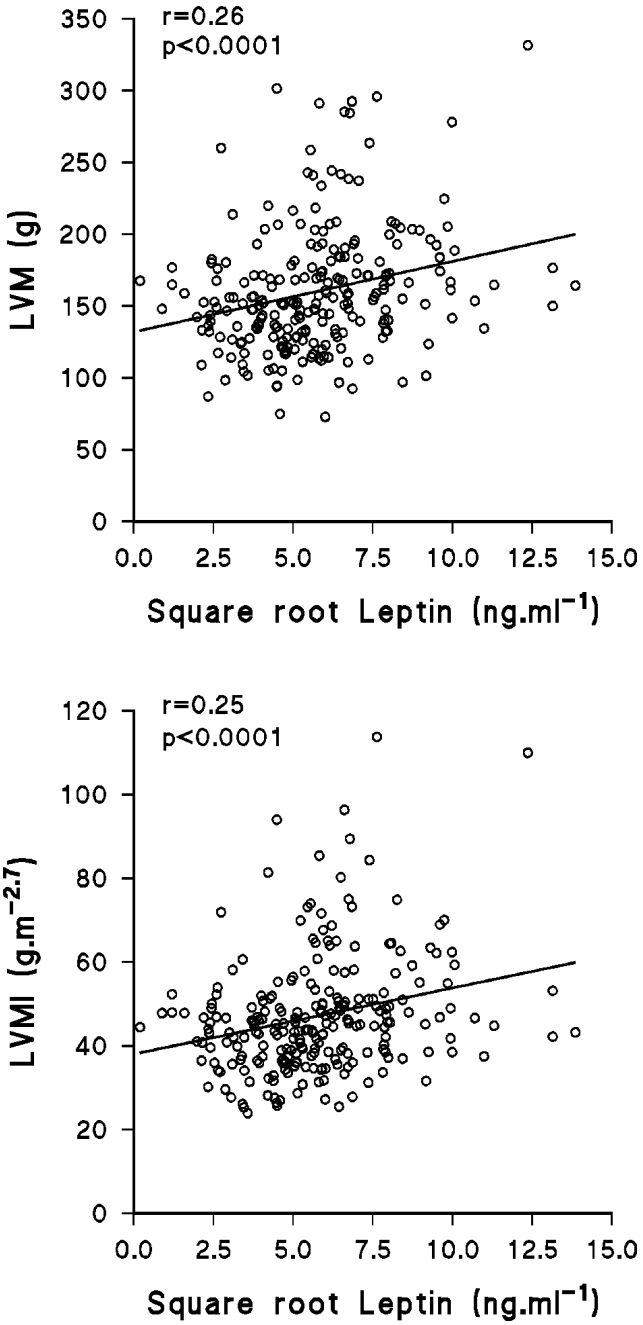


Figure 3.9. Correlations between plasma leptin concentrations and left ventricular mass (LVM) (upper panel) and LVM indexed to height$^{2.7}$ (LVMI) (lower panel) in women from the study sample.

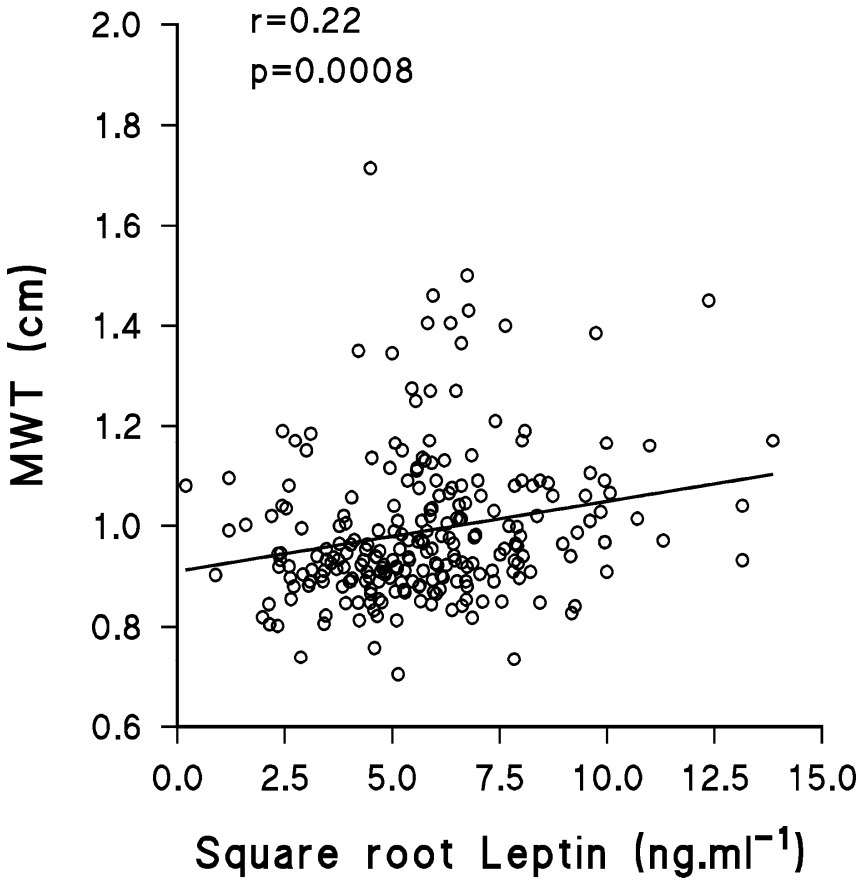


Figure 3.10. Correlations between plasma leptin concentrations and left ventricular mean wall thickness (MWT) in women from the study sample.

