Chapter 1

Introduction

Cardiac dilatation and heart failure:

Current controversies

1.1 Introduction

Heart failure is a major cause of morbidity and mortality in many communities (reviewed by guidelines committees-Hunt et al 2001, Cohn et al 2000). Irrespective of current therapeutic approaches, this disease process may have an average survival rate of 2-to-3 years from the time of diagnosis, often matching that of the deadliest malignancies (Hobbs 2004, Stewart et al 2001, Cohn et al 2000, Massie et al 1997, Lenfant 1994;). Heart failure is a complex syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill or eject blood (Hunt et al 2001). However, an important prognostic feature of heart failure is the development of cardiac dilatation, with a greater cavity size predicting a worse outcome (Cohn et al 2000, Levine et al 2000, Lee et al 1993).

As will be further discussed in the introduction and literature review to the present dissertation, cardiac chamber dilatation contributes to pump dysfunction and subsequent end stage heart failure (de Kam et al 2002, Cohn et al 2000, Mann et al 1999, Cohn et al 1995). Chamber dilatation heralds a poor outcome in heart failure in-part because pharmacological interventions may have little effect on advanced increases in cardiac cavity size (Levine et al 2000). Indeed, therapeutic agents may only partially reduce cardiac chamber dimensions (Levine et al 2000). To develop novel pharmacological approaches to cardiac chamber dilatation, an understanding of the mechanisms involved is required. This dissertation is aimed at providing further insight into the fundamental mechanisms responsible for cardiac dilatation. The current understanding of the role of cardiac dilatation in contributing toward heart failure and the mechanisms of cardiac

dilatation will therefore be reviewed in this chapter. The outstanding issues with respect to the mechanisms of cardiac dilatation that were addressed in this dissertation will be underscored.

As will be highlighted in subsequent discussion, neurohumoral activation, which often accompanies heart failure, appears to play a central role in inducing increases in cavity size in cardiac disease. As will be discussed, most of the clinical evidence to support this notion has been indirect. Moreover, the impact of neurohumoral activation on cardiac dilatation has generally been perceived to be an effect secondary to myocardial contractile disturbances. Consequently, there is an opinion that to target cardiac dilatation therapeutically, the mechanisms that should be sought are those of contractile disturbances. Our group have nevertheless provided the first direct evidence to indicate that chronic β-adrenoreceptor activation induces pump dysfunction or promotes the transition from compensated cardiac hypertrophy to pump dysfunction, principally through chamber dilatation rather than myocardial dysfunction (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a, Woodiwiss et al 2001). Moreover, the primary abnormality involved appears to be independent of contractile disturbances (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a). As the animal model of cardiac dilatation studied in the present dissertation is that previously developed by our group (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a), in the introduction to this dissertation I will also review the current literature on the role of neurohumoral activation in heart failure, the potential mechanisms by which neurohumoral activation may lead to cardiac dilatation, and the potential mechanisms

responsible for the transition from compensated cardiac hypertrophy to heart failure in pressure overload states.

As will be discussed, the progression to heart failure or cardiac dysfunction is characterized by myocardial fibrosis and/or alterations in the phenotypic characteristics of myocardial collagen (Veliotes et al 2005, Hein et al 2003, Badenhorst et al 2003a, Badenhorst et al 2003b, Qin et al 2003, Lombardi et al 2003, Woodiwiss et al 2001, Fielitz et al 2001, Shirani et al 2000, Brooks et al 1997, Brilla et al 1996, Gunja-Smith et al 1996, Conrad et al 1995, Weber et al 1990). An increased myocardial collagen synthesis may occur in cardiac disease as a consequence of myocardial repair following cardiomyocyte damage, as well as through activation of synthesis without tissue damage, through neurohumoral stimulation (Veliotes et al 2005, Badenhorst et al 2003a, Weber 1999, Weber and Brilla 1991, Weber et al 1988b). The accumulation of collagen in the cross-linked form appears to be more closely associated with myocardial stiffness, diastolic dysfunction, and hence diastolic heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001, Norton et al 1997, Spinale et al 1991, Iomoto et al 1988). In contrast, if fibrosis is associated with an accumulation of collagen of the non-cross-linked form, the matrix will become susceptible to degradation by collagenases, the ability of collagen to tether myocytes will potentially be reduced, myocyte side-to-side slippage will occur and cardiac chamber dilatation will result (Veliotes et al 2005, Badenhorst et al 2003a, Badenhorst et al 2003b, Woodiwiss et al 2001). Indeed, collagen that is resistant to cleavage protects the heart from cardiac dilatation and hence from reductions in pump function (Lindsey et al 2003). Neurohumoral activation in cardiac disease may however result in the accumulation of myocardial collagen of both the cross-linked and the non-

cross-linked form. The conundrum that was addressed in the present dissertation was the functional consequences of neurohumoral-induced increases in collagen of the non-cross-linked form in an animal model with pre-existing increases in collagen of the cross-linked form. Consequently as part of the introduction to this dissertation I will also review the current literature on our understanding of diastolic heart failure, the stimuli for collagen synthesis in the myocardium and the role of collagen in mediating cardiac dysfunction in myocardial diseases.

1.2.0 Pathophysiology of heart failure.

From a pathophysiological perspective, the modern classification of heart failure includes high output heart failure, low output heart failure due to systolic functional abnormalities (pump dysfunction) and diastolic functional abnormalities that result in ventricular filling problems. High output cardiac failure generally occurs as a result of a high venous return which produces an increase in cardiac filling and hence an enhanced cardiac output (Frank-Starling effect). Even though the cardiac output is high, it is either inadequate for the body's energy requirements during exercise (because heart function is already working at peak performance) or the disease process, through increases in cardiac filling results in excessive back-pressures and increases in venous and capillary hydrostatic pressures in both the lungs and the systemic circulation to cause the clinical symptoms and signs of heart failure.

In contrast to high output heart failure where the heart muscle may have normal function or relatively normal function, there are two broad groups of pathophysiological

abnormalities that affect heart muscle function *per se* that can lead to heart failure. These are diastolic and systolic functional changes. The mechanisms of these changes will be discussed in subsequent sections.

1.2.1 Diastolic dysfunction

The period of cardiac diastole encompasses that time during which the myocardium loses its ability to generate force and returns to an unstressed length. Filling of the ventricle occurs during this time period. Diastolic dysfunction occurs when these processes are prolonged, slowed or incomplete. Measurements that reflect changes in normal diastolic function generally depend on the onset, rate, and extent of ventricular pressure decline and filling and the relationship between pressure (P) and volume (V) or stress and strain during diastole (Gilbert and Glanz, 1989). If diastolic function is truly normal, these measurements must remain normal both at rest and during the stress of a variable heart rate, stroke volume, end diastolic volume and blood pressure. Diastolic cardiac abnormalities are generally associated with a decreased cardiac chamber compliance. Cardiac chamber compliance is the change in volume for a given unit change in pressure during ventricular diastole. If the ventricle becomes stiff (less compliant) for any reason, the relationship between diastolic P and V is left shifted with an increased slope of the relationship and for a given diastolic volume there is an associated increase in diastolic pressure in the ventricle (Figure 1.1).

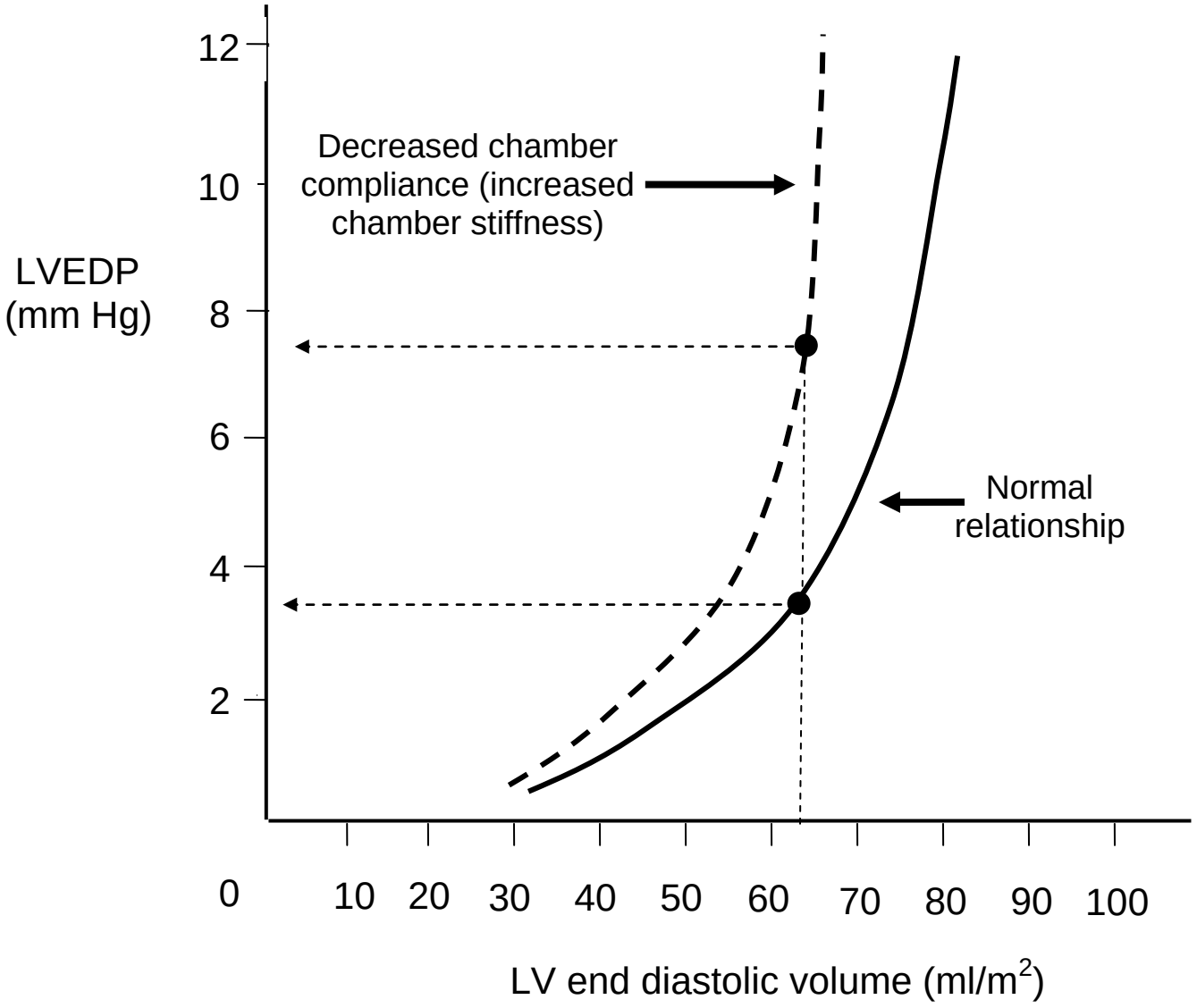

Figure 1.1. Left ventricular end diastolic pressure (LVEDP)-volume relationship, showing a normal relationship (continuous line) and the change in the relationship when chamber compliance decreases or stiffness increases (dashed line). The effect of a decreased compliance on diastolic pressure is illustrated by the dashed arrows.

In general, causes of a decreased cardiac chamber compliance include diseases that affect either active or passive (material) properties of the myocardium, including the triad of aging, diabetes mellitus and hypertension (Zile et al 2004, Norton et al 1997, Norton et al 1996), ventricular hypertrophy including hypertrophic cardiomyopathy (Lombardi et al 2003, Shirani et al 2000), and infiltrative disease of the myocardium. These diseases alter compliance as either the ventricular wall is thickened (it is harder to fill a thick walled chamber than a thin walled chamber), myocardial elasticity is reduced (changes in passive myocardial properties occur), or decreases in myocardial relaxation occur (changes in active myocardial properties result). There are of course a number of other factors that may influence cardiac chamber compliance, including viscoelastic forces, coronary vascular engorgement, pleuro-pericardial interactions, ventricular interactions and pericardial disease (Gilbert and Glanz 1989). However, a discussion of these changes goes beyond the scope of this dissertation. Nevertheless, they have been reviewed by Gilbert and Glanz (1989). The most common causes of a decreased cardiac chamber compliance (increased chamber stiffness) are alterations in the properties of the myocardium.

1.2.1.1 Myocardial properties that contribute toward diastolic dysfunction.

Myocardial properties that contribute toward diastolic dysfunction include an inability of the heart to relax, as well as alterations in the intrinsic material properties (passive properties) of the myocardium. As indicated in the above discussion, changes in the ability of the myocardium to relax may contribute toward diastolic dysfunction and

possibly diastolic heart failure. There are a number of factors that influence myocardial relaxation, but this discussion also goes beyond the scope of this dissertation. These have largely been reviewed by Gilbert and Glanz (1989) and involve changes in myocardial contractility and the ability of cardiomyocytes to sequester calcium during relaxation, a function which itself is an active process requiring adenosine triphosphate. Importantly, relaxation abnormalities may impact on the rate of pressure change during isovolumic relaxation and at the end of relaxation (-dP/dt or tau), or P-V relations measured at either the end of isovolumic relaxation or during early filling of the ventricle (Gilbert and Glanz, 1989). In contrast, alterations in myocardial material properties may influence P-V relations at any time in the diastolic period of the cardiac cycle, but they particularly affect that period of the cardiac cycle when the ventricle as maximally filled (end diastole)(Gilbert and Glanz, 1989). Alterations in myocardial material properties can only be determined by accounting for the impact of geometric changes in the ventricle on diastolic function. The impact of myocardial material properties on diastolic function are therefore assessed using either isolated muscle strips, where length-tension relations for a given muscle volume are assessed in resting muscle (Brooks et al 1997, Bing et al 1995, Conrad et al 1995), or in the whole heart by assessing stress-strain relations in diastole (Norton et al 1997, Norton et al 1996, Woodiwiss et al 1995, Weber et al 1990). The impact of material properties of the myocardium on diastolic function are then assessed from stiffness constants calculated from these relationships.

Myocardial material properties are largely dependent on interstitial changes in the myocardium. Previously it was thought that a simple accumulation of myocardial collagen was sufficient to reduce myocardial compliance (increased myocardial

stiffness). However, a normal myocardial stiffness (Thiedermann et al 1983) or only modest increases in myocardial stiffness (Norton et al 2002) are both associated with increments in myocardial collagen concentrations. Moreover, pharmacological-induced improvements in stiffness may occur without decreases in collagen concentrations (Norton et al 1997); and decrements in collagen concentrations do not necessarily reduce stiffness (Motz and Strauer 1989). Thus, alternative mechanisms have been sought to explain changes in the material properties of the myocardium.

Consistent with earlier studies (Spinale et al 1991, Iomoto et al 1988), our group have provided substantial evidence to indicate that increases in the cross-linked properties of myocardial collagen determine myocardial material properties and stiffness (Badenhorst et al 2003, Norton et al 1997, Norton et al 1996). Increases in myocardial cross-linking, produced by the production of advanced Amadori products of myocardial collagen (excess irreversible glycosylation of collagen) are associated with diabetes mellitus (Norton et al 1996). Moreover, through an as yet unidentified mechanism, collagen cross-linking increases in hypertension and left ventricular hypertrophy (Badenhorst et al 2003b, Norton et al 1997, Satoshi et al 1995). An enhanced myocardial collagen cross-linking is associated with an increased passive stiffness of the myocardium, a change that results in a decreased cardiac chamber compliance (Badenhorst et al 2003b). In pressure overload hypertrophy, although further increases in myocardial collagen in the late stages of hypertrophy, when the transition to pump dysfunction occurs, are of the non-cross-linked phenotype, the earlier increases in myocardial collagen of the cross-linked phenotype are also maintained (Badenhorst et al 2003b, Tsotetsi et al 2001). The result is that even in the late stages of hypertrophy when

pump dysfunction occurs there is an increased passive stiffness of the myocardium, a change that still translates into a decreased cardiac chamber compliance (Badenhorst et al 2003b).

1.2.1.2 Diastolic heart failure

Whereas diastolic cardiac dysfunction describes an abnormal mechanical property of the heart, diastolic heart failure is a clinical syndrome. From a clinical perspective, diastolic heart failure refers to a condition in which abnormalities in diastolic mechanical function occur in the presence of a clinical syndrome of heart failure and a preserved ejection fraction (index of systolic function) (Zile et al 2004). It has become apparent that up to 50% of patients with heart failure may have diastolic heart failure (Vasan et al 1999). The predominant risk factors for diastolic heart failure are old age, hypertension and diabetes mellitus (Vasan et al 1999). This may not be surprising considering that the material properties of the myocardium are fundamentally altered by increases in myocardial collagen cross-linking associated with diabetes mellitus (Norton et al 1996) as well as hypertension and left ventricular hypertrophy (Badenhorst et al 2003b, Norton et al 1997).

The mechanisms by which diastolic dysfunction translates into heart failure are relatively simple to understand. Increased ventricular diastolic pressures produced by a stiff chamber (Figure 1.1) will augment venous pressures in the pulmonary and systemic circulation. The result would be pulmonary congestion and peripheral oedema. Moreover, an increased chamber stiffness increases the resistance to cardiac filling, and ultimately

leads to an attenuation of the increase in cardiac output that normally occurs during exercise. The exercise-induced increase in cardiac output is normally achieved by an enhanced venous return and recruitment of the Frank-Starling effect. A restricted chamber filling mediated by an increased stiffness will limit the ability to recruit the Frank-Starling effect. An attenuation of the increase in cardiac output that normally occurs during exercise with diastolic dysfunction will result in a reduced exercise capacity.

1.2.2 Pump dysfunction

With respect to changes in heart muscle that result in pump dysfunction, three general mechanisms explain pump dysfunction. These are an increased ventricular afterload, decreases in the intrinsic contractile properties of the myocardium, and cardiac dilatation. An increase in ventricular afterload has in the past been considered to be an increase in the resistance to flow from the ventricle, a change that may occur in hypertension or ventricular outlet tract obstruction (e.g. stenotic valvular lesions, hypertrophic obstructive cardiomyopathy). However, the physical definition of afterload relates to the law of LaPlace, where afterload is wall tension or stress, and tension is proportional to the product of pressure and radius and inversely proportional to wall thickness (see following discussion). Thus, afterload includes anything that influences the pressure in the ventricle or the geometry of the ventricle and should not just be restricted to factors that influence just the resistance to flow. This issue will be further discussed under the section on cardiac dilatation. The impact of alterations in intrinsic myocardial

contractile function and cardiac dilatation will be discussed in the following sections. However, before doing so it is first worth defining the terms “pump dysfunction” or “pump failure”.

The functional changes that accompany cardiac failure due to pump failure are best explained with respect to the Frank-Starling relation. Figure 1.2 illustrates the normal Frank-Starling relationship and the changes that occur in association with either an enhanced pump function or a decreased pump function (“pump failure”).

Movement of the ventricular function curve to the left of normal occurs when either intrinsic myocardial contractility increases (e.g. increased circulating catecholamines as may occur with exercise or shock); afterload decreases (such as following vasodilatation); or the relationship between wall thickness and internal radius increases (such as with concentric cardiac hypertrophy), changes that enhance pump function. In contrast, as in the case in most forms of heart failure due to systolic functional abnormalities, displacement to the right and downward from the normal occurs when either ventricular contractility is depressed, afterload is increased or the heart dilates. Importantly however, even though acute catecholamine effects increase pump function, there is now substantial evidence to support a role for chronic sympathetic activation as a major determinant of progressive pump dysfunction.

1.2.2.1 Chronic sympathetic effects in heart failure

The evidence in favour of over-activation of the sympathetic nervous system contributing toward the progression of heart failure includes the following. Plasma

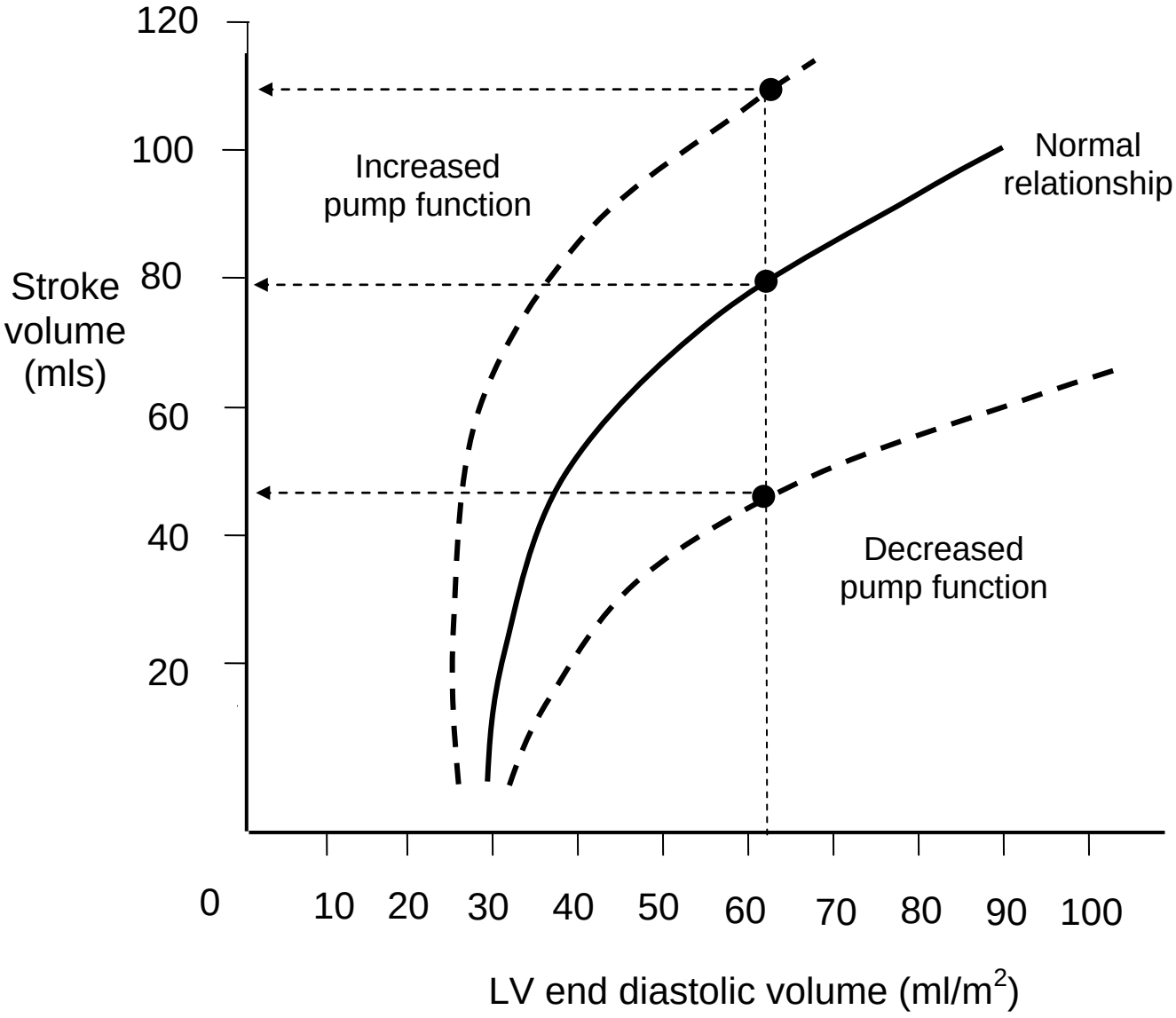

Figure 1.2. The normal relationship between left ventricular end diastolic volume and stroke volume (continuous line) and the change in the relationship when pump function either improves (left sided dashed line) or declines (pump failure)(right sided dashed line). The impact of changes in pump function on stroke volume is illustrated by the dashed arrows.

noradrenaline concentrations are increased in heart failure and predict the severity of heart failure (symptoms based on the New York Heart Association classification), pump dysfunction in heart failure and mortality in heart failure (Anand et al 2003, Sigurdsson et al 1994, Francis et al 1993, Swedberg et al 1990, Hasking et al 1986, Cohn et al 1984, Kluger et al 1982). Moreover, myocardial noradrenaline levels are enhanced in patients with chronic heart failure and these changes predict mortality in heart failure (Esler et al 1997, Kaye et al 1995). Further, in randomized-controlled clinical trials, blockade of a major myocardial receptor target of the sympathetic nervous system, namely β-adrenergic receptors, improves pump function and decreases morbidity and mortality in patients with mild-to-moderate and severe heart failure (Flather et al 2005, Toyama et al 2003, Waagstein et al 2003, Pool-Wilson et al 2003, Gerson et al 2002, Packer et al 2001, MERIT-HF 1999, CIBIS-II 1999, Packer et al 1996, Waagstein et al 1993). It is based on these findings that all guidelines for the diagnosis and treatment of heart failure indicate a high level of evidence in favour of using β-adrenoreceptor blockers as standard care in mild-to-severe forms of compensated cardiac failure (Hunt et al 2001).

Despite the overwhelming evidence indicating that chronic β-adrenoreceptor activation reduces pump function in chronic heart failure, the mechanisms responsible for this adverse effect still remain obscure. The potential mechanisms could include either decreases in intrinsic myocardial contractility or cardiac chamber dilatation or both. These potential mechanisms will be discussed in subsequent sections.

1.2.2.2 Myocardial contractility and pump dysfunction

Intrinsic myocardial function has always been difficult to either define or measure. However, it is important to note that the intrinsic contractile properties of cardiac muscle are independent of the force-frequency relationship (heart rate), external loading produced by increases in resistance of the systemic or pulmonary vasculature or of the outlet tracts to the systemic and pulmonary vasculature (afterload), loading due to cardiac dilatation, or loading due to alterations in filling volumes (Frank-Starling effects) or filling pressures (preload). A reduction in intrinsic myocardial contractility may occur if there is a loss of the number of viable myocytes remaining in the myocardium or if the function of individual viable myocytes is reduced. Obviously disease processes that result in acute myocardial tissue damage, such as occlusion of coronary vessels in myocardial infarction, or infective diseases of the myocardium, such as myocarditis, will result in cell death and intrinsic myocardial contractile disturbances. However, it has also become clear that progressive cell death and dysfunction also accompanies chronic diseases of the heart (Sabbah 2000, Singh et al 2000, Narula et al 1996).

With respect to the possibility that progressive cardiomyocyte death accompanies chronic heart failure, there are many cellular and molecular changes that mediate cell death and dysfunction in association with chronic cardiac pathology. However, the most well studied cellular changes that accompany chronic heart failure have been those changes that are associated with neurohumoral activation, particularly activation of β-adrenoreceptors (Singh et al 2000, Colucci et al 2000). Importantly, as I studied the potential mechanisms by which chronic β-adrenoreceptor activation contributes toward

pump dysfunction, I will briefly review the evidence to support the notion that chronic β-adrenoreceptor activation contributes toward intrinsic myocardial contractile disturbances.

1.2.2.2.1 Chronic β-adrenoreceptor activation and intrinsic myocardial contractile disturbances

A well established mechanism by which chronic β-adrenoreceptor activation is thought to promote progressive decreases in cardiac contraction, is through a reduced inotropic sensitivity of the β-adrenoreceptor-cyclic adenosine monophosphate system to agonist stimulation. The reduction in inotropic sensitivity of this system in chronic cardiac disease is thought to occur through a number of mechanisms. Of particular importance, in failing hearts a decrease in β_1-adrenoreceptor density may occur in response to sustained elevations in sympathetic activation (Tevaearai and Koch 2004, Schotten et al 2000, Steinfath et al 1991, Brodde et al 1991, Brodde et al 1989, Bohm et al 1994, Brodde et al 1986, Bristow et al 1986, Bristow et al 1982). Furthermore, attenuated β_1-adrenoreceptor-mediated inotropic responsiveness in progressive heart failure may also occur in-part through upregulation of inhibitory G proteins (Gi) which reduce intracellular cyclic adenosine monophosphate concentrations (Eschenhangen et al 1992, Böhm et al 1990, Feldman et al 1988, Neumann et al 1988).

Like the β_1-adrenoreceptor, the β_2-adrenoreceptor mediates contractile effects (Molenaar and Parsonage 2005, Bristow et al 1986, Bristow et al 2000), but in contrast to the β_1-adrenoreceptor where there is extensive evidence to support a role of this receptor

in mediating heart failure, the role of the β_2-adrenoreceptor is controversial. Indeed, in transgenic animals, although a 15-fold increase in β_1-adrenoreceptor expression results in heart failure (Molenaar and Parsonage 2005), a substantially greater increase (60-fold) in β_2-adrenoreceptor expression does not produce the same effects (Liggett et al 2000). Thus, chronic β_2-adrenoreceptor activation in response to sustained increases in sympathetic drive in human heart failure, are unlikely to contribute to adverse effects.

A reduced cardiac contractility following chronic β-adrenoreceptor activation may occur not only through downregulation of adrenergic signaling pathways, but also through decreases in cell survival. Indeed, possibly via an imbalance between oxygen demand-to-supply ratios (sympathetic activation increases heart rate and cardiac contractility, but does not necessarily promote increases in coronary flow) and by direct effects on cardiac myocytes (Mann et al 1992) activation of the sympathetic nervous may stimulate cardiomyocyte necrosis. Moreover, via β_1-adrenoreceptors, chronic adrenergic stimulation may promote increases in cell death by activation of apoptotic signaling pathways induced in-part by stress activated-mitochondrial pathways (Remondino et al 2003, Singh et al 2000, Colucci et al 2000, Communal et al 1998). In contrast to the pro-apoptotic effect of β_1-adrenoreceptors, β_2-adrenoreceptors are thought to be anti-apoptotic (Communal et al 1999). This may in-part explain the ability of a 15-fold increase in β_1-adrenoreceptor over-expression to cause heart failure (Molenaar and Parsonage 2005), whilst a substantially greater increase (60-fold) in β_2-adrenoreceptor over-expression does not produce the same effects (Liggett et al 2000). However, adrenergic-induced cardiomyocyte apoptosis can be blocked by both β_1 and β_2 adrenoreceptor antagonists (Singh et al 2001, Communal et al 1999).

1.2.2.3 Cardiac chamber dilatation and pump dysfunction

Cardiac chamber dilatation refers to an increase in chamber cavity volumes or dimensions as a consequence of right shifts in diastolic P-V relations (Figure 1.3). Cardiac dilatation is not simply an increase in cavity volume alone, as this may result from an enhanced blood volume or venous return without necessarily a right shift in the diastolic P-V relation. The right shift in the diastolic P-V relation is fundamentally the opposite of what occurs when the compliance of the ventricle is reduced, but generally does not involve alterations in the slope of the relationship (Figure 1.3).

For many years cardiac chamber dilatation was perceived as a compensatory change in heart failure (Ertl et al 1991). This notion was based on the well recognized fact that in heart failure, fluid accumulation and a reduced cardiac contractility increase ventricular filling volumes (Patterson and Adams 1996). Although pump dysfunction is reduced, increased ventricular filling volumes may maintain a normal ejection volume (stroke volume) through the Frank-Starling effect. Only when marked pump dysfunction occurs is the increase in filling volume inadequate to maintain stroke volume. The obvious negative impact of increased filling volumes is an increased filling pressure with subsequent “backward” heart failure (if filling pressures in the left ventricle increase, pulmonary capillary hydrostatic pressures increase and pulmonary congestion occurs). To allow the heart to accommodate greater volumes at normal filling pressures it would obviously be useful to shift the diastolic P-V relation to the right, where a greater filling volume occurs at much lower filling pressure, thus relieving pulmonary congestion (Figure 1.3). Although these arguments are not necessarily incorrect, the more recent

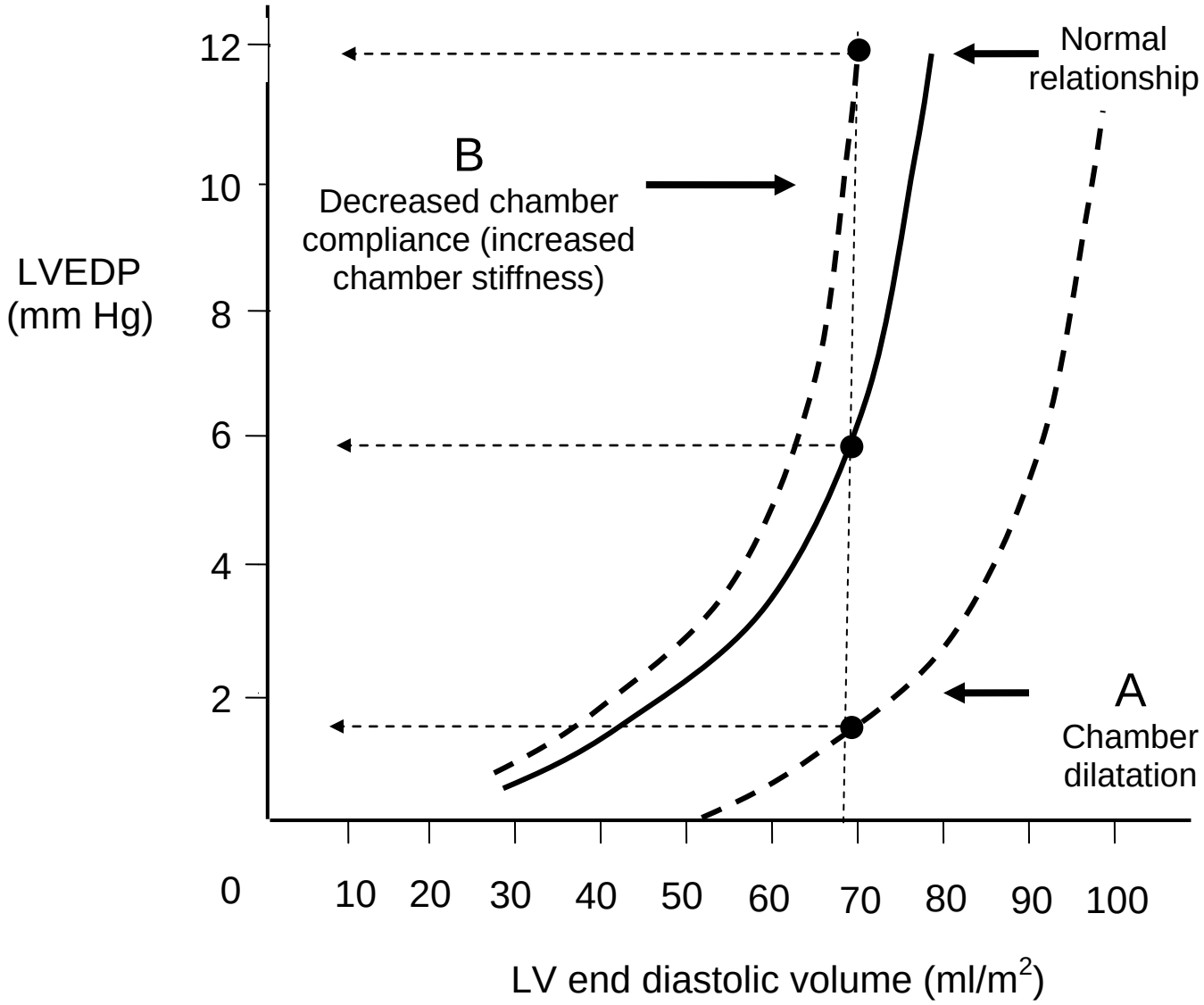

Figure 1.3. Left ventricular end diastolic pressure (LVEDP)-volume relationship, showing a normal relationship (continuous line) and the change in the relationship with cardiac chamber dilatation (dashed line-A). This curve (dashed line A) is compared with a curve resulting from a decrease in chamber compliance (dashed line B). The effect of a cardiac dilatation on diastolic pressure is illustrated by the arrows.

view of cardiac dilatation is that it is causally related to heart failure. The following outlines these arguments.

There is now substantial evidence to indicate that cardiac dilatation is a precursor of left ventricular dysfunction and clinical heart failure (Vasan et al 1997, Gaudron et al 1993; Pfeffer et al 1992). Moreover, as indicated in the introduction to this chapter, cardiac dilatation is a major risk factor for mortality in heart failure (Foley et al 1995, Lee et al 1993, Nestico et al 1985). Indeed there is a strong relationship between the degree of ventricular dilatation and one-year mortality in heart failure (Gadsboll et al 1990). Moreover, with the treatment of heart failure, reductions in cardiac chamber volumes and dimensions are associated with better long-term outcomes, including survival (Levine et al 2000, Sharpe and Doughty 1998, Doughty et al 1997). Furthermore, patients predicted to be at risk for long-term left ventricular dilatation have an increased risk of mortality and heart failure at 6 months (de Kam et al 2002). Moreover, recent evidence produced by members of our laboratory indicates that pump dysfunction in heart failure is more closely associated with cardiac dilatation than with abnormalities in intrinsic myocardial function (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a, Norton et al 2002). Nevertheless, these data may still represent an epiphenomenon rather than a cause-effect relationship in heart failure. If cardiac dilatation does indeed cause heart failure through pump dysfunction, the obvious question is through what mechanisms does this occur?

1.2.2.3.1 Mechanisms of the impact of cardiac dilatation on pump function

La Place's law as applied to the heart could in-part explain the impact of cardiac dilatation on pump function. Indeed, chamber dilatation is associated with an increased cavity volume and hence radius, and a reduced wall thickness, effects that will, according to La Place's law, increase wall tension or stress (Laskey et al 1984). As wall stress determines myocardial oxygen consumption, a dilated ventricle may increase the myocardial oxygen demand-to-supply ratio. A demand-to-supply mismatch may subsequently decrease cardiac contraction. Despite the lack of objective evidence to support this notion, the premise on which it is based is attractive and hence this mechanism has largely been the hypothesis to which most subscribe.