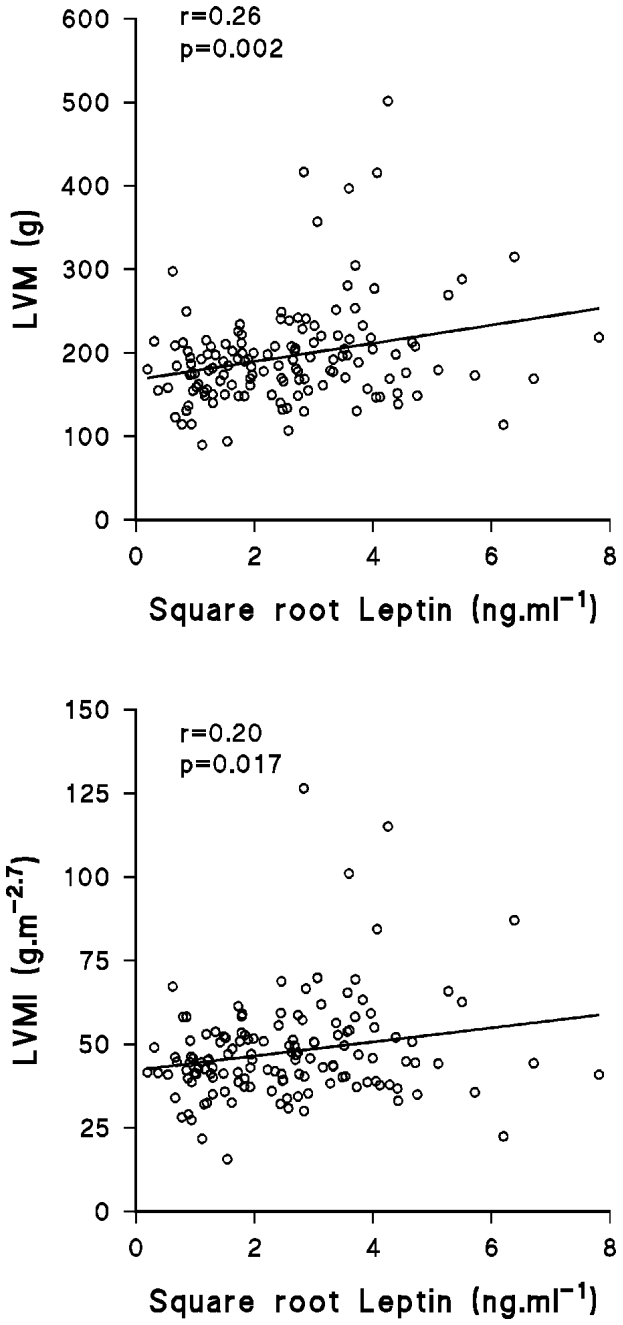


Figure 3.11. Correlations between plasma leptin concentrations and left ventricular mass (LVM) (upper panel) and LVM indexed to height$^{2.7}$ (LVMI) (lower panel) in men from the study sample.

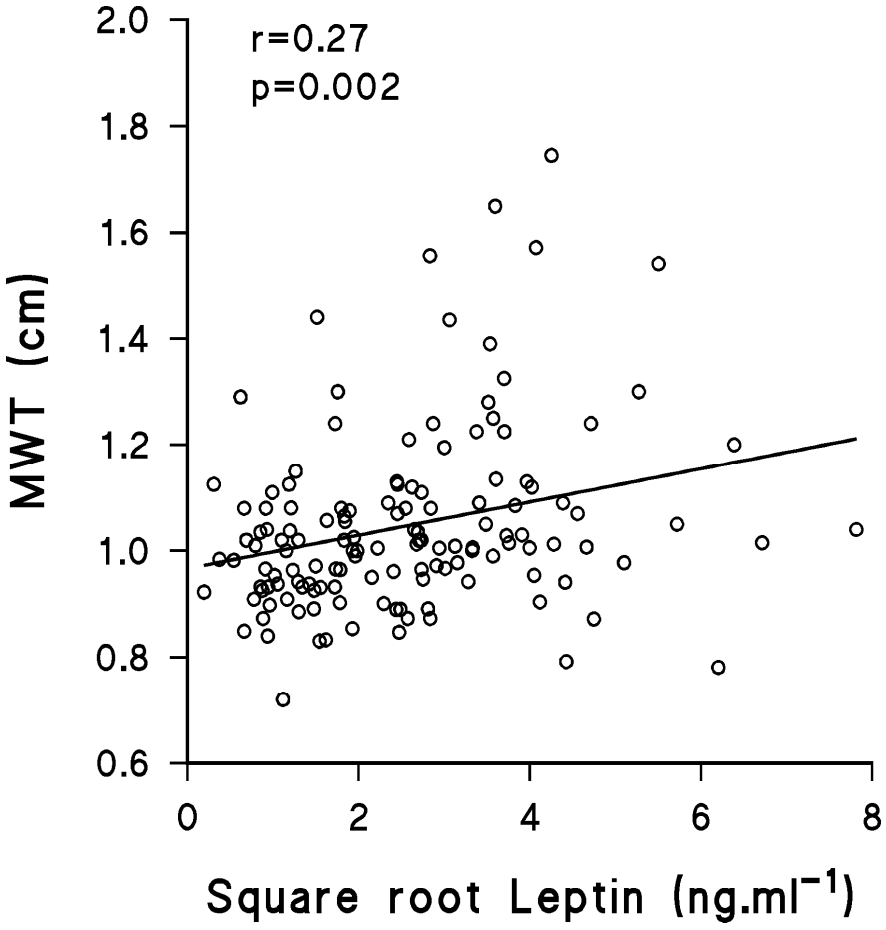


Figure 3.12. Correlations between plasma leptin concentrations and left ventricular mean wall thickness (MWT) in men from the study sample.

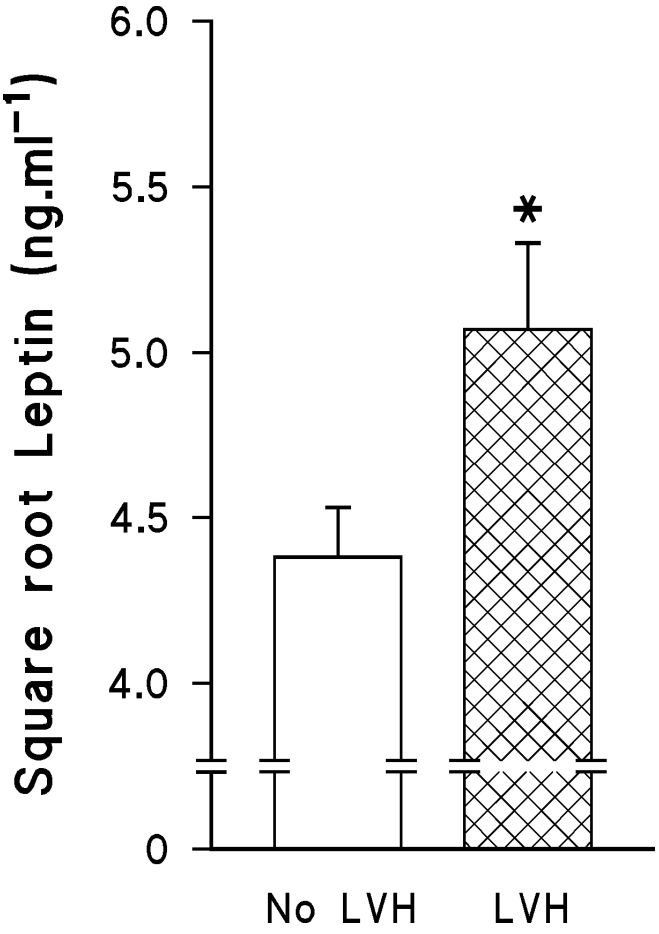


Figure 3.13. Plasma leptin concentrations in women and men with and without an increased left ventricular mass indexed to height$^{2.7}$ (with and without left ventricular hypertrophy [LVH]) (*p<0.001 vs no LVH on an unpaired Student's t-test).

3.9 Independent predictors of left ventricular mass index and geometry.

Tables 3.5-to-3.7 shows the factors independently associated with LVM (Table 3.5), LVMI (Table 3.6) and LV wall thickness (Table 3.7) in sex-specific groups with waist circumference employed as an index of adiposity in one model and BMI in another. In multivariate adjusted regression models the major predictors of LVM, LVMI, and LV wall thickness were waist circumference or BMI in both men and women, systolic blood pressure in women, treatment for hyprtension in men when assessing LV wall thickness (Table 3.7) and age in men and women when assessing LVMI (Table 3.6). Despite relatively strong univariate associations between plasma leptin concentrations and LVM, LVMI and mean wall thickness (Figures 3.9 to 3.12), plasma leptin concentrations were not independent predictors of either LVM, LVMI, or LV mean wall thickness in either women or in men (Tables 3.5 to 3.7).

Table 3.8 shows the factors independently associated with LVH in sex-specific groups with waist circumference employed as the index of adiposity in one model and BMI in another. In multivariate adjusted regression models the major predictors of LVH were BMI in both men and systolic blood pressure in women. Despite the increased plasma leptin concentrations noted in participants with LVH (Figures 3.13), plasma leptin concentrations were not independent predictors of LVH in either women or in men (Table 3.8).

Table 3.5. Predictors of left ventricular mass from multivariate regression analysis in sex-specific groups..