However, when systolic function is measured using a stress (or load)-independent measure of pump function (end systolic elastance as defined by the slope of the systolic P-V relation) (Sugawa et al 1988) in an animal model of congestive cardiac failure and pump dysfunction associated with massive cardiac dilatation (Norton et al 2002), pump dysfunction was noted to be reduced without parallel changes in myocardial contractility. These data would suggest that a mechanism unrelated to a stress or load-induced effect contributes to pump dysfunction in cardiac dilatation. One potential explanation is that since remodelling of the chamber occurs in cardiac dilatation, alterations in the shape of the chamber (Douglas et al 1989) result in inappropriate force transduction in dilated ventricles. In other words, changes in the shape of the ventricular chamber may reduce the direction of the forces normally generated during torsion of the chamber during myocyte contraction, which in-turn leads to pump dysfunction. Alternatively, a dilated

chamber could simply be remodelled so that larger chamber volumes are required to produce cardiomyocyte stretch and hence recruit the Frank-Starling effect.

Irrespective of the mechanism by which cardiac dilatation leads to pump dysfunction, there is nevertheless substantial evidence to indicate that chamber dilatation reduces pump function. Hence, the mechanisms of this effect need to be considered.

1.2.2.3.2 Cellular mechanisms responsible for cardiac dilatation

A number of cellular mechanisms have been proposed to explain the development of cardiac dilatation. The initial proposal was that cardiac dilatation is the consequence of an inappropriate hypertrophic process occurring in cardiomyocytes, where excessive increases in myocyte length relative to increases in cell width result in a dilated chamber (Gerdes et al 1992). This notion was further supported by data obtained in ischaemic dilated cardiomyopathy (Anand et al 1997, Gerdes and Capasso 1995, Zimmer et al 1990), hypertensive heart failure (Tamura et al 1998) and other animal models of heart failure (Spinale et al 1991b). However, as cardiomyoctes lie at a variety of different angles to the endocardial surface of the chamber, including at right angles, it is difficult to see how this would favour one form of chamber geometry over another simply by increasing cell length as opposed to width.

A more recently favoured view of the mechanisms of chamber dilatation is that the altered geometry of the heart is the consequence of side-to-side slippage of cardiomyocytes as previously suggested (Olivetti et al 1990). Indeed, myocyte slippage in end stage dilated cardiomyopathy has been well documented (Beltrami et al 1995,

Linzbach 1960), but the measurement approaches have not been reproduced by Gerdes et al (1995) or by our group. The potential mechanisms involved in side-to-side slippage are underscored in subsequent discussion.

Cardiomyocytes are tethered by collagen struts which maintain the integrity of the myocardium. Degradation of collagen may contribute to left ventricular dilatation by reducing cardiomyocyte attachments and encouraging side-to-side slippage of cardiomyocytes. Degradation of myocardial collagen may occur as a consequence of activation of collagenases or matrix metalloproteinases (MMPs). Matrix metalloproteinases are an endogenous family of proteolytic enzymes that degrade all components of the myocardial extracellular matrix (Gunasinghe et al 2001). An increased myocardial expression and activation of MMPs has been demonstrated in patients with congestive heart failure or in patients with a reduced systolic function (Reddy et al 2004, Polyakova et al 2004, Li et al 2001, Li et al 1998; Spinale et al 2002; Spinale 2000) and in animal models of pump dysfunction (Sakata et al 2004, Mukherjee et al 2003, King et al 2003, Peterson et al 2001, Rohde et al 1999, Mujumdar et al 1999, Spinale et al 1998). MMP inhibition has been shown to attenuate left ventricular remodeling in animal models of pacing-induced heart failure (Spinale et al 1999), myocardial infarction (Mukherjee et al 2003, Rohde et al 1999) and heart failure in the spontaneously hypertensive rat (Peterson et al 2001). Moreover, a loss of MMP inhibitory control of MMPs, through a gene deletion of the tissue inhibitor of the matrix metalloproteinase-type 1 (TIMP-1) leads to left ventricular dilatation in mice (Roten et al 2000).

Although many forms of cardiac dilatation are accompanied by increases in myocardial collagen concentrations, the role of increased myocardial collagen

concentrations as a determinant of cardiac dilatation was unclear until our group provided further clarity on this issue. As discussed above, increases in myocardial collagen concentrations are thought to alter myocardial material properties and thus determine left and not right shifts in chamber diastolic P-V relations. Moreover, reductions in cardiac cavity dimensions following the use of assist devices is generally accompanied by increases and not decreases in myocardial collagen concentrations (Madigan et al 2001, Li et al 2001, McCarthy et al 1995, Scheinin et al 1992). In addition, pacing-induced cardiac dilatation (Spinale et al 1996, 1991b) and β-adrenergic-induced cardiac dilatation (Woodiwiss et al 2001) are accompanied by decreases rather than increases in myocardial collagen concentrations. A more recent view of how increases in myocardial collagen synthesis could contribute toward chamber dilatation is nevertheless through the production of collagen that is susceptible to degradation (Badenhorst et al 2003b). In this regard, collagen of the non-cross-linked phenotype is associated with systolic dysfunction and cardiac dilatation (Woodiwiss et al 2001, Spinale et al 1996, Gunja-Smith et al 1996, Capasso et al 1989). Moreover, by genetically decreasing the susceptibility of collagen to degradation, a reduced degree of dilatation accompanies pressure-overload states (Lindsey et al 2003).

In pressure overload states, the functional impact of increases in myocardial collagen concentrations appears to depend largely on whether the collagen that accumulates is of the cross-linked or non-cross-linked form (Badenhorst et al 2003b). Increases in myocardial collagen of the cross-linked form are associated with an increase in myocardial stiffness. Increases in myocardial collagen of the non-cross-linked form are associated with chamber dilatation. Moreover, increases in myocardial collagen of both

the cross-linked and non-cross-linked form are associated with increases in myocardial stiffness and chamber dilatation (Badenhorst et al 2003b).

Cardiomyocyte cell death, through either apoptosis or necrosis is also thought to promote the development of cardiac dilatation through a decrease in the integrity of myofibrils. However, as this was not a major component of my studies I shall only discuss this with reference to the impact of chronic sympathetic activation (see subsequent sections).

1.2.2.3.3 Chronic β-adrenoreceptor activation and cardiac dilatation: Association with pump dysfunction or cause of pump dysfunction?

There is indirect evidence to suggest that sympathetic activation promotes the development of pump dysfunction through cardiac dilatation. Indeed, transgenic animal models with decreased adrenergic activation are protected against the development of dilatation and heart failure when exposed to pressure-overloads (Esposito et al 2002). Moreover, blockade of β-adrenoreceptors prevents the transition from cardiac hypertrophy to a dilated ventricle in hypertension (Chan et al 2004), and a genetically modified 15-fold increase in β_1-adrenoreceptor expression results in heart failure and cardiac dilatation (Molenaar and Parsonage 2005). Measures of neurohumoral activation are closely associated with cardiac dimensions in patients with heart failure (Davila et al 2000, Patten et al 1998). Moreover, treatment of patients with β-adrenoreceptor blockers results in decreases in cardiac dimensions (Sharpe and Doughty 1998, Doughty et al 1997; Lechat et al 1997). However, the impact of β-adrenoreceptor activation or β-

adrenoreceptor blockade on cardiac dimensions is still generally perceived to be secondary to changes in cardiac contractility and subsequent alterations in cardiac preloads. What has not been given due consideration is that a primary target of β-adrenergic receptor activation or blockade is the cellular mechanisms responsible for cardiac dilatation.

Our group have recently shown that chronic β-adrenoreceptor activation contributes to cardiac dilatation through direct cellular mechanisms, rather than through indirect effects mediated by changes in cardiac contractility and subsequent alterations in cardiac preloads (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a). Indeed, in these studies (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a) load-independent measures of intrinsic myocardial contractility (the slope of the left ventricular developed stress-strain relationship) and load-dependent measures of intrinsic myocardial function (left ventricular midwall fractional shortening) were unchanged, despite a marked decrease in chamber systolic function (decreased slope of the left ventricular pressure-volume relationship as well as a decrease in left ventricular ejection fraction) (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a). The reduced chamber systolic function could only be attributed to an increase in left ventricular cavity dimensions and a right shift in the left ventricular diastolic pressure-volume relationship (left ventricular dilatation) (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a).

The obvious question that arises from studies performed by our group (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a) is how intrinsic myocardial contractility remains unchanged following chronic β-adrenoreceptor activation, when

there is substantial evidence to indicate that chronic β-adrenoreceptor activation promotes downregulation of β-adrenoreceptor-mediated contractile responses and apoptosis and necrosis (see section 1.2.2.2.1). Indeed, although the dose of the β-adrenoreceptor agonist used by us on a daily basis to promote cardiac dilatation and pump dysfunction does not induce necrosis (Badenhorst et al 2003a), this dose used over prolonged periods is able to attenuate β-adrenoreceptor-mediated contractile responses and to induce apoptosis (Osdachii et al 2007, Osadchii et al 2005). The answer to this conundrum has been provided by us in recent studies. We have been able to show that attenuated β-adrenoreceptor-mediated contractile responses either translate into reduced norepinephrine-induced contractile responses at supraphysiological norepinephrine concentrations (Osadchii et al 2005), or that intrinsic myocardial contractility is sustained through enhanced myocardial α-adrenoreceptor mediated contractile responses and an increased presynaptic myocardial norepinephrine release (Osadchii et al 2007).

1.2.3.3.4 Chronic β-adrenoreceptor activation and cardiac dilatation: Potential cellular mechanisms

As indicated in the above discussion (section 1.2.2.3.2) the cellular mechanisms by which chronic β-adrenoreceptor activation could produce cardiac dilatation include increases in cardiomyocyte cell lengthening, or side-to-side slippage of cardiomyocytes. Although it is well recognized that chronic β-adrenoreceptor activation promotes cardiac hypertrophy (Osadchii et al 2007, Veliotes et al 2005, Osadchii et al 2005, Badenhorst et al 2003a, Woodiwiss et al 2001), presently there are no published studies that have

explored whether chronic β-adrenoreceptor activation mediates this effect through cardiomyocyte cell lengthening. However, unpublished data from our laboratory (Correa et al, personal communications), indicates that the transition from compensated hypertrophy to cardiac dilatation mediated by chronic β-adrenoreceptor activation cannot be attributed to increases in cardiomyocyte length-to-width ratios.

With respect to side-to-side slippage of cardiomyocytes, there are two potential interstitial changes that may account for β-adrenoreceptor-induced cardiac dilatation, independent of effects on intrinsic myocardial contractility. First, stimulation of cultured cardiomyocytes by β-adrenoreceptor agonists, activates MMPs (Menon et al 2005, Coker et al 2001), a change that could reduce cardiomyocyte attachments through degradation of intercellular collagen struts. Our group have provided evidence to demonstrate that MMP activation does indeed occur in vivo as opposed to in cell culture, in response to chronic β-adrenoreceptor activation (Veliotes et al, in-preparation). Second, in association with cardiac dilatation, chronic β-adrenoreceptor activation promotes the synthesis of myocardial collagen of preferentially the non-cross-linked phenotype (Veliotes et al 2005, Badenhorst et al 2003a). As the non-cross-linked collagen phenotype is susceptible to MMP degradation (Woodiwiss et al 2001), its preferential accumulation may also result in excessive breaks and tears in intercellular collagen struts and thus side-to-side slippage of cardiomyocytes. Certainly the preferential accumulation of myocardial collagen of the non-cross-linked phenotype is associated with chronic β-adrenoreceptor-mediated cardiac chamber dilatation, independent of effects on intrinsic myocardial contractility (Veliotes et al 2005, Badenhorst et al 2003a).

With respect to side-to-side slippage of cardiomyocytes, apoptotic changes may also account for β-adrenoreceptor-induced cardiac dilatation, independent of effects on intrinsic myocardial contractility. Indeed, as indicated in the above discussion, via β_1-adrenoreceptors, chronic adrenergic stimulation may promote increases in cell death by activation of apoptotic signaling pathways (Singh et al 2001, Communal et al 1998, Communal et al 1999). Moreover, our group have also recently demonstrated that the doses of the β-adrenoreceptor agonist used by us on a daily basis to promote cardiac dilatation and pump dysfunction are associated with substantial cardiomyocyte apoptotic effects (Osadchii et al 2007). However, we have also recently observed that β-adrenoreceptor-mediated cardiac dilatation can be prevented by aldosterone receptor blockade (Veliotes et al 2005) without a similar beneficial effect of aldosterone receptor blockade on β-adrenoreceptor agonist-induced apoptosis (Mielke et al in-preparation). These data therefore cast doubt on the relevance of β-adrenoreceptor agonist-induced apoptosis in mediating cardiac dilatation.

1.3.0 Can changes in myocardial material properties explain right shifts in diastolic P-V relations induced by chronic β-adrenoreceptor activation?

As indicated in the above discussion, diastolic P-V relations are well recognized as being determined by both changes in chamber geometry and size (chamber remodeling) as well as by modifications in myocardial material properties (see sections 1.2.1.1 and 1.2.2.3 above). Moreover, reductions in the cross-linked properties of myocardial collagen are thought to be major determinants of cardiac chamber remodeling

as well as myocardial material properties (see sections 1.2.1.1 and 1.2.3.3.4 above). Reductions in cross-linking of collagen could alter the diastolic P-V relationship by either reducing the slope of the myocardial stress-strain relationship (decrease myocardial stiffness or increased myocardial compliance)(see section 1.2.1.1) and/or shift the diastolic P-V relationship to the right without altering the slope of the stress-strain relationship (chamber dilatation)(see section 1.2.3.3.4). Importantly, we have provided the first direct evidence to indicate that chronic β-adrenoreceptor activation promotes a right shift in the diastolic P-V relationship in association with reductions in the cross-linked properties of myocardial collagen (Osadchii et al 2007, Veliotes et al 2005, Badenhorst et al 2003a, Woodiwiss et al 2001). Intuitively therefore, the question arises as to whether β-adrenoreceptor-mediated right shifts in cardiac diastolic P-V relationships occur through alterations in just adverse chamber remodeling or whether changes in myocardial material properties also contribute?

A material property effect produced by decreases in myocardial collagen cross-linking should theoretically alter the slope of the diastolic P-V relation and hence easily be detected. As we have previously shown that the slope of the diastolic P-V relationship is not fundamentally altered by chronic β-adrenoreceptor stimulation (Badenhorst et al 2003a), we have assumed that changes in myocardial material properties do not contribute toward β-adrenoreceptor-mediated right shifts in diastolic P-V relationships. However, we have not accounted for the potential impact of geometric changes produced by adverse chamber remodeling on diastolic P-V relationships. As indicated in section 2.1.1 the impact of myocardial material properties on diastolic P-V relations must account for concomitant geometric changes in the cardiac chamber. To assess material

property changes, myocardial diastolic stress-strain relations should be constructed and the stiffness constant determined (see section 1.2.1.1). In the present dissertation I therefore sought to determine the relative contribution of adverse chamber remodeling versus alterations in myocardial material properties toward rightward shifts in left ventricular diastolic P-V relations mediated by chronic β-adrenoreceptor activation.

As indicated in the above discussion (section 1.2.1.1) the impact of interstitial changes on myocardial material properties is most obvious in pressure overload hypertrophy (Badenhorst et al 2003b, Tsotetsi et al 2001, Norton et al 1997). Consequently, I studied the role of myocardial material properties on diastolic P-V relations induced by chronic β-adrenoreceptor activation in an animal model of pressure overload cardiac hypertrophy. Thus, a review of the current literature regarding the importance of cardiac hypertrophy in cardiovascular disease is required before describing the study conducted in this dissertation and its outcomes.

1.4.0 Cardiac hypertrophy is not necessarily a compensatory change.

Most forms of cardiac pathology ultimately result in cardiac hypertrophy. The identification of cardiac hypertrophy using clinical assessments, chest X-Rays, electrocardiography, echocardiography or other more sophisticated tools in patients with suspected cardiac pathology is useful for diagnostic and prognostic purposes. However, a cause-effect relationship between cardiac hypertrophy and cardiovascular disease is still controversial. For many years left ventricular hypertrophy (LVH) was considered a compensatory response to a chronic pressure load on the left ventricle. In accordance with the law of Laplace, an increased wall thickness in LVH maintains a normal left

ventricular wall stress in the face of increments in pressure generated within the left ventricular cavity. Based on the principle that left ventricular geometry and wall thickness change to maintain systolic stress within normal limits (Grossman et al 1976), LVH was considered an adaptive reaction to a pressure overload with a more favorable outcome predicted (Grossman et al 1976). However, LVH can no longer be considered a compensatory change. Indeed, many studies now support the notion that LVH is a predictor of cardiovascular risk independent of conventional blood pressure (BP) measurements (Drazner et al 2004, Okin et al 2004; Devereux et al 2004, Mathew et al 2001, Verdecchia et al 2001, Gardin et al 2001, Ghali et al 1998, Verdecchia et al 1998, Levy et al 1994, Koren et al 1991, Levy et al 1990, Casale et al 1986). Importantly, LVH predicts the development of a decrease in left ventricular ejection fraction (a preload-independent index of pump function) or diastolic function independent of traditional risk factors including conventional BP (Drazner et al 2004). Thus, LVH predicts the transition to cardiac dysfunction. However, a challenge in cardiovascular medicine is to determine the mechanisms that mediate the transition from compensated LVH to cardiac dysfunction independent of conventional BP.

1.4.1 The role of the sympathetic nervous system in promoting the transition from compensated hypertrophy to cardiac dysfunction.

The mechanisms responsible for the progression from concentric LVH to left ventricular failure are beginning to emerging. With respect to the present dissertation, what is conceptually important is a potential role of sympathetic activation in cardiac

hypertrophy. In this regard, a number of lines of evidence now support the notion that sympathetic over-activation may contribute toward the transition to heart failure in LVH. First, increased myocardial noradrenaline concentrations are measured in the coronary sinus in patients with hypertensive hypertrophy prior to the development of heart failure (Schlaich et al 2003, Kelm et al 1996, Agabiti-Rosei et al 1987). Our group have further established the finding of an increased myocardial norepinephrine release into the coronary effluent in an animal model of compensated LVH due to pressure overload, namely the spontaneously hypertensive rat (SHR) (Veliotes et al 2005). Myocardial norepinephrine release increases in compensated LVH because of both a reduced norepinephrine reuptake as well as an increased sympathetic nervous system activity (Rumantir et al 2000, Simpson et al 1991). Second, transgenic animal models with decreased adrenergic activation are protected against the development of cardiac dilatation, pump dysfunction and heart failure when the left ventricle is exposed to a pressure-overload (Esposito et al 2002). Third, in compensated hypertensive hypertrophy excessive adrenergic activation is associated with down-regulation of β-adrenergic systems (Osadchii et al 2007, Bohm et al 1995, Bohm et al 1994, Castellano et al 1993, Limas and Limas 1978), changes that could promote the development of contractile dysfunction. Indeed, the postulate that prolonged sympathetic activation could lead to β-adrenergic desensitization and hence promote the transition from cardiac hypertrophy to cardiac failure was hypothesised at least a decade ago (Böhm et al 1994). Fourth, blockade of β-adrenoreceptors prevents the transition from cardiac hypertrophy to heart failure in SHR independent of blood pressure effects (Chan et al 2004). Fifth, our group has demonstrated that SHR with compensated LVH are particularly susceptible to the

ability of chronic β-adrenoreceptor activation to promote the transition to cardiac dilatation and pump dysfunction (Badenhorst et al 2003a). Consequently in studying the potential role of alterations in myocardial material properties to β-adrenoreceptor-mediated right shifts in diastolic P-V relations, I selected to study this effect in an animal model of compensated LVH, namely the SHR.