	Men		Women	
Independent variable	β-coefficient*	p value	β-coefficient*	p value
Model with waist circumference.				
Height	0.266	0.7620	11.004	**0.0132**
Waist circumference	2.001	**<0.0001**	0.631	**<0.0001**
Systolic blood pressure	0.407	0.3264	0.716	**<0.0001**
Age	-0.296	0.3795	0.214	0.3067
Treatment for hypertension	20.372	0.1937	3.134	0.6645
Smoking	-1.403	0.8346	6.643	0.1871
Leptin	-3.759	0.3955	0.423	0.7407
Alcohol	5.236	0.5064	1.514	0.7527
Diabetes mellitus or HbA1c>6.1%	-5.731	0.7350	11.804	0.2484
Model with body mass index				
Body mass index	4.484	**<0.0001**	1.546	**<0.0001**
Systolic blood pressure	0.596	0.0501	0.562	**<0.0001**
Age	-0.159	0.7150	0.217	0.3458
Treatment for hypertension	21.083	0.1459	0.939	0.8920
Smoking	6.224	0.2572	6.644	0.1359
Leptin	-1.498	0.7069	-0.371	0.7711
Alcohol	4.091	0.6239	3.288	0.4846
Diabetes mellitus orHbA1c>6.1%	-5.038	0.7789	9.429	0.3786

* Additional adjustments include pulse rate, menopausal status and oestrogen contraception. Probability values were further adjusted for non-independence of family members.

Table 3.6. Predictors of left ventricular mass index ($g/m^{2.7}$) from multivariate regression analysis in sex-specific groups.

	Men		Women	
	β-coefficient	p value	β-coefficient	p value
Models with waist circumference				
Waist circumference	0.32	**<0.0001**	0.12	**0.0017**
Systolic blood pressure	0.06	0.4592	0.27	**<0.0001**
Age	0.08	**0.0472**	0.11	**0.0472**
Treatment for hypertension	4.05	0.2768	1.52	0.5044
Smoking	-1.65	0.4093	2.21	0.2823
Leptin	- 0.93	0.4316	0.29	0.5542
Alcohol	1.15	0.5679	-0.81	0.5928
Diabetes mellitus or HbA1c>6.1%	- 1.53	0.7335	3.35	0.2750
Models with body mass index				
Body mass index	1.07	**<0.0001**	0.37	**0.0001**
Systolic blood pressure	0.10	0.3184	0.26	**<0.0001**
Age	0.08	**0.0126**	0.12	**0.0261**
Treatment for hypertension	4 .30	0.2402	1.15	0.5834
Smoking	-0.20	0.9033	2.27	0.2216
Leptin	-1.28	0.3165	-0.02.	0.9612
Alcohol	-0.93	0.6500	-0.81	0.6051
Diabetes mellitus or HbA1c>6.1%	-0.58	0.8996	3.06	0.3295

* Additional adjustments include pulse rate, menopausal status and oestrogen contraception. Probability values were further adjusted for non-independence of family members.

Table 3.7. Predictors of left ventricular wall thickness from multivariate regression analysis in sex-specific groups.

	Men		Women	
Independent variable	β-coefficient	p value	β-coefficient	p value
Model with waist circumference				
Waist circumference	0.01	**<0.0001**	0.001	**0.0002**
Systolic blood pressure	0.001	0.2876	0.002	**<0.0001**
Age	0.001	0.2941	0.001	0.0578
Treatment for hypertension	0.10	**0.0363**	0.0002	0.9914
Smoking	0.01	0.4688	0.01	0.3693
Leptin	- 0.001	0.3334	0.003	0.4927
Alcohol	-0.01	0.7708	0.01	0.2040
Diabetes mellitus or HbA1c>6.1%	-0.01	0.7788	-0.02	0.4393
Model with body mass index				
Body mass index	0.01	**<0.0001**	0.004	**0.0009**
Systolic blood pressure	0.002	0.0508	0.002	**<0.0001**
Age	-0.001	0.4939	0.001	**0.0140**
Treatment for hypertension	0.10	**0.0205**	-0.001	0.9659
Smoking	0.03	0.1268	0.01	0.4342
Leptin	-0.003	0.8079	-0.004	0.3645
Alcohol	-0.01	0.7682	0.01	0.1933
Diabetes mellitus or HbA1c>6.1%	-0.01	0.7615	-0.03	0.2634

* Additional adjustments include pulse rate, menopausal status and oestrogen contraception. Probability values were further adjusted for non-independence of family members.

Table 3.8. Predictors of left ventricular hypertrophy from multivariate logistic regression analysis in sex-specific groups.

	Men		Women	
Independent variable	β-coefficient	p value	β-coefficient	p value
Model with waist circumference.				
Waist circumference	-0.026	0.1966	-0.006	0.7154
Systolic blood pressure	-0.008	0.5502	-0.033	**0.0013**
Age	-0.026	0.1415	-0.028	0.0764
Treatment for hypertension	0.506	0.4355	-0.330	0.4508
Smoking	0.548	0.0773	-0.045	0.9089
Leptin	0.150	0.4105	-0.101	0.2610
Alcohol	-0.322	0.3618	-0.096	0.7709
Diabetes mellitus or HbA1c>6.1%	0.439	0.5188	-0.294	0.6269
Model with body mass index				
Body mass index	-0.142	**0.0095**	-0.015	0.0962
Systolic blood pressure	-0.015	0.2760	-0.030	**0.0030**
Age	-0.021	0.2045	-0.028	0.0648
Treatment for hypertension	0.395	0.5496	-0.218	0.6161
Smoking	0.317	0.2835	-0.051	0.8958
Leptin	0.286	0.1343	-0.032	0.7360
Alcohol	-0.321	0.3662	-0.059	0.8593
Diabetes mellitus or HbA1c>6.1%	0.231	0.7388	-0.312	0.6024

* Additional adjustments include pulse rate, menopausal status and oestrogen contraception. Probability values were further adjusted for non-independence of family members.

Chapter 4

Discussion

4.1 Main outcomes of the present study

The main finding in the present study is that in a population sample with a high frequency of excess adiposity (~69%) and of LVH (~28%), with strong associations noted between indices of adiposity and either LVM, LVMI, LV wall thickness, or LVH, although on univariate analysis plasma leptin concentrations were strongly correlated with LVM, LVMI, LV wall thickness and LVH, plasma leptin concentrations were not an independent predictor of LVM, LVMI, LV wall thickness or LVH in either women or men. The inclusion of indices of adiposity in regression models relating plasma leptin concentrations to LVM, LVMI, LV wall thickness and LVH were largely responsible for abolishing these associations.

4.2 Comparison with previous studies

Previous studies assessing the relationship between plasma leptin concentrations and LVM, LVMI, wall thickness and LVH have provided inconsistent outcomes. In contrast to the present study conducted in 241 women and 137 men where plasma leptin concentrations were not independently associated with LV wall thickness, in 40 insulin resistant hypertensive men and 15 apparently healthy men an association between plasma leptin concentrations and LV wall thickness was noted independent of indices of adiposity or blood pressure (Paolisso et al 1999). Furthermore, in contrast to the present study, where plasma leptin concentrations were not related to LVH, plasma leptin concentrations were associated with LVH in 31 morbidly obese participants both before and after weight loss following bariatric surgery (Perego et al 2005).

An independent relationship between plasma leptin concentrations and LV wall thickness or LVH in 55 (Paolisso et al 1999) and in 31 (Perego et al 2005) participants

could represent false positive outcomes. However, the independent relationship between changes in plasma leptin concentrations and LVH following weight loss (Perego et al 2005) provides prospective data to support the hypothesis. Differences in the study groups assessed could explain the apparent discrepancies between these previous studies (Perego et al 2005, Paolisso et al 1999) and the present study. In these previous studies (Perego et al 2005, Paolisso et al 1999), select subgroups of obese participants were studied. In this regard, in these prior studies only hypertensive participants with insulin resistance (Paolisso et al 1999), or morbidly obese (Perego et al 2005) participants were studied. In contrast, in the present study, a random selection of participants, only 22% of whom had a BMI>35 kg/m^2, and many of whom may not have had insulin resistance, were studied. It is possible that either the presence of insulin resistance is required to show an independent relationship between plasma leptin concentrations and LVM or wall thickness or an independent relationship is noted only when severe or morbid obesity occurs.

In a previous study showing an independent relationship between plasma leptin concentrations and LV wall thickness (Paolisso et al 1999), the male participants had a mean BMI of ~26.9 kg/m^2. Hence in the men in this prior study (Paolisso et al 1999) there may have been a greater sensitivity to detect an independent relationship between plasma leptin concentrations and LV wall thickness than in the men in the present study whose mean BMI was ~25.8 kg/m^2. Nevertheless, if this argument holds, then this effect has to be sex-specific, as in the present study the 241 women had a mean BMI of ~31.3 kg/m^2 and no independent relationship between plasma leptin concentrations and LVM, LVMI or wall thickness were noted in women.

Consistent with the present study, in a previous study conducted in 191 participants with a BMI range of 24.8 and 27.1 kg/m^2, plasma leptin concentrations were not independently associated with LV wall thickness or LVM after adjustments for body

size (Malmqvist et al 2002). However, it could be argued that a lack of independent relationship between plasma leptin concentrations and LVM or wall thickness in participants with a BMI range of 24.8 and 27.1 kg/m^2 (Malmqvist et al 2002) may represent a low sensitivity to detect an independent relationship in a population with a low prevalence of excess adiposity. Indeed, on univariate analysis plasma leptin concentrations correlated only with LV relative wall thickness and not with LVMI in this prior study (Malmqvist et al 2002). However, the present study provides clear evidence at least in women with a high prevalence of excess adiposity (77%), that plasma leptin concentrations are not independently associated with LVM, LVMI or LV wall thickness. Indeed, in the present study fairly strong univariate correlations were noted between plasma leptin concentrations and either LVM, LVMI or LV wall thickness prior to adjustments for confounders, but after appropriate adjustments, including adjustments for adiposity indices, no independent relationships between plasma leptin concentrations and LVM, LVMI, LV wall thickness or LVH were noted.

In contrast to the outcomes of the present study, or to those outcomes noted in previous studies (Perego et al 2005, Malmqvist et al 2002, Paolisso et al 1999), in a cross-sectional study of 410 participants, plasma leptin concentrations were inversely associated with LVMI and wall thickness (Pladevall et al 2003). This inverse relationship may reflect a protective effect of leptin on the heart as described in preclinical studies (Barouch et al 2003). The apparent discrepancy in the outcomes of this study (Pladevall et al 2003) as compared to other studies (Perego et al 2005, Malmqvist et al 2002, Paolisso et al 1999), may reflect differences in the populations sampled. For example, in this previous study (Pladevall et al 2003), the population sampled had a mean BMI of ~26.3 kg/m^2, thus representing a low prevalence of excess adiposity. In this regard, in cross-sectional studies with a low prevalence of excess adiposity, the prevalence of insulin resistance and thus presumably also leptin resistance may be low. Consequently,