1.5.0 Aims of the dissertation and importance of this aim

The primary aim of the present dissertation was therefore to determine the relative contribution of adverse chamber remodelling versus alterations in myocardial material properties toward rightward shifts in left ventricular diastolic P-V relations mediated by chronic β-adrenoreceptor activation in a rat model of compensated LVH (SHR). The importance of this aim is ultimately to establish the fundamental mechanism that determines pump dysfunction following chronic β-adrenoreceptor activation in cardiac pathology. By establishing this mechanism, if indeed one mechanism supersedes others, more logical approaches to pharmacological therapy may be developed in the prevention and treatment of heart failure. This is of particular importance as β-adrenoreceptor blockers may not be used in many patients with chronic heart failure. Indeed, it is estimated that a significant proportion of patients with heart failure do not receive β-adrenoreceptor blockers as they are either intolerant to these agents because of their negative inotropic and chronotropic actions or because they are simply not prescribed and that in about 20% of elderly patients β-adrenoreceptor blockers are contraindicated because of the presence of obstructive lung disease (Sturm et al 2007, Bongers et al 2006,

Murphy et al 2004, Rutten et al 2003, Pont et al 2003). Moreover, β-adrenoreceptor blockers are contraindicated in patients with heart block or other bradyarrhthmias. These side effect profiles of β-adrenoreceptor blockers are mediated by receptor effects and signal transduction systems that control myocardial pacemaker tissue, myocardial contractility and bronchiolar smooth muscle function. Identifying a downstream target responsible for pump dysfunction which does not influence receptor effects and signal transduction systems that control myocardial pacemaker tissue, myocardial contractility and bronchiolar smooth muscle function could lead to the development of a novel drug target that mediates the same benefits of β-adrenoreceptor blockers, but does not carry the same side effect profile.

Chapter 2

Methods

2.1 Groups and treatment regimen.

The rodent model studied in this dissertation was that induced by chronic administration of the β-adrenoreceptor agonist, isoproterenol (ISO) to spontaneously hypertensive rats (SHR) with compensated LVH (Badenhorst et al 2003). Rats with compensated LVH were utilized as rats without LVH are relatively insensitive to changes in LV diastolic and systolic P-V relations following chronic β-adrenoreceptor activation (Badenhorst et al 2003) and the impact of interstitial changes on myocardial material properties is most obvious in SHR (Norton et al 1997, Badenhorst et al 2003).

To ultimately assess whether there is an age-dependent effect of chronic β-adrenoreceptor activation in compensated LVH, in the present study older SHR were studied as compared to those previously studied (rats of 7-to-11 months of age were previously studied) (Badenhorst et al 2003). In the present study therefore, 12-month old SHR and age-matched Wistar Kyoto (WKY) controls were randomised and subsequently studied over a time period (12-to-19 months of age) prior to that when LV decompensation occurs in the SHR colony bred by the Central Animal Services of the University of the Witwatersrand (Tsotetsi et al 2001). SHR were assigned to groups that received either twice daily intraperitoneal injections of ISO (Imuprel: Adcock Ingram, 0.02 $mg.kg^{-1}$ per injection subcutaneously ≈0.2 ml) for 7 months or twice daily intraperitoneal injections of the saline vehicle for 7 months. Twelve-month-old WKY rats received twice daily intraperitoneal injections of the saline vehicle of ISO for 7 months prior to data collection.

In the study assessing the impact of ISO on SHR, ISO was not given to WKY rats as the number of breeding pairs of WKY rats, and the litters produced by the WKY breeding pairs available, were insufficient to provide an appropriate number of WKY rats born within the week of SHRs, to allocate these rats to two as opposed to one group. However, we have previously shown that ISO has no effect on LV geometry and function in younger WKY control rats (Badenhorst et al 2003a). Nevertheless it may be argued that an interaction between ageing and adrenergic activation may promote cardiac dilatation or produce alterations in myocardial material properties. Thus, to ensure that ISO is not able to promote cardiac dilatation or changes in myocardial material properties (myocardial stiffness) in older WKY rats, i.e. of a similar age as that assessed in the present study (12 months old at the beginning of the study and 19 months old after 7 months of ISO or vehicle administration) , in a parallel study I assessed the impact of 7 months of ISO or vehicle administration on cardiac chamber dimensions and myocardial material properties in WKY rats of 11-14 months of age (when the interventions were initiated). In this parallel study WKY rats were assigned to either an ISO (n=7) or a vehicle (n=6) intervention group according to their ages in order to achieve age-matching. ISO and the saline vehicle were given as described above.

To ensure that ISO injections consistently produced haemodynamic effects with intraperitoneal injections, a pilot study was performed to determine acute changes in left ventricular endocardial fractional shortening (FS_{end})(chamber systolic function) and midwall fractions shortening (FS_{mid}) in response to intraperitoneal injections of ISO in anaesthetized rats. The measurement techniques and the anaesthetic approach are described in section 2.3. FS_{end} and FS_{mid} were determined at 15 minute intervals for an

hour post ISO injection. Data obtained in response to acute ISO injections were compared with data obtained with injections of the saline vehicle in a separate group of rats.

2.2 Tail cuff blood pressures

Although our group has previously shown that the model of cardiac dilatation and pump dysfunction induced by chronic β-adrenoreceptor activation in SHR is not attributed to BP effects, this was demonstrated in SHR from 7-to-11 months of age (Badenhorst et al 2003). To exclude a potential confounding effect of BP changes in older SHR (12-to-19 months of age) in the present study, non-invasive systolic BP was assessed on three separate occasions in each group using a tail-cuff technique. These measurements were obtained at least 24-hours after the last dose of ISO. The experimental setup for these studies is depicted in Figure 2.1. Rats were placed in restrainers, the tail pre-warmed and the tail cuff inflated every 15 minutes (see Figure 2.1) for an hour a day on five separate days prior to the first measurement in order to habituate them to the procedure.

In order to determine BP, rat tails were pre-warmed until the tail artery pulse could be detected with a photoelectric diode coupled to a Model 29 pulse amplifier (IITC Inc.)(Figure 2.2). A tail-cuff coupled to a pressure transducer was placed on the rat tail proximal to the photoelectric diode and inflated until the tail pulse disappeared (Figure 2.2). The tail cuff pressure was then slowly released until the tail artery pulse returned. Systolic BP was taken as the cuff BP at which the tail artery pulse returned (Figure 2.2). Recordings of the tail cuff pressure and the pulse were made on a Beckman dynograph

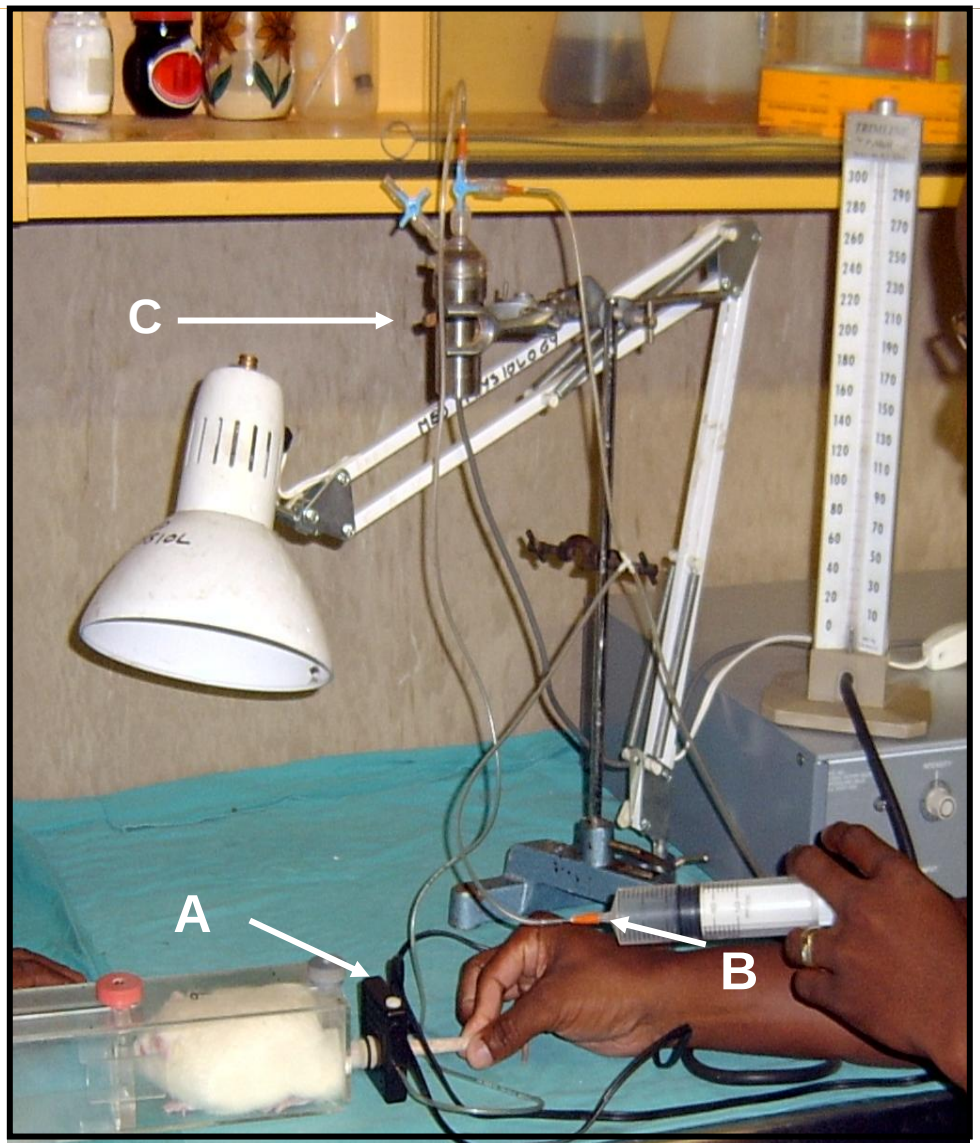

Figure 2.1 Approach used to measure tail artery systolic blood pressures in rats. Note the rat in a restrainer on the left with a photoelectric diode and tail cuff around the tail (A). The investigator on the right is injecting air via a syringe (B) into an air filled system comprising a pressure transducer (C) coupled to both the tail cuff and a mercury manometer (in the background).

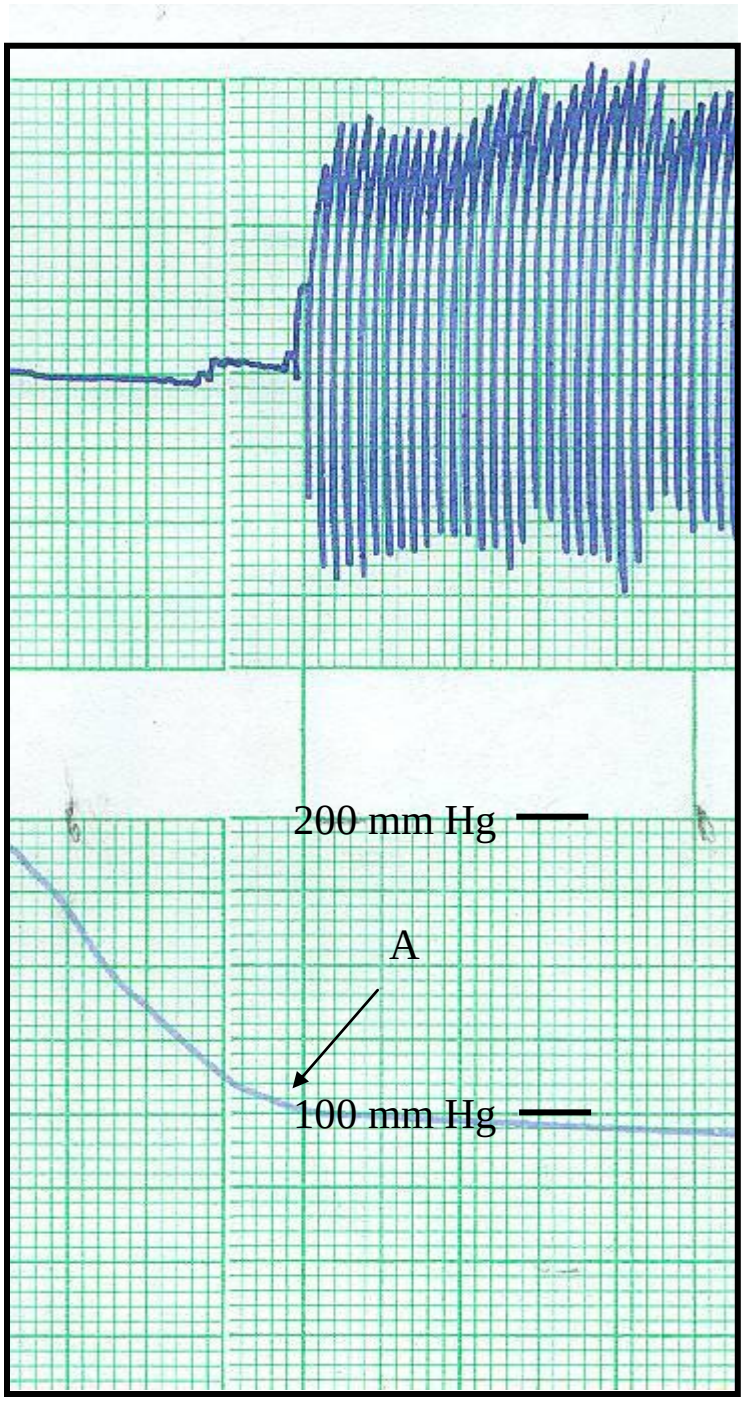

Figure 2.2 Typical recordings obtained to determine tail cuff blood pressure in rats. The upper trace shows the tail pulse and the lower trace the pressure in the tail-cuff proximal to where the tail pulse is being measured. Point A represents the pressure in the tail cuff at which the tail pulse reappears after releasing the pressure in the tail cuff (Systolic blood pressure).

model R511A recorder. A calibration of the pressures recorded in the tail cuff was made both before and after each recording.

2.3 Echocardiography.

A week before termination of rats and at least 24-hours after the last dose of ISO, echocardiography was performed in the study assessing the impact of ISO on SHR, using a 7.5 MHz transducer and a Hewlett Packard Sonos 2000 sector scanner (Figure 2.3), as previously outlined (Norton et al 2002, Woodiwiss et al 2002, Chung et al 1998). Rats were anaesthetized with ketamine ($75mg.kg^{-1}$) and xylazine ($15mg.kg^{-1}$). The rat's chest was shaved and the rat was placed in the prone position in a container with an open window over which the chest area was positioned. The echocardiograph probe was inserted through this window in order to obtain echocardiograph images. Two-dimensional targeted M-mode echocardiography was employed. A two dimensional image was obtained in the short axis of the left ventricle at the level of the papillary muscle. The sector scanner was positioned to obtain clear images across the maximal diameter of the short axis of the left ventricle. An M-mode image was obtained which was considered a high quality image if the endocardial surfaces of both the anterior (septal) wall and the posterior wall were clearly visible throughout systole and diastole (Figure 2.4). Care was taken not to include the endocardial surface of the papillary muscle in the posterior wall measurements. On-line recordings were obtained and recordings were also obtained for later off-line analysis to ensure quality control. The left

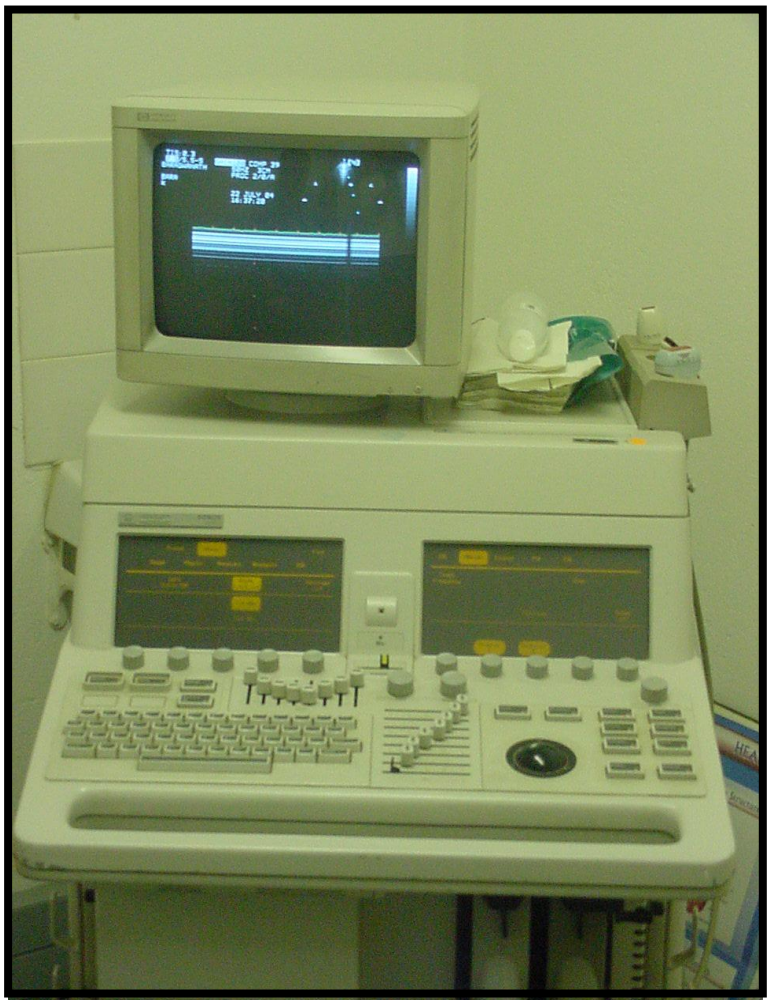

Figure 2.3. Hewlett Packard echocardiograph used to obtain in-vivo measures of left ventricular structure and function in anaesthetised rats.