increases in leptin release from adipose tissue may mediate anti-hypertrophic effects on LVM thus revealing an inverse relationship between plasma leptin concentrations and LVMI or LV wall thickness such as noted in the study by Pladevall et al (2003). However, in studies where patients are specifically selected for either the presence of insulin resistance or morbid obesity where presumably a high prevalence if insulin resistance would be noted (Perego et al 2005, Paolisso et al 1999), these patients may also have a high prevalence of leptin resistance. Under these circumstances, leptin's effects on sympathetic activation and subsequently LVM, may over-ride the anti-hypertrophic benefits of leptin (Barouch et al 2003). If this argument holds, it is also possible that the pro-hypertrophic sympathetic effects of leptin on the heart may compete with the antihypertrophic effects of leptin on the heart (Barouch et al 2003), thus rendering an overall lack of relationship between plasma leptin concentrations and either LVM, LVMI or LV wall thickness as noted in the present study and in the study by Malmqvist et al (2002). In this regard, further large studies are therefore required to compare the relationship between plasma leptin concentrations and LVM, LVMI or LV wall thickness in leptin resistant versus leptin sensitive obese patients.

An additional potential explanation for the differences in outcomes of the present and of prior studies (Perego et al 2005, Pladevall et al 2003, Paolisso et al 1999), is that in the present population study, our group has employed standardized and high quality conventional blood pressure measurements (Libhaber et al 2008, Majane et al 2007, Majane et al 2007, Shiburi et al 2006), whereas other groups have not commented on the quality of the blood pressure measurements employed. As indicated in the introductory chapter to the present dissertation, blood pressure is a major determinant of LVM and the quality of the blood pressure measurement is likely to affect the relationship between blood pressure and LVM noted. Indeed, our group has recently demonstrated that the conventional blood pressure measurements obtained in the

present study are as closely associated with multiple target organ changes as are 24 hour ambulatory blood pressures (Woodiwiss et al 2008). In this regard, as leptin promotes increases in blood pressure through activation of the sympathetic nervous system, and this may in-part mediate leptin-induced increases in LVM, adjustments for high quality blood pressure measurements may mask relations between plasma leptin concentrations and LVMI or LV wall thickness. However, although this argument may hold true for women in the present study, where blood pressure was closely associated with LVMI and wall thickness, the same argument is unlikely to hold for men in whom blood pressure was not independently associated with LVMI or LV wall thickness.

4.3 Plasma leptin concentrations in the population studied.

It may be argued that a lack of relationship between plasma leptin concentrations and either LVM, LVMI, LV wall thickness or LVH in the present study may reflect measurement error in plasma leptin concentrations. Indeed, there is some controversy as to whether plasma leptin concentrations should be measured under fasting conditions (Raben and Astrup 2000), and in the present study evidence obtained from questionnaire data indicated that approximately half the participants had not had an appropriate 8 hour fast prior to blood samples being obtained. Importantly, however, plasma leptin concentrations were no different between fasted and non-fasted participants, suggesting that the fasting state has no impact on the variability of plasma leptin concentrations. Furthermore, in support of an appropriate measurement technique in the present study, consistent with previous studies (Perego et al 2005, Pladeval et al 2003), I showed a marked difference in plasma leptin concentrations between women and men, with strikingly higher concentrations noted in women even after adjustments for differences in body size (data not shown). The exact mechanism of this sex effect

has not been elucidated. Moreover, in further support of appropriate measurement technique in the present study, I was able to show a conspicuous univariate relation between indices of adiposity and plasma leptin concentrations, with the strength of these relations equivalent to previously published relations (Pladeval et al 2003, Leyva et al 1997). As with previously published data (Paolisso et al 1999, Pladeval et al 2003, Leyva et al 1997) these relations between adiposity indices and plasma leptin concentrations were noted for all adiposity indices. The strength of the relations between adiposity indices and plasma leptin concentrations are unlikely to reflect a propensity of obese participants to ignore the request to fast. Indeed, as indicated in the aforementioned discussion, I noted no differences in plasma leptin concentrations between fasting and non-fasting participants in the present study. Indeed, to my knowledge there is no data to indicate that plasma leptin concentrations increase with food intake.

4.4 Left ventricular hypertrophy in the population sample studied.

It may be argued that, in contrast to other studies where independent associations between plasma leptin concentrations and either LVMI, LV wall thickness or LVH have been noted (Perego et al 2005, Pladevall et al 2003, Paolisso et al 1999), an inability to show independent relations between plasma leptin concentrations and LVMI or wall thickness may relate to unique characteristics of LV growth in groups of African descent. Indeed, in the present study, the prevalence of LVH was noted to be remarkably high. Is there evidence to suggest biologically-based ethnic differences in LVM?