A - LV end diastolic internal diameter

B - LV end systolic internal diameter

C - LV end diastolic posterior wall thickness

D - LV end systolic posterior wall thickness

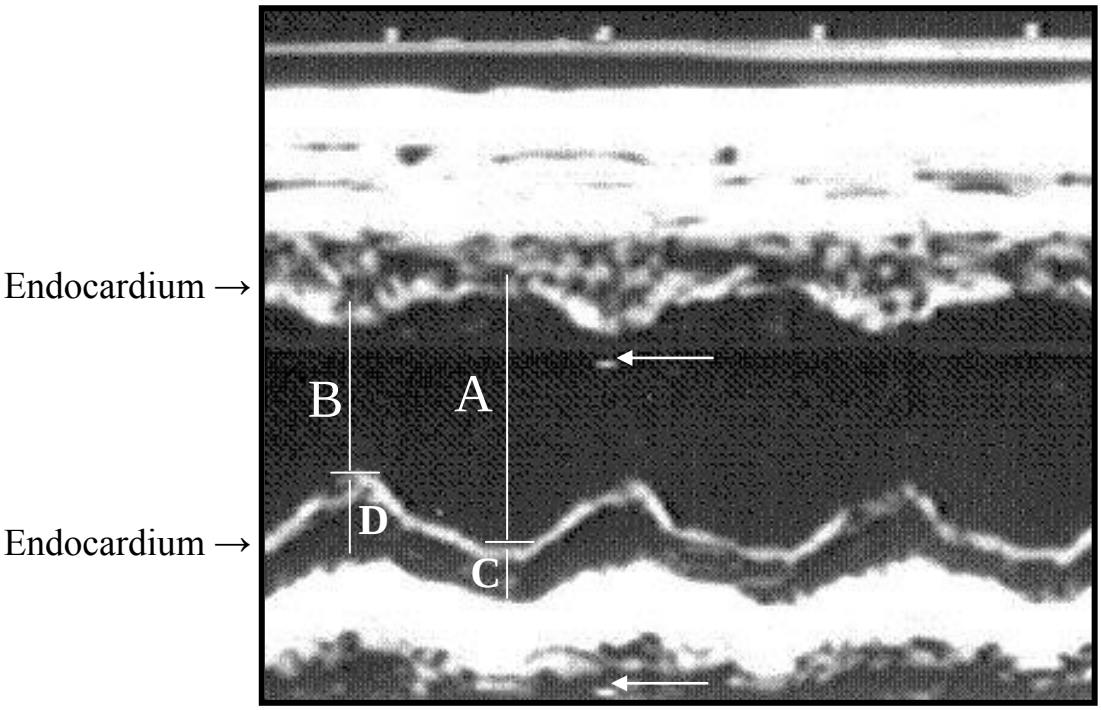

Figure 2.4. Typical recordings obtained to determine left ventricular dimensions using two-dimensional guided M-mode echocardiography. The white arrows are pointing to calibration markers that indicate a distance of 1 cm.

ventricular internal dimensions and posterior wall thickness were measured at the end of systole and at the end of diastole according to the American Society for Echocardiography's leading edge method (Sahn et al 1978) (Figure 2.4). Left ventricular anterior wall thickness was not determined as the right ventricular surface of the septal wall was seldom clearly evident. Anterior wall thickness was therefore assumed to be equivalent to posterior wall thickness for all calculations. An example of measurements made from actual recordings is provided in Figure 2.4. Measurements were made from 3 consecutive beats.

Left ventricular endocardial (FS_{end}) and midwall (FS_{mid}) fractional shortening were determined from the following equations. For the calculations of FS_{mid}, anterior wall thickness was assumed to be equivalent to posterior wall thickness. Hence ½ LV PWT + ½ LV anterior wall thickness was assumed to be equivalent to LV PWT.

FS_{end} = LV EDD-LV ESD/LVEDD x 100

Where

LV EDD = left ventricular end diastolic internal diameter

LV ESD = left ventricular end systolic internal diameter

FS_{mid} = (LVEDD + LVED PWT)- (LVESD + LVES PWT)/ (LVEDD + LVED PWT) x 100

Where

LVED PWT = left ventricular end diastolic posterior wall thickness

LVES PWT = left ventricular end systolic posterior wall thickness.

Left ventricular endocardial and midwall fractional shortening were utilized as indices of chamber and myocardial function respectively (Norton et al 2002, Chung et al 1998). Importantly, left ventricular ejection fraction was not used as an index of left ventricular chamber systolic function as clear endocardial surface images were not always available throughout the whole circumference of the heart on two-dimensional imaging. Diastolic function (transmitral early-to-late velocity ratios) was not assessed using echocardiography as the reproducibility of this measurement in our hands is poor. Neither endocardial nor midwall fractional shortening are independent of afterload or heart rate, but like left ventricular ejection fraction, they do account for preload-dependent Frank-Starling effects.

2.4 Isolated, perfused heart preparations

The degree of left ventricular remodeling as assessed from diastolic dimension measurements obtained over a range of filling pressures was determined in an isolated, perfused organ system (Badenhorst et al 2003, Norton et al 2002, Woodiwiss et al 2001). Moreover to obtain load-independent measures of systolic and diastolic chamber and myocardial function, an isolated, perfused organ system was also employed (Badenhorst et al 2003, Badenhorst et al 2003b, Norton et al 2002, Woodwiss et al 2001). Rats were anaesthetized with ketamine ($75mg.kg^{-1}$) and xylazine ($15mg.kg^{-1}$). A thoracotomy was performed and hearts were immediately excised and placed in an ice-cold perfusion solution (see description of solution in subsequent discussion) to maintain their viability prior to perfusion. Hearts were subsequently placed on a Langendorff apparatus and

retrogradely perfused via the aorta. Using this approach, perfusion fluid travels down the aorta toward the heart, the aortic valve stays in a closed position because of the pressure gradient produced across the aortic valve, and perfusion fluid flows down the coronary arteries only. Thus myocardial viability is maintained via constant coronary perfusion without relying on the function of the left ventricular chamber to determine coronary flow and myocardial tissue viability. The perfusion solution consisted of (in mM) 118.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 1.2 $MgSO_4$ and 10.0 glucose with a pH of 7.4. The solution was saturated with 95% O_2 and 5% CO_2 gas and carefully filtered through a size 0.45μm Millipore Durapore membrane filter before assessing the pH.

The isolated, perfused organ system is depicted in Figures 2.5 and 2.6. The perfusion solution was constantly gassed with 95% O_2 and 5% CO_2 for the duration of each study. Hearts were perfused retrogradely at a constant flow of 12 $ml.min^{-1}$.g wet heart weight. This was achieved by first obtaining a crude heart weight (heart weight with left ventricle, right ventricle, atria and large vessels) before mounting the heart on the perfusion apparatus and then setting the speed on a peristaltic pump to achieve the appropriate coronary flow. Coronary flow rate was assessed from timed samples obtained of the coronary effluent. A standard proportion of left ventricular weight to crude heart weight was assumed for each preparation and then checked at the end of the study. The perfusion apparatus maintained a constant temperature of the perfusion solution by passing the perfusion solution through a tube surrounded by a water jacket through which heated water flowed (Figure 2.5). The temperature of the coronary effluent was maintained at 37^oC by controlling the temperature of the water flowing through the water

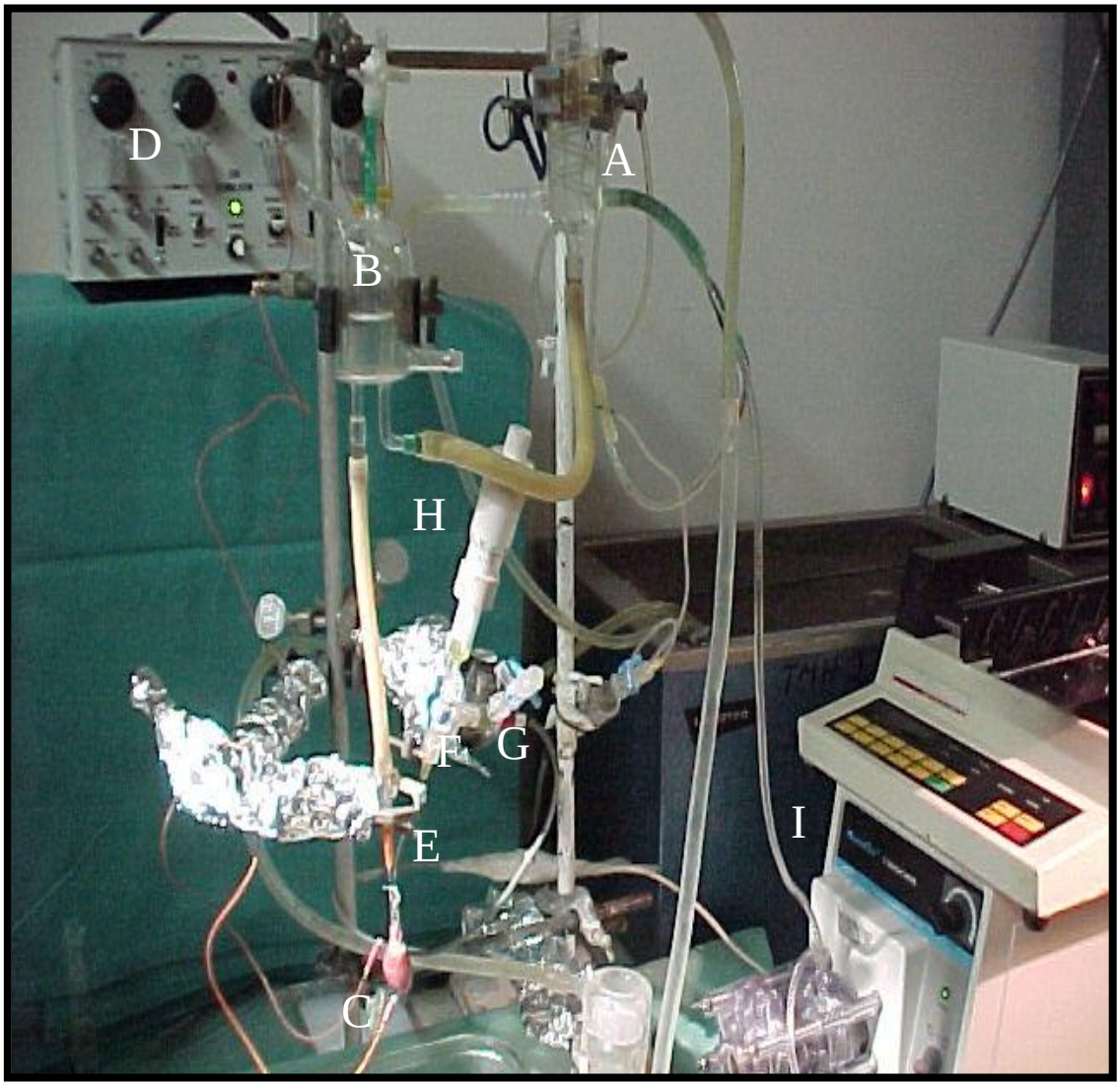

Figure 2.5. Apparatus for isolated, perfused heart studies. A, water jacket; B, bubble trap; C, platinum electrodes attached to isolated heart; D, pacing device; E, fluid filled catheter with balloon attached which has been inserted into the left ventricular lumen; F, three way tap open to E, G and H; G, pressure transducer; H, micromanipulator; I, peristaltic pump

jacket. To avoid air bubbles entering the coronary arteries, a bubble trap was placed proximal to the heart (Figure 2.5). Once the heart was mounted on the perfusion apparatus, platinum electrodes were attached to the right atrium and the apex of the heart and the heart was paced at 360 beats.min^{-1} using a Grass (Astro Med Inc.) model SD9 stimulator (Figure 2.5). All hearts were paced at the same rates in order to avoid having to account for an impact of heart rate on functional measurements. The heart rate selected for these studies was much lower than what we have measured in conscious restrained rats *in vivo* (400-500 beats.min^{-1}). This lower heart rate was employed as this is a crystalloid perfused preparation rather than a blood perfused preparation. It is only in blood perfused preparations in which physiological heart rates can be employed because of the presence of a greater arterial oxygen content. The heart rate selected for the study was one aimed to achieve maximal systolic function through the "Treppe" and other effects without producing demand-induced ischemia. To ensure that rat hearts are not ischemic at 360 beats.min^{-1} previous studies have been performed to assess the pacing rate at which diastolic pressures in the rat heart begin to increase. Demand-induced ischemia characteristically results in an increase in diastolic left ventricular pressures, whereas low-flow ischemia decreases contractile function (Umeda et al 2003). In our hands, we have found that 360 beats.min^{-1} is well below the rate at which left ventricular diastolic pressures first begin to increase in crystalloid perfused rat hearts. Hearts were paced at a voltage that was estimated to be 10% above threshold for spontaneous excitation (Norton et al 2002).

In order to measure left ventricular developed pressures and diastolic pressures a latex balloon was placed through the mitral valve into the left ventricular chamber

(Figure 2.5 and 2.6). The latex balloon was coupled via fluid-filled catheters and a three way tap to both a pressure transducer and a micromanipulator (Figure 2.6). The lumen of the latex balloon was sufficiently large to accommodate volumes well beyond the maximal left ventricular volume of a normal rat or a rat with a dilated ventricle. Indeed, the balloon had a pressure-volume relationship where the pressure in the balloon only started to increase well beyond that of the maximal left ventricular volume of a normal rat or a rat with a dilated ventricle. In the assessment of intraventricular volumes, the balloon material was included as part of volume. The volume of the balloon material was calculated using a volume displacement technique employing larger quantities of the same material and calibrating against the weight of the material. Before inserting the balloon into the left ventricular cavity, the balloon was emptied by removing the micromanipulator, opening the three way tap to atmosphere, squeezing the balloon and allowing fluid to move from the balloon, up the catheter and out of the tap, and then closing the three way tap to the balloon (Figure 2.6).

Left ventricular pressures were determined at as many multiple small increments in volume as were practically possible. The micromanipulator has a Vernier scale (Figure 2.6) that allows for 0.005-0.01 ml increments in volume to be injected into the balloon. The micromanupilator was regularly calibrated by weighing 0.005-to-0.01 ml increments of volumes of fluid. Left ventricular developed pressures and diastolic pressures were measured and recorded on a Hellige recorder (Figure 2.7). Left ventricular developed pressures were recorded on a different channel to left ventricular diastolic pressures (Figure 2.7). An amplified calibration scale was used to assess left ventricular diastolic pressure to ensure the accuracy of recordings (Figure 2.7). The channel used to record left

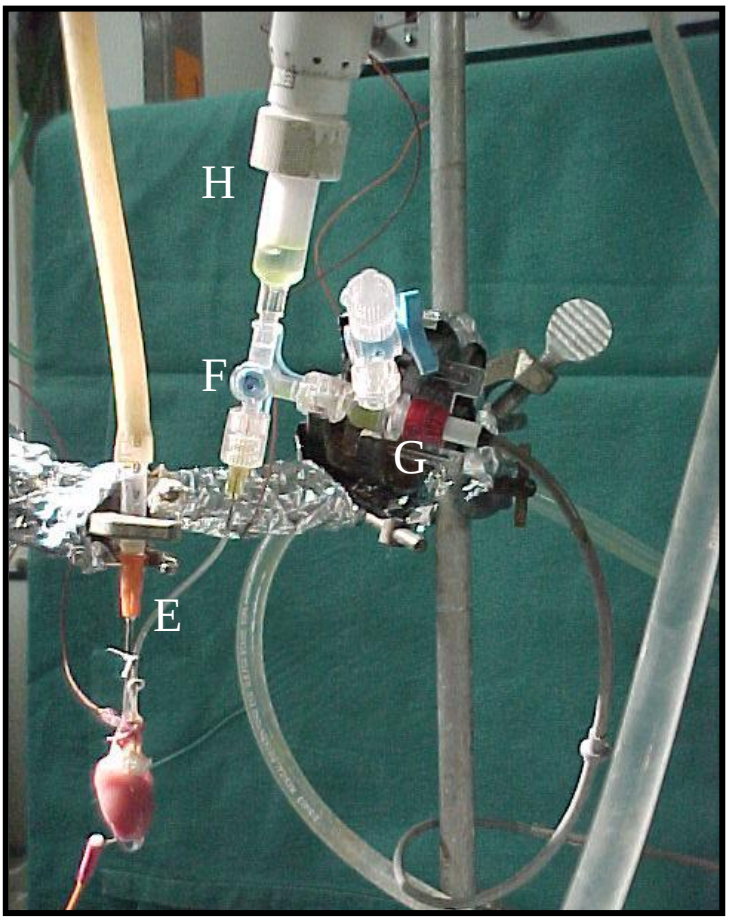

Figure 2.6. Enlargement of a portion of the experimental approach shown in figure 2.5. This figure better shows E, the fluid filled catheter with balloon attached which has been inserted into the left ventricular lumen; F, three way tap G, pressure transducer; and H, micromanipulator.

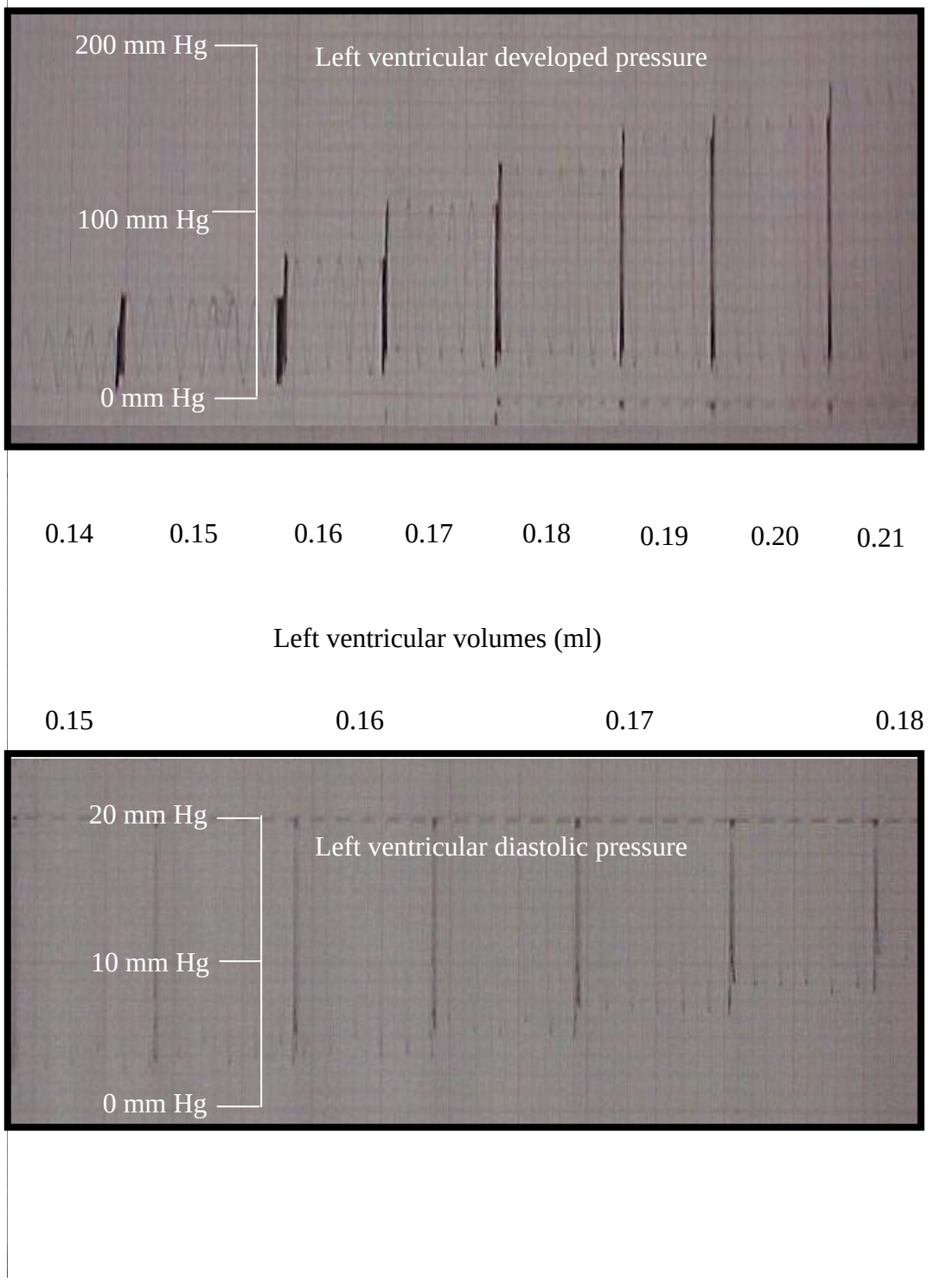

Figure 2.7. Typical recordings obtained of left ventricular developed pressures and left ventricular diastolic pressures in isolated, perfused heart preparations.

ventricular developed pressures was calibrated using a mercury manometer. The channel used to record left ventricular diastolic pressures was calibrated with a water-filled U-tube system designed to calibrate low pressure systems (Norton et al 1996). Calibrations for both left ventricular developed pressure and diastolic pressure recordings were performed both before and after each heart preparation.