Whilst data from some early studies suggests that after adjusting for blood pressure, ethnicity is not associated with an increased prevalence of LVH (Gottdiener et al 1994, Hammond et al 1984) other early studies indicate that LVM index is increased in

African-Americans as compared to Caucasians after blood pressure adjustments (Dunn et al 1983). More recent studies have now clarified this issue. It is now well documented that LVH is more prevalent amongst subjects of African ancestry than in Caucasian subjects (Dekkers et al 2002, Drazner et al 2005, Kizer et al 2004, Rodriguez et al 2004, Skelton et al 2003, Lorber et al 2003, Zabalgoitia et al 1998, Gardin et al 1995, Koren et al 1993). Importantly, in the Dallas Heart Study, conducted in over 2000 individuals, where LVM was determined using magnetic resonance imaging, African-Americans had an increased LVM even after adjustments for other determinants of LVM, including fat mass, fat-free mass, systolic blood pressure, age, gender and measures of socioeconomic status (Drazner et al 2005). These findings are in agreement with the Hypertension Genetic Epidemiology Network Study, where over 1000 African-Americans were studied and noted to have a greater LVM and LV relative wall thickness than 580 Caucasians after adjustment for confounding variables (Kizer et al 2004). In a study comparing African-American to European-American youth, ethnic effects on LVM appeared in early adolescence, and persisted even when controlling for anthropometric factors, haemodynamic variables and socioeconomic status (Dekkers et al 2002).

What determines ethnic disparities in LVM? The impact of ethnicity on LVM appears to be stronger in subjects with higher blood pressure values (Drazner et al 2005), suggesting that blood pressure may still be the major factor influencing the impact of ethnicity on LVM. Assuming that LVM may represent the long-term consequences of abnormal blood pressure control, is there evidence for worse blood pressure control in groups of African descent? Indeed, the prevalence of hypertension in groups of African ancestry living outside of Africa has been reported to be 40.5%, as compared to the lower hypertension prevalence rates of 27.4% reported to occur in those of European descent (Glover et al 2005). These figures represent an increasing rather than declining difference in prevalence of hypertension between ethnic groups than that previously

reported on (Ashaye and Giles, 2003). Even in the context of equivalent health promotion services between ethnic groups, people of African ancestry between the ages of 45-to-64 years are still five-six times more likely to be hospitalised for hypertension than their Caucasian counterparts (Holmes et al 2005). Moreover, higher prevalence rates of hypertension persist in those of African descent when adjusting for income and educational level (Howard et al 2000). Thus, ethnic differences in LVM are more likely to reflect the accrual of long-term differences in blood pressure control between ethnic groups as opposed to true biological differences.

However, there are no studies that have been conducted in groups of African descent living in Africa that have attempted to determine whether LVM is characteristically higher in these groups as well after controlling for disparities in blood pressure control. Nevertheless, the thresholds (partition values) for LVM indexed to body surface area in clinically normal individuals (without hypertension) from randomly selected families in an urban developing community of African descent in South Africa have recently been shown to be only marginally higher than other population samples (Libhaber et al 2007). Thus, the high prevalence of LVH in the present study is likely to reflect the high prevalence of both uncontrolled hypertension and an excessively high prevalence of excess adiposity, rather than unique characteristics of LV growth in the ethnic group studied. Hence, the data obtained in the present study with respect to the lack of independent association between plasma leptin concentrations and LVM or LV wall thickness is likely to apply to other ethnic groups as well. However, further studies are nevertheless required in other ethnic groups before this claim is justified.

4.5 Blood pressure in the population sample studied.

In the present study a relatively high proportion of hypertensive participants were not receiving therapy. As indicated in the introduction to this dissertation, blood pressure is a major determinant of LVM. Consequently, blood pressure-mediated increases in LVM may have partly masked the independent impact of leptin on LVM or LV wall thickness. However, as indicated in the aforementioned discussion, this argument can only hold true for women in the present study, where blood pressure was closely associated with LVMI and wall thickness. However, the same argument is unlikely to hold for men in whom blood pressure was not independently associated with LVMI or LV wall thickness.

With respect to blood pressure in the population studied, it is also important to consider the fact that arterial stiffness may influence central blood pressure and that in the present population sample, indices of arterial stiffness are strongly associated with LVM beyond other factors related to LVM including conventional or 24-hour brachial artery blood pressure (Libhaber et al 2008). It is therefore also possible that obesity-induced effects on arterial stiffness and hence central artery blood pressure could also have masked the impact of leptin on LVM, LVMI, LV wall thickness and LVH. Importantly however, independent effects of arterial stiffness on LVM are noted only in women, and not in men in the present population (Libhaber et al 2008). Consequently, arterial stiffness effects on LVM are unlikely to have masked any relationship between plasma leptin concentrations and LVM in men in the present study.

4.6 Potential limitations of the present study

The results of the present study must be interpreted in light of the study limitations. First, although the number of participants approached who agreed to the study was fairly high for a study of this nature, of these only a proportion agreed to having echocardiography performed on them. The potential of a selection bias therefore needs to be considered. Importantly however, as indicated in the results section, those who agreed to echocardiography were remarkably similar in their demographic and clinical characteristics as the whole group. Thus, the data obtained from echocardiography are highly likely to be representative of all participants enrolled in the study and hence the chances of a selection bias reduced.

Second, the present study was a cross-sectional and not a prospective study and hence I was unable to evaluate whether an independent temporal relationship exists between plasma leptin concentrations and LVM. Further prospective, observational studies are required assessing whether plasma leptin concentrations determined at baseline or changes in plasma leptin concentrations over time are able to independently predict LVM at follow up. In this regard, as indicated in the aforementioned discussion, plasma leptin concentrations and their changes during weight loss were associated with LVH over time in 31 morbidly obese patients (Perego et al 2005). The small study sample and the use of morbidly obese patients nevertheless makes this study (Perego et al 2005) difficult to interpret in the context of overweight or moderately obese patients.