Left ventricular pressures were determined at incremental volumes in the absence of an inotropic stimulus, and then following exposure of the heart to 10^{-7} M ISO added to the perfusate to assess adrenergic-inotropic reserve. As the isolated, perfused heart preparation used in this dissertation is isovolumic, left ventricular minimum pressures (diastolic pressures) were assumed to be the equivalent of left ventricular end diastolic pressure in a heart with volume changes that correspond to a normal cardiac cycle.

2.4.1 Assessment of adverse structural remodeling (dilatation) from data obtained in isolated, perfused hearts.

Left ventricular diastolic pressure-volume relations were constructed to assess the degree of left ventricular dilatation. For statistical comparisons, left ventricular dilatation (remodeling) was assessed by comparing left ventricular volumes obtained at a left ventricular diastolic pressure of 0 mm Hg (volume intercept of the left ventricular diastolic pressure-volume relationship-LV V_0) (Veliotes et al 2005, Badenhorst et al 2003a, Badenhorst et al 2003b, Norton et al 2002, Woodiwiss et al 2001).

To further assess the degree of adverse left ventricular chamber remodeling, left ventricular end diastolic relative wall thickness (LVED wall thickness-to-radius ratio

[LVED h/r]) values were assessed over a range of left ventricular filling pressures and the LVED h/r intercept of the LVED h/r-left ventricular diastolic pressure relationship compared. That latter comparison was made to ensure that the increases in left ventricular cavity size or volumes noted were truly representative of adverse structural remodeling (dilatation), where adverse structural remodeling is either the consequence of cardiomoycyte lengthening or side-to-side cardiomyocyte slippage. With either of these changes, a concomitant increase in wall thickness would not occur in conjunction with increases in internal dimensions, and hence the relationship between wall thickness and internal diameter would be reduced.

Left ventricular end diastolic wall thickness (LVED h) and internal radius (LVED r) were calculated from previously described formulae (Weber et al 1988a) as follows assuming a thick wall spherical geometry:

$$\text{LVED r} = \sqrt[3]{\text{LVEDV} \times (3/4\ \pi)}$$

Where LVEDV is left ventricular end diastolic volume.

$$\text{LVED h} = \frac{\text{LVEDD} - 2r}{2}$$

Where LVEDD is left ventricular end diastolic diameter which is calculated from

$$\text{LVEDD} = 2 \times \sqrt[3]{\text{LVEDV} + (0.943 \times \text{LV mass}) \times (3/4\ \pi)}$$

Where 0.943 is the density of myocardial tssue.

2.4.2 Myocardial material properties from data obtained in isolated, perfused hearts.

Myocardial material properties were assessed from the myocardial diastolic stiffness constant determined from the slope of the linearized diastolic stress-strain relation (myocardial *k*) (Badenhorst et al 2003b, Norton et al 2002). By converting pressures and volumes into stress and strain data, the impact of alterations in chamber geometry on diastolic function are eliminated. Left ventricular diastolic stress and strain were calculated from previously described formulae (Badenhorst et al 2003b, Norton et al 2002, Weber et al 1988a) assuming a thick-walled spherical geometry of the left ventricle as follows:

$$\text{Left ventricular diastolic stress} = \frac{1.36 \times \text{LVED pressure} \times (\text{LVEDV})^{2/3}}{[\text{LVEDV} + (0943 \times \text{LV mass})]^{2/3} - \text{LVEDV}^{2/3}}$$

$$\text{Left ventricular diastolic strain} = \frac{\{\text{LVEDV}^{1/3} + [\text{LVEDV} + (0.943 \times \text{LV mass})]^{1/3}] - 1}{\text{LV V0}^{1/3} + [\text{LV V0} + (0.943 \times \text{LV mass})]^{1/3}}$$

2.4.3 Systolic chamber and myocardial function from data obtained in isolated, perfused hearts.

Unlike indices of left ventricular pump (chamber) and myocardial function obtained *in vivo* (FS_{end} and FS_{mid}), which are afterload-dependent, indices of systolic chamber and myocardial function used by our group as obtained in isolated perfused heart preparations are afterload and preload-independent.

To determine systolic chamber function the slope of the linear portion of the systolic developed pressure-volume relationship was calculated (systolic chamber elastance-E). This is the equivalent of left ventricular end systolic elastance in an ejecting and filling left ventricle. Left ventricular end systolic elastance is the slope of the end systolic pressure-volume relationship. Left ventricular end systolic elastance has been well established as being afterload and preload-independent (Sugawa et al 1988). The linear portion of the systolic developed pressure-volume relationship in an isovolumic preparation is considered to be the equivalent of end systolic elastance in a heart that ejects and fills during the cardiac cycle, as peak systolic pressures in an isovolumic preparation are the same as end systole pressures. Data points were included in the peak systolic pressure-volume relationship if on linear regression analysis for individual rats, the r^2 value with each point included was 0.95 or more. Using this approach I could include the first five left ventricular developed pressures in the left ventricular developed pressure-volume relationship for all rats for baseline measurements.

To determine intrinsic systolic myocardial function, the slope of the systolic developed stress-strain relationship was calculated (myocardial systolic elastance-En)

(Veliotes et al 2005, Badenhorst et al 2003a, Norton et al 2002). By converting pressures and volumes into stress and strain data, the impact of alterations in left ventricular chamber geometry on systolic function are eliminated (Weber et al 1988a). Thus, En is the myocardial equivalent of E and hence is also afterload and preload-independent. En is a linear relationship and hence substantially more data points were included in the analysis than five data points. However, to ensure that calculations of En were representative of data obtained for E, En was also recalculated from only the first five left ventricular developed pressure and volume data and provided essentially the same outcomes. This is not surprising as the relationship is linear. Left ventricular developed stress and strain values were calculated using the same equations used to calculate diastolic stress and strain except that the pressures and volumes used in the calculations were for developed pressures and not diastolic pressures (see above equations).

2.5 Cardiac weights

After completion of data collection from isolated, perfused hearts, hearts were removed from the apparatus and blotted dry. Both the left and the right atria as well as the aorta and pulmonary artery were removed. The free wall of the right ventricle was trimmed off the septum of the heart. The left ventricle with the septum was then weighed and left ventricular weight was expressed as an absolute value as well as a proportion of body weight to account for variations in the growth of rats. Importantly, daily ISO administration at the doses employed do not modify body weight in SHR, but SHR have lower body weights than WKY controls (Badenhorst et al 2003a). A sample of tissue

from the left ventricular free wall near the base of the heart was weighed and dried in an oven at 80^{o}C overnight and then reweighed to determine dry heart weight.

2.6 Myocardial collagen

To determine the interstitial changes that may modify myocardial material properties or contribute toward right shifts in left ventricular diastolic pressure-volume relations, samples of left ventricular tissue were then weighed and stored at –70^{o}C prior to tissue analysis. The samples used to assess these characteristics were matched to the area of the heart assessed, with all analysis being done on the left ventricular antero-lateral wall in the mid-portion of the longitudinal axis. Myocardial hydroxyproline concentration ([HPRO]) was determined after acid (HCl) hydrolysis using previously described techniques (Stegemann and Stalder 1967) as previously performed (Badenhorst et al 2003b, Norton et al 2002, Tsotetsi et al 2001, Woodiwiss et al 2001, Norton et al 1997).

Myocardial collagen was also extracted and digested with cyanogen bromide (CNBr) (Norton et al 1997; Tsotetsi et al 2001; Woodiwiss et al 2001; Badenhorst et al 2003b). A portion of the CNBr digested collagen sample was subjected to acid hydrolysis and [HPRO] determination. The amounts of non-cross-linked (soluble) and cross-linked (insoluble) collagen in the myocardium were ascertained based on the solubility of myocardial collagen to CNBr digestion (Norton et al 1997; Tsotetsi et al 2001; Woodiwiss et al 2001; Badenhorst et al 2003b).

Using the remaining portion of the CNBr digested sample, polyacrylamide gel electrophoresis was subsequently performed on vertical gels by stacking and separation of gel concentrations of 3% and 12.5% respectively using a previously described technique (Laurent et al 1981, Mukherjee and Sen 1993). The type I-to-III collagen ratio was determined following gel scanning (Woodiwiss et al 2001, Tsotetsi et al 2001, Norton et al 1997). Myocardial type I and III collagen concentrations were assessed from type I-to-III ratios and the [HPRO] in myocardial tissue (Woodiwiss et al 2001, Tsotetsi et al 2001, Norton et al 1997). Representative examples of polyacrylamide electrophoretic gels used to determine collagen type I and III ratios and concentrations are illustrated in Figure 2.8. Electrophoretic patterns of myocardial collagen extracts were obtained and the bands corresponding to collagens type I and III identified from collagen type I (Sigma) and III (Calbiochem) standards. The relative amounts of type I:III collagen were determined from the relationship between the quantity of collagen applied to the gel and the relative area under the densitometry curve corresponding to bands G [α_1(I)-CB-8] and H [α_2(I)-CB-3] (type I) (Laurent et al 1981) and band M [α_1(III)-CB-5 plus α_1(III)-CB-9] (type III)(Laurent et al 1981, Mukherjee and Sen 1993). Bands G, H and M were chosen because they contain very little interference from co-migrating peptides of other collagen types.

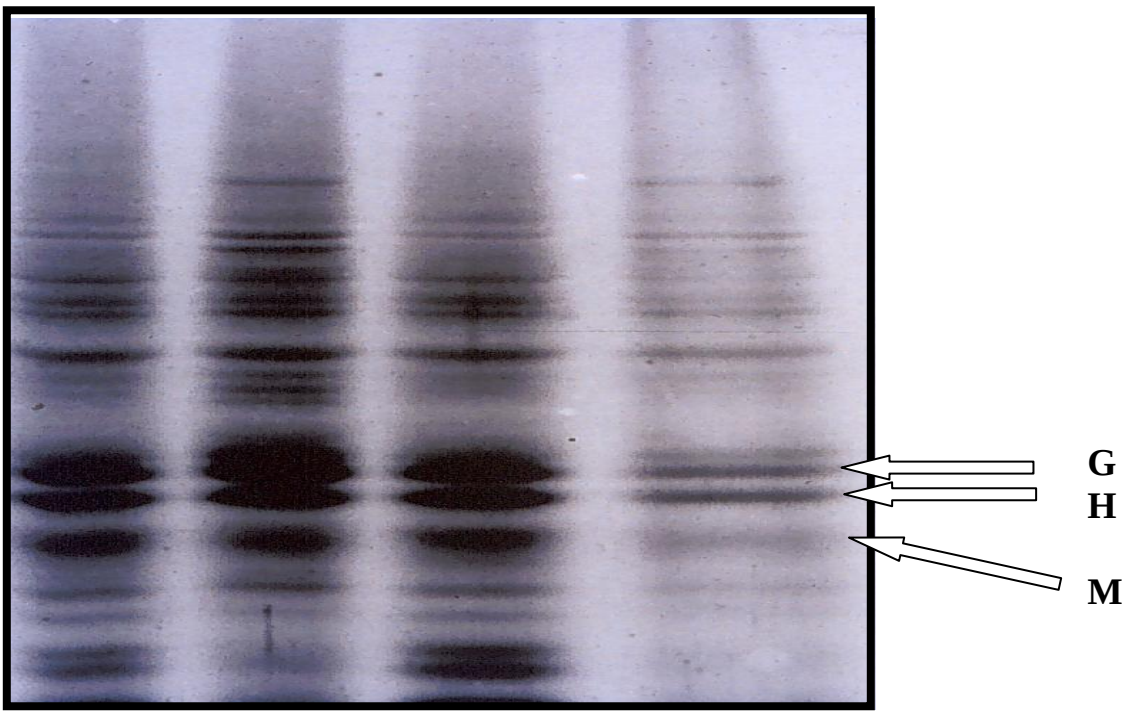

Figure 2.8. Polyacrylamide electrophoretic gel showing typical banding patterns for myocardial collagen. See text for explanation.

2.7 Pathological score

Before storing tissue for biochemical assessment, a longitudinal slice of the left ventricle from the apex to the base through the left ventricular free wall was obtained from all rats for histology. Left ventricular tissue was stored in formalin for subsequent histology. Left ventricular tissue was processed routinely for light microscopy and 50 μm-thick sections of the long axis circumference were cut through the full thickness of the left ventricular wall. Ten slices were obtained at 1-mm intervals and stained with van Gieson's stain. After staining, a pathological grade was assigned, where 0 indicates no damage; 1 and 2, patchy fibrosis in less than or more than 20% of the field respectively; 3 and 4, diffuse contiguous subendocardial fibrosis in less than or more than 50% of the field respectively and 5 and 6, full thickness fibrosis in less than or more than 50% of the field respectively (Woodiwiss et al 2001, Teerlink et al 1994). Slides showing fibrotic and normal areas are illustrated in Figure 2.9.

2.8 Data analysis.

Regression analysis was used to determine the lines of best fit for the cardiac function and other relations. Differences in left ventricular weight, internal dimensions, relative wall thickness, indices of performance, myocardial collagen biochemical analyses, and myocardial fibrosis between groups were assessed by a one-factor ANOVA followed by a Tukey *post hoc* test. A two way ANOVA was used to assess differences between groups in blood pressure over the seven month period of the study and in

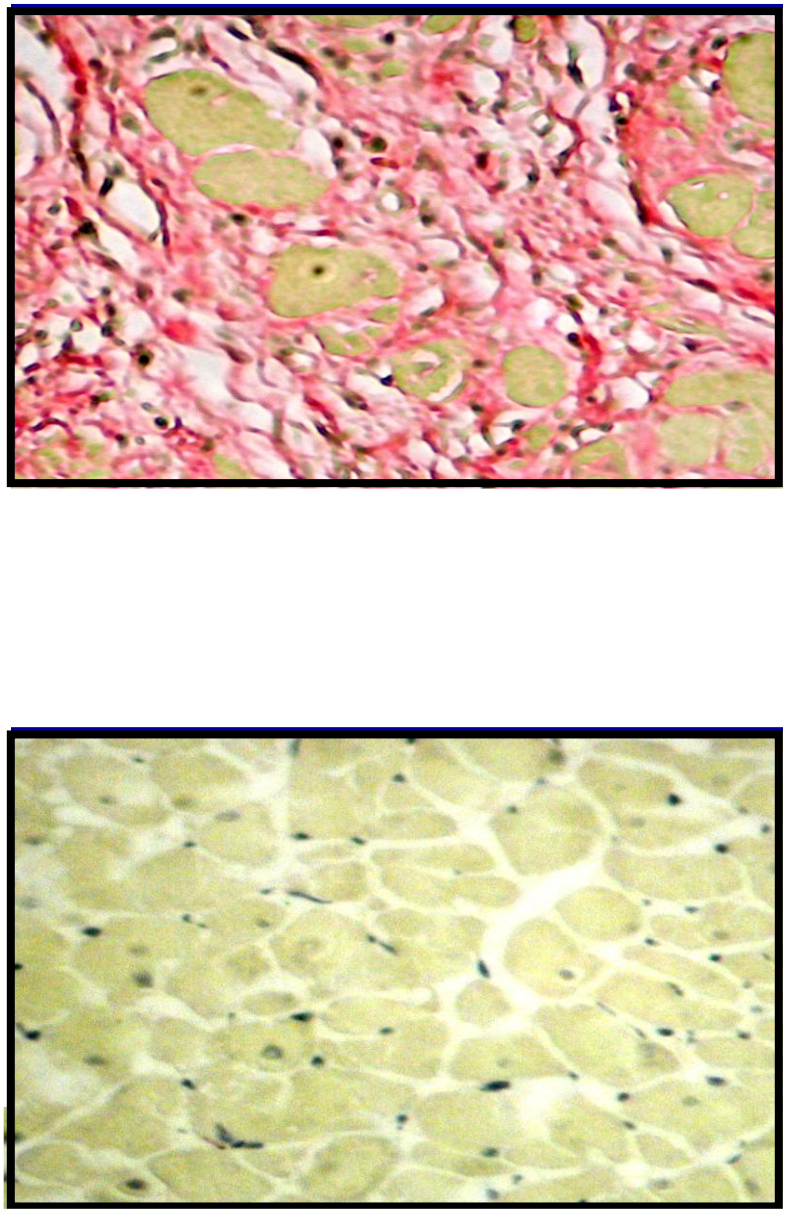

Figure 2.9. Histological images obtained using light microscopy from cross-sections of myocardial tissue stained with van Gieson's stain. The slides show evidence of tissue necrosis and diffuse fibrosis (upper panel) as compared to normal areas (lower panels). The myocardium of the rat in the upper panel was scored as 3.5 (diffuse subendocardial fibrosis in less than 50% of the field).

systolic function over time after acute ISO injections. All values in the text are represented as mean ± SEM.

Chapter 3

Results

3.1 Haemodynamic effects of acute intraperitoneal injections of isoproterenol.

Figures 3.1 and 3.2 show the impact of acute ISO injections on left ventricular systolic function as determined using echocardiography. Within a few minutes of the intraperitoneal injection a marked increase in systolic function occurred, with increases in both chamber (FSend) and myocardial (FSmid) function noted. Figure 3.1 shows an example of an echocardiograph at 15 minutes and Figure 3.2 shows the mean values and SEM of all rat's data at 30 minutes as compared to baseline. These haemodynamic effects are sustained for up to forty five minutes after injection (data not shown). Not calculated but clearly evident in Figure 3.1 is the increase in heart rate in response to ISO administration. In all rats assessed the effect on pump function was as profound as that illustrated in Figure 3.1. Although data from SHR are not shown, the haemodynamic response was the same.

3.2 Blood pressures

At all times during the course of the study that blood pressures were determined, SHR had markedly greater systolic blood pressures (SBP) as compared to WKY controls as assessed using a non-invasive tail-cuff technique in conscious, restrained rats (Figure 3.3). Importantly ISO administration failed to significantly modify systolic blood pressure in either SHR (Figure 3.3) or in WKY (SBP in mm Hg after 7 months from a parallel study conducted in WKY rats; WKY-ISO [n=7]=136±8 mm Hg, WKY-saline

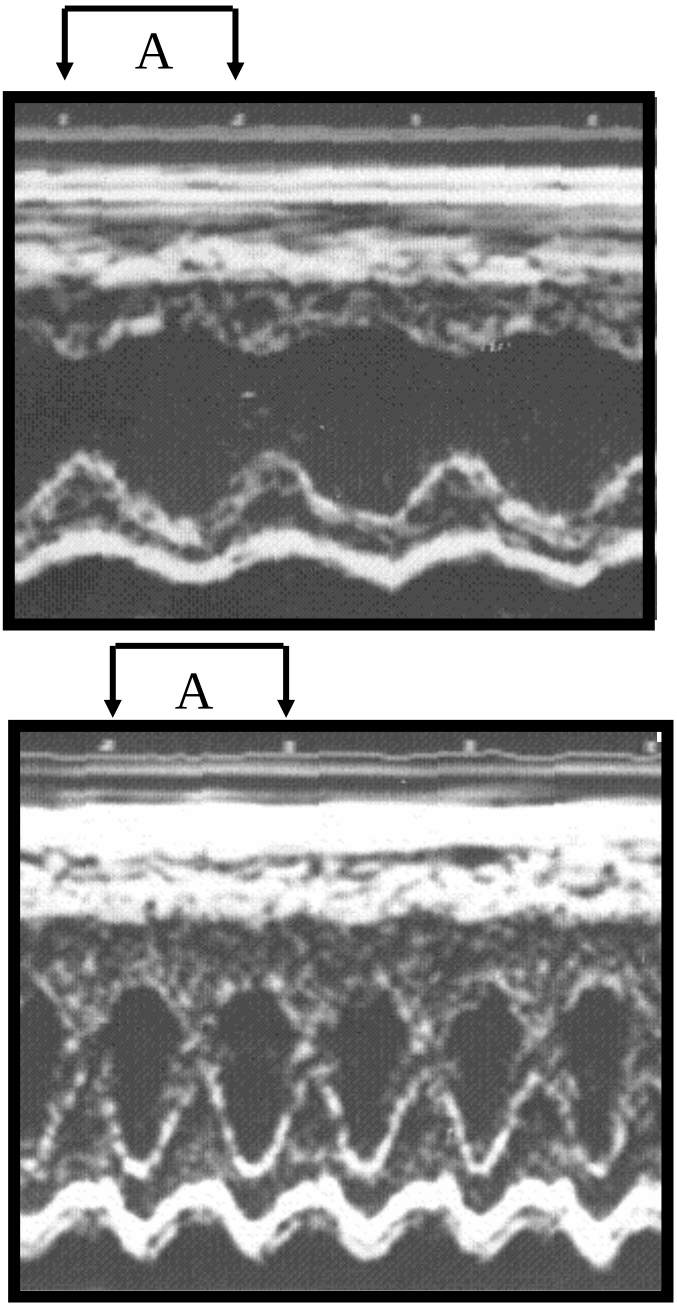

Figure 3.1. Typical M-mode echocardiographic recordings obtained before (upper panel) and after (lower panel) an acute intraperitoneal injection of isoproterenol (ISO) at the dose injected daily in rats for 7 months. This recording is from a WKY rat. Mean data for the group of WKY rats is shown in Figure 3.2. Note the distance between the timing markers (A) indicating that these recordings have been made at the same sector speed.

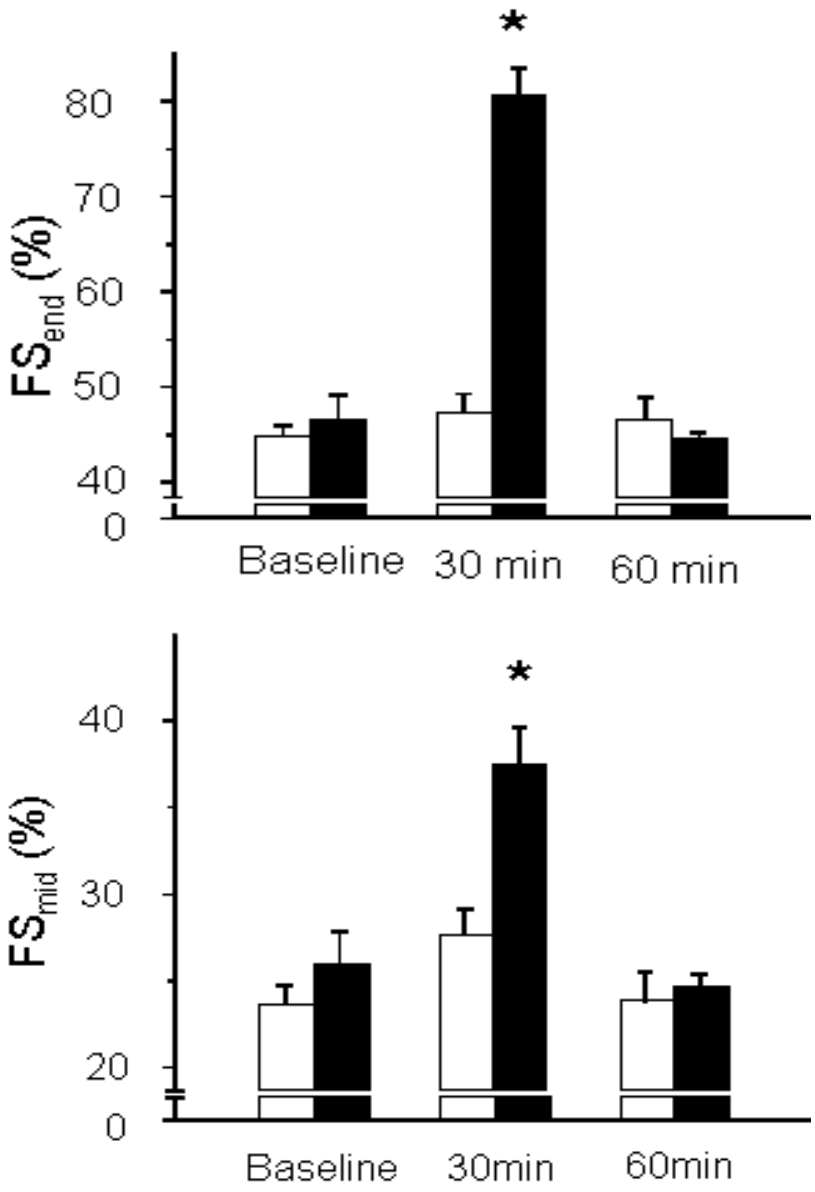

Figure 3.2. Left ventricular endocardial fractional shortening (FS_{end})(upper panel) and midwall fractional shortening (FS_{mid})(lower panel) at baseline and at 30 and 60 minutes after acute intraperitoneal injections of isoproterenol (ISO) (closed bars) and the saline vehicle (open bars) at the dose injected daily in rats for 7 months. These data were obtained in WKY rats (n=10 per group).

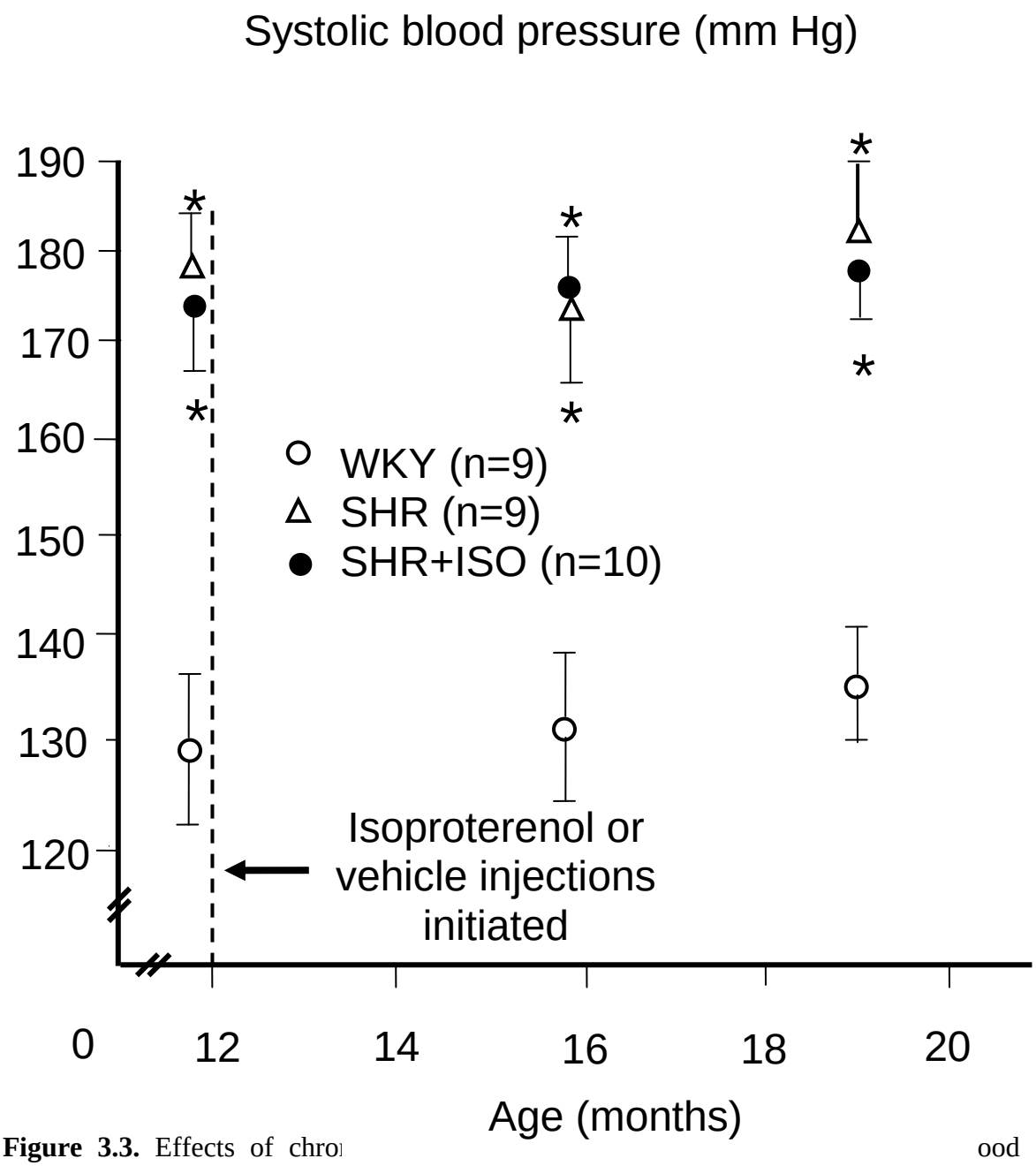

Figure 3.3. Effects of chro ood pressure, measured using a non-invasive tail cuff technique, in conscious restrained spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. * $p<0.001$ versus WKY group. No changes over time were noted.

vehicle [n=6]=128±10 mm Hg) at any time during the study that blood pressures were measured (Figure 3.3) 24-hours or more after the last dose of isoproterenol.

3.3 Body weight and left ventricular weight

Table 3.1 shows the effect of chronic ISO administration on body weight and left ventricular weights in SHR. Spontaneously hypertensive rats had much lower body weights than age-matched WKY controls. Despite having a lower body weight, SHR had a markedly greater absolute left ventricular weight as compared to WKY controls (Table 3.1). Moreover, SHR had a greater left ventricular weight-to-body weight ratio (Table 3.1). Daily administration of ISO augmented the increase in absolute left ventricular weight and the left ventricular weight-to-body weight ratio noted in SHR (Table 3.1). The ratio of wet-to-dry left ventricular weight was the same between the groups (data not shown) and in comparison to wet-to-dry left ventricular weights of hearts that have not undergone crystalloid perfusion obtained from prior studies performed by our group (Norton et al 1997, Tsotetsi et al 2001).

The body weights (WKY-saline [n=6]=405±21g, WKY-ISO [n=7]=422±24g) and left ventricular weights (WKY-saline [n=6]=0.99±0.05g, WKY-ISO [n=7]=1.08±0.06g) of 11-14 month old WKY rats receiving either the vehicle or ISO for 7 months were similar to those of the WKY rats shown in Table 3.1. Importantly, ISO had no effect on body weight (data given above), left ventricular weight (data given above) or left ventricular weight-to-body weight ratio (data not shown).

Table 3.1. Effects of chronic isoproterenol administration (ISO) on body weight and left ventricular weight in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY).

	WKY n=9	SHR n=9	SHR+ISO n=10
Body weight (g)	421±14	355±5†	351±7†
Left ventricular (LV) weight (g)	1.04±0.02	1.29±0.05*	1.52±0.08†#
LV/body weight x 10^4	2.48±0.05	3.64±0.13†	4.31±0.22†#

SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. *$p<0.05$, † $p<0.01$ versus WKY group; # $p<0.05$ versus SHR group.

3.4 Left ventricular internal dimensions assessed *in vivo*.

Figure 3.4 shows the effect of chronic ISO administration on left ventricular internal dimensions in SHR. As assessed using echocardiography, SHR at 19 months of age had similar left ventricular end diastolic internal diameters (LVEDD) as compared to WKY controls (Figure 3.4). However, SHR receiving ISO from 12-to-19 months of age had increases in both left ventricular end diastolic internal diameter and left ventricular end systolic internal diameter as compared to WKY controls and untreated SHR (Figure 3.4).

3.5 Left ventricular diastolic pressure-volume relations.

Figure 3.5 shows the effect of chronic ISO administration on left ventricular diastolic pressure-volume relations and the volume intercept of the relationship (see continuation of figure over the page). Although there was a trend for an increased slope of the relationship, untreated SHR at 19 months of age had a similar left ventricular diastolic pressure-volume relationship as WKY controls (Figure 3.5). Importantly, untreated SHR at 19 months of age had the same volume intercept of the relationship (LV V_0) as WKY controls (Figure 3.5, second figure). However, ISO administered to SHR for 5 months resulted in a marked right shift in the left ventricular diastolic pressure-volume relationship (Figure 3.5, first figure) and an increase in LV V_0 (Figure 3.5, second figure), changes consistent with chamber dilatation.

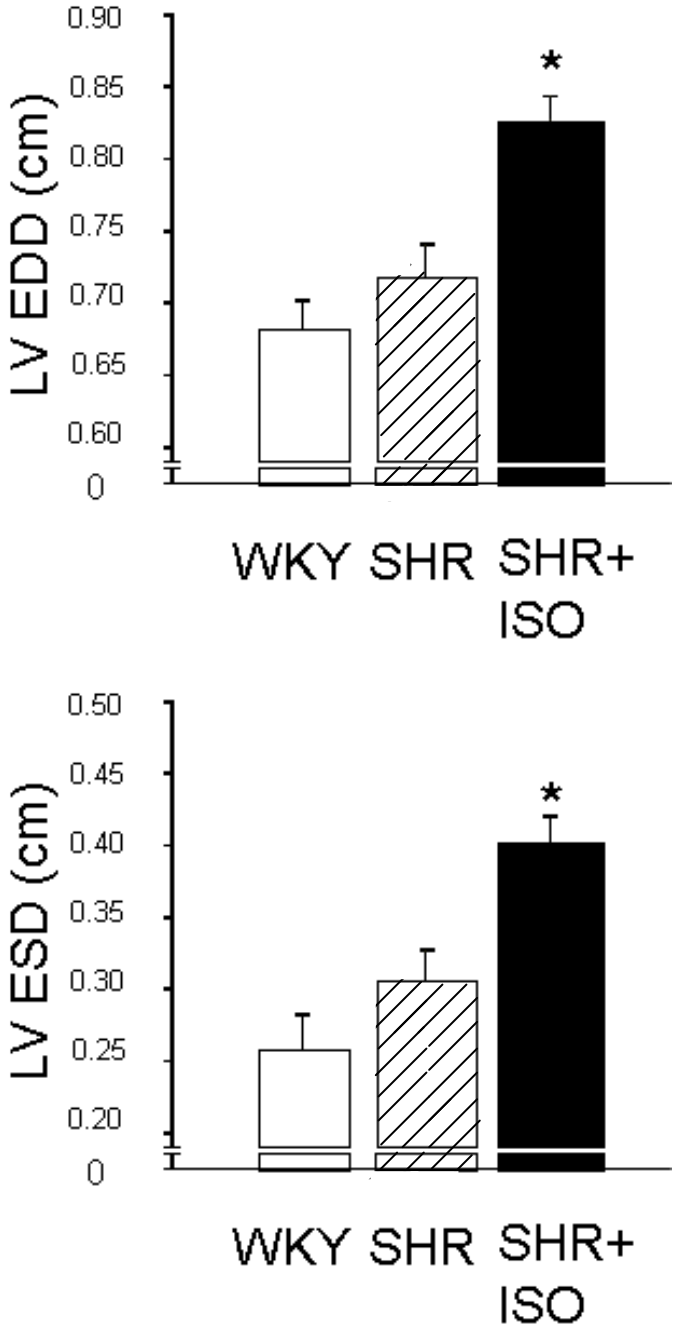

Figure 3.4. Effects of chronic isoproterenol (ISO) administration on left ventricular end diastolic (LVEDD) and end systolic (LVESD) diameters in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol * $p<0.05$ versus other groups. See Table 3.1 for sample sizes.