A third potential limitation of the present study is that obesity in the study participants was predominantly in females. As a prior study showing an independent relationship between plasma leptin concentrations and LV wall thickness was conducted in men only (Paolisso et al 1999), it is possible that independent relations between plasma leptin concentrations and LVM or wall thickness may have been masked in the

present study by a low prevalence of obesity in men. Consequently, I cannot exclude the possibility of an independent relationship between plasma leptin concentrations and LVM in obese men.

A fourth potential limitation of the present study is that I did not categorize participants into subgroups based on potential mechanisms through which leptin is thought to mediate adverse or beneficial effects. For example I could have identified participants with a high sympathetic drive by measuring indices of sympathetic nervous system activity, such as heart rate variability, plasma catecholamine concentrations, or 24-hour urinary excretion rates of catecholamine metabolites. It is possible that a relationship between plasma leptin concentrations and LVM may have been noted in patients in whom leptin had augmented sympathetic activity. Furthermore, I could have identified patients with and those without insulin resistance through measurements of fasting glucose and insulin concentrations and calculating the homeostasis model of insulin resistance. By doing this I could have identified those patients most likely to have leptin resistance and subsequently assessed the relationships between plasma leptin concentrations and LVM, LVMI, LV wall thickness or LVH in participants with versus those without insulin and hence probably leptin resistance. Importantly, however, the present study was not designed to answer these questions as in order to answer these questions a much larger study sample would have been required. These questions are nevertheless questions that are presently being addressed under the umbrella of the present study which is ongoing and intended to reach the final study sample size in the year 2012.

A fifth potential study limitation is that plasma leptin concentrations were assessed at only one visit. However in large population-based studies, the opportunity to obtain blood samples more than once over short time periods is limited.

Last, echocardiography is a subjective assessment. Importantly, although I repeated many of the measurements myself to ensure that I was proficient in the measurement, the data from the present study that were analyzed for dissertation and potential publication purposes was that obtained by an highly experienced echocardiographer and cardiologist (Dr. Carlos Libhaber), who was able to demonstrate a low degree of variability on two separate measurements and a high degree of reproducibility in a pilot study conducted prior to initiating the present study.

4.7 Implications of the present study

Importantly, the outcomes of the present study do not suggest that leptin plays little role in moderating LVM, LVMI, LV wall thickness and LVH. As indicated in above discussion, it is possible that leptin's impact on LVM in the present population sample is through blood pressure effects, at least in women, in whom blood pressure was independently associated with LVM, LVMI, LV wall thickness and LVH. If this is true then adjusting for blood pressure as a confounding variable will mask the impact of leptin on LVM in women.

Second, as the correlation coefficients between indices of adiposity and plasma leptin concentrations were strong, it is possible that even if leptin is the major factor responsible for the impact of excess adiposity on LV growth, that adjusting for adiposity indices will eliminate the relationship between plasma leptin concentrations and LVM, LVMI, LV wall thickness and LVH. In these circumstances, the only method of testing the hypothesis that leptin mediates changes in LV growth is to specifically target leptin as a cardiac growth promoting or inhibitory signal in obese individuals. Presently, there are no agents to my knowledge that could possibly qualify for this study design.

Alternatively, as indicated in aforementioned sections, it is possible that leptin's pro-hypertrophic effect on LVM through either direct myocardial effects (Rajapurohitam et al 2003), blood pressure changes, sympathetic effects or other alterations, is negated by the anti-hypertrophic actions of plasma leptin as described in pre-clinical studies (Barouch et al 2003), the overall effect being little change in LVM. Thus, although leptin may play a major role in mediating LVM, this hypothesis can only be tested in subgroups or participants with either insulin and hence presumably leptin resistance or with evidence of sympathetic activation.

Although the outcomes of the present study provide few insights into leptin's role in mediating LV growth, the outcomes do provide important insights into the use of leptin as a biomarker for identifying people who are likely to have LVH. In this regard, from the present study there is no doubt that the measurement of plasma leptin concentrations provides no prophetic power beyond that of adiposity indices in predicting increases in LVM or wall thickness. As an extension to this argument, it is therefore unlikely that the independent relationship between plasma leptin concentrations and stroke, myocardial infarction and CVD in general (Solderberg et al 1999a, Solderberg et al 1999b, Wallace et al 2001) can be explained by an independent relationship between plasma leptin concentrations and LVM.

4.8 Conclusions

In conclusion, in 378 randomly recruited participants from a population sample with a high prevalence of excess adiposity, despite strong univariate correlations between plasma leptin concentrations and LVMI and geometry, these relations were not independent of adiposity indices or blood pressure. The present study therefore suggests that the independent relations between plasma leptin concentrations and

cardiovascular morbidity and mortality (Solderberg et al 1999a, Solderberg et al 1999b, Wallace et al 2001) are unlikely to be explained by an independent relationship between plasma leptin concentrations and LVM and wall thickness. Moreover, the present study suggests that plasma leptin concentrations are unlikely to be useful as a biomarker for persons with an increased LVM.

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