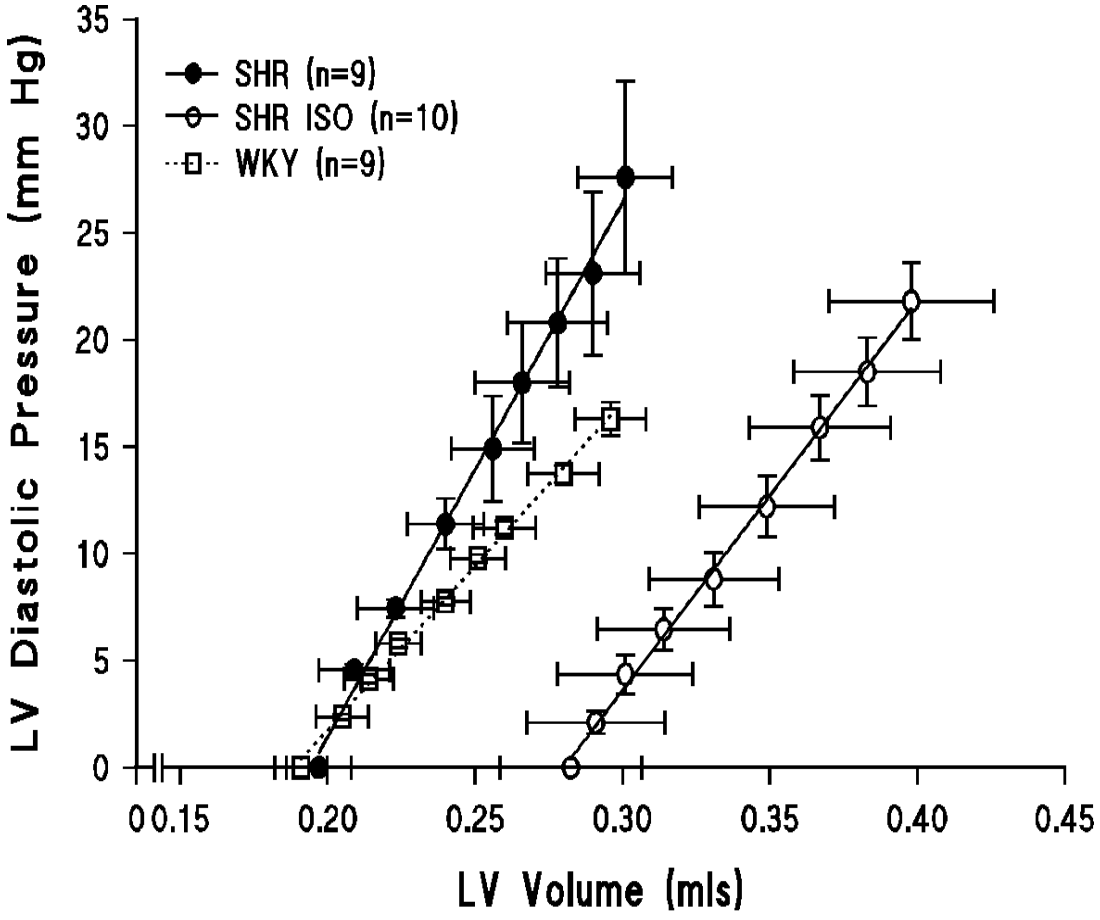

Figure 3.5 (see next page for continuation of figure). Effects of chronic isoproterenol (ISO) administration on left ventricular (LV) diastolic pressure-volume relations in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. Statistical comparisons of the volume intercepts of these relations are made in the next figure.

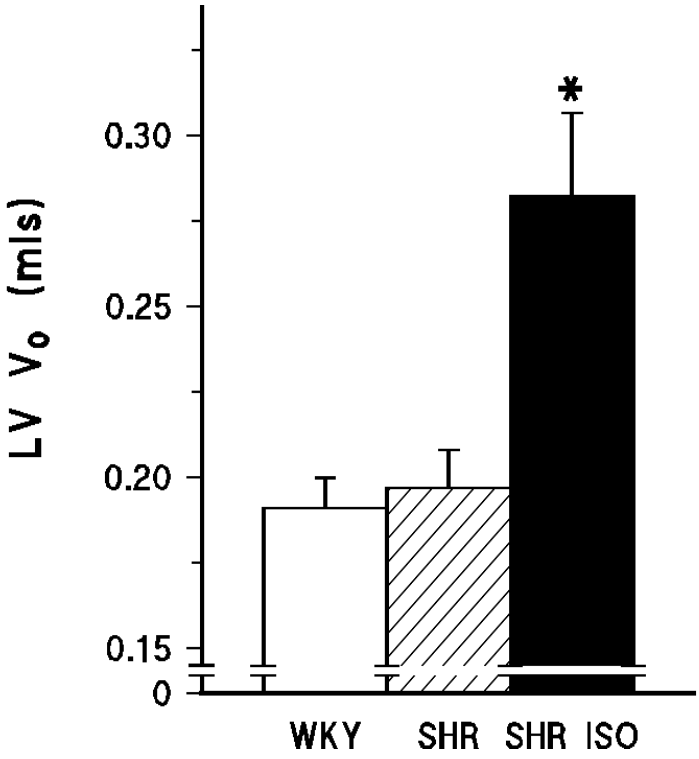

Figure 3.5 continued. Effects of chronic isoproterenol (ISO) administration on the volume intercept (LV V_0) of the left ventricular diastolic pressure-volume relations (shown in the previous page) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. * $p<0.01$ versus other groups.

Left ventricular diastolic pressure-volume relations, and the volume intercept at 0 mm Hg (LV V_0) in 11-14 month old WKY rats receiving either ISO or the vehicle of ISO for 7 months were similar to those of the WKY rats shown in Figure 3.5 (LV V_0 in mls; WKY-saline [n=6]=0.20±0.01, WKY-ISO [n=7]=0.199±0.01). Importantly, ISO had no effect on LV V_0 (data given above).

3.6 Left ventricular diastolic relative wall thickness-filling pressure relations.

Figure 3.6 shows the effect of chronic ISO administration on the relationship between left ventricular end diastolic pressure (LVEDP) and left ventricular end diastolic wall thickness (h)-to-radius (r) ratio (LVED h/r) (first figure) and the LVED h/r intercept of these relations (LVED h/r_0)(see continuation of the figure over the page). Consistent with concentric left ventricular hypertrophy, untreated SHR at 19 months of age had an increased LVED h/r (relative wall thickness) as compared to age-matched WKY controls (Figure 3.6). However, chronic ISO administration to SHR resulted in relative wall thinning, with marked decreases in LVED h/r noted over a range of LVEDP values (Figure 3.6) despite further increases in LV weight (Table 3.1).

Left ventricular end diastolic h/r in 11-14 month old WKY rats receiving either ISO or the vehicle of ISO for 7 months (data not shown) was similar to that of the WKY rats shown in Figure 3.5 (data not shown). Importantly, ISO had no effect on LVED h/r (data not shown).

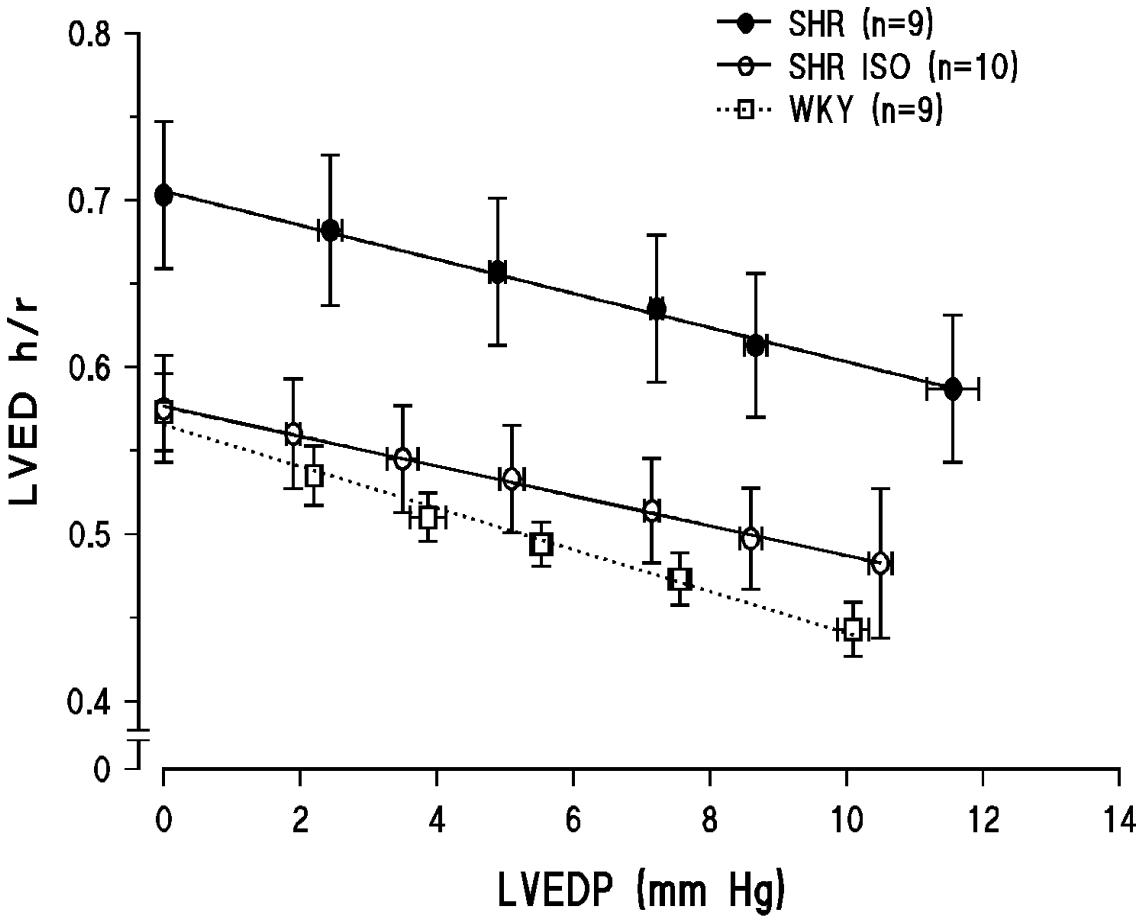

Figure 3.6 (see next page for continuation of figure). Effects of chronic isoproterenol (ISO) administration on the relationship between left ventricular end diastolic pressure (LVEDP) and left ventricular end diastolic wall thickness (h)-to-radius (r) ratio (LVED h/r) in spontaneously hypertensive rats (SHR). Statistical comparisons of the LVED h/r intercept of these relations (LVED h/r_0) are shown over the page Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the vehicle; SHR+ISO, SHR receiving isoproterenol

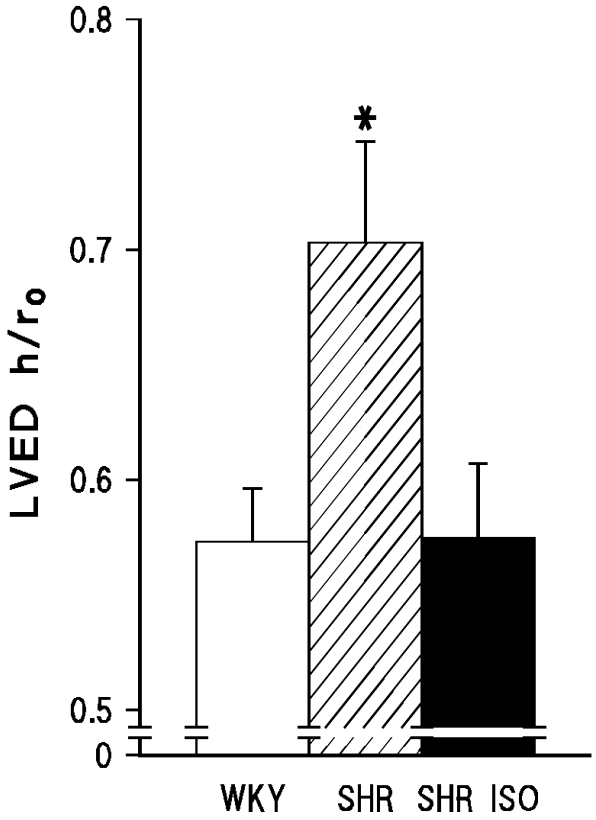

Figure 3.6 continued. Effects of chronic isoproterenol (ISO) administration on the left ventricular end diastolic h/r intercept (LVED h/r_0) of the relationships between left ventricular end diastolic pressure (LVEDP) and left ventricular end diastolic wall thickness (h)-to-radius (r) ratio (LVED h/r) (shown on the previous page) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. * $p<0.01$ versus other groups.

3.7 Myocardial diastolic stiffness (myocardial material properties).

Figure 3.7 shows the effect of chronic ISO administration on the relationship between left ventricular diastolic midwall stress and strain (first figure) and the slope (*k*) of the linearised midwall stress-strain relationship (second figure over the page) in spontaneously hypertensive rats. SHR at 19 months of age had a marked left shift in left ventricular diastolic midwall stress-strain relations and an increase in the slope (myocardial *k*) of these relations as compared to WKY rats (Figure 3.7). These data indicate an increase in myocardial stiffness in SHR. Chronic ISO administration to SHR failed to modify either LV diastolic midwall stress-strain relations or myocardial *k* ($p>0.05$ compared to SHR receiving the saline vehicle) (Figure 3.7). Thus, essentially chronic ISO administration, despite producing marked right shifts in left ventricular diastolic P-V relations in SHR (Figure 3.5), failed to modify the increased myocardial stiffness (change in material properties) noted in SHR.

Left ventricular diastolic midwall stress and strain relations (data not shown) and the slope (*k*) of the linearised midwall stress-strain relationship (WKY-saline [n=6]=14.6±0.8, WKY-ISO [n=7]=0.14.8±0.9) in 11-14 month old WKY rats receiving either ISO or the vehicle of ISO for 7 months, were similar to those of the WKY rats shown in Figure 3.7. Importantly, ISO had no effect on myocardial *k* (data given above).

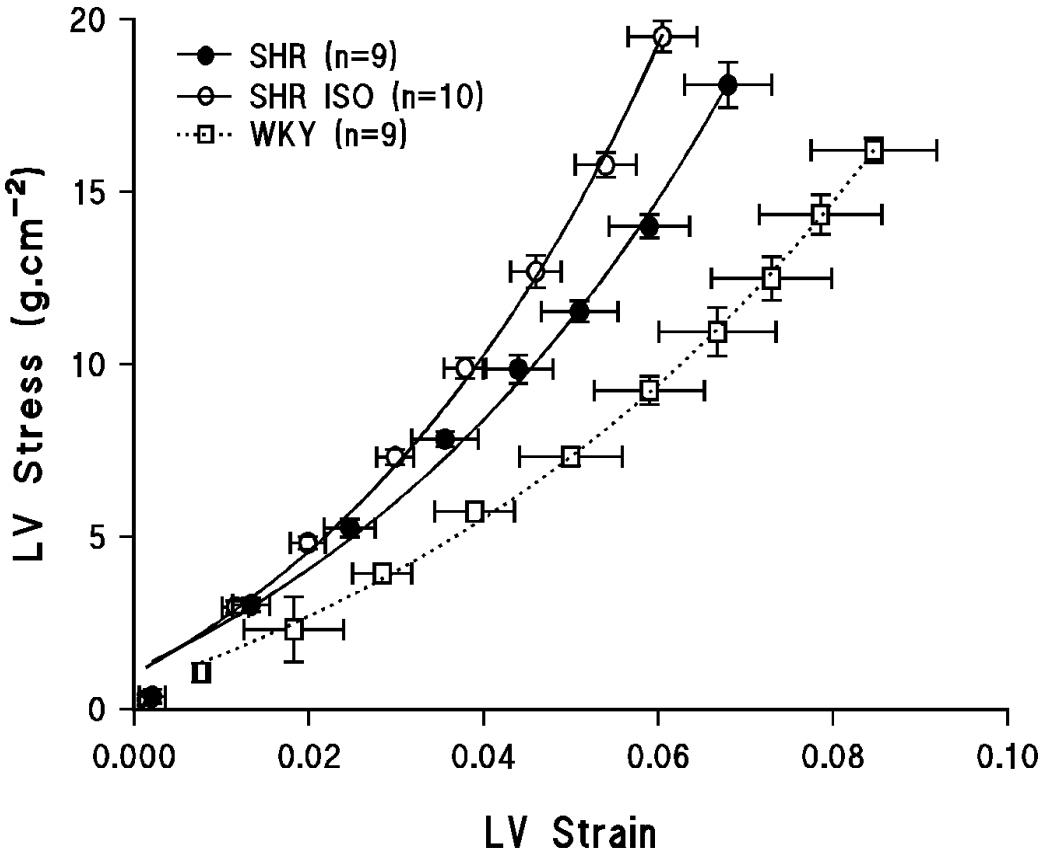

Figure 3.7 (see continuation of figure over the page). Effects of chronic isoproterenol administration (ISO) on the relationship between left ventricular (LV) diastolic midwall stress and strain in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. The slope (*k*) of the linearised midwall stress-strain relationships are compared statistically over the page.

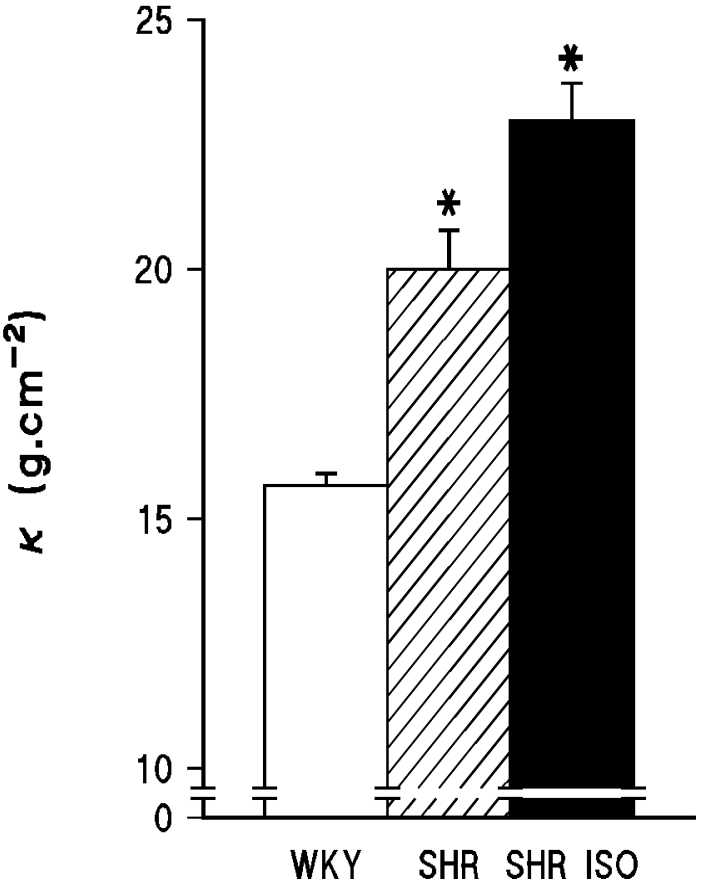

Figure 3.7 continued. Effects of chronic isoproterenol administration (ISO) on the slope (*k*) of the linearised relationship between left ventricular (LV) diastolic midwall stress and strain (shown on the previous page) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. * $p<0.01$ versus WKY group

3.8 Systolic chamber function.

Figures 3.8 (upper panel) and 3.9 show the effect of chronic ISO administration to SHR on a load-dependent measure of systolic chamber function measured *in vivo* (Figure 3.8, upper panel) and a load-independent measure of systolic chamber function measured *ex vivo* (Figure 3.9). As assessed both *in vivo* (Figure 3.8, lower panel-LV FS_{end}) and *ex vivo* (Figure 3.9, E in the absence of an inotropic stimulus), chamber systolic function (pump function) was maintained in untreated SHR at 19 months of age. However, chronic ISO administration to SHR promoted a decrease in chamber systolic function as assessed both *in vivo* (Figure 3.8, lower panel-LV FS_{end}) and *ex vivo* (Figure 3.9, E in the absence of an inotropic stimulus). Data obtained *ex vivo* in the presence of an adrenergic stimulus (ISO) produced essentially the same outcomes. However, only three values could be included for each rat in the calculation of E, as the linear portion of the curve was very steep and hence only the first three values fulfilled the pre-specified quality control criteria of providing an r^2 value >0.95 on linear regression analysis.

Left ventricular end systolic elastance (E) in 11-14 month old WKY rats receiving either ISO or the vehicle of ISO for 7 months (data not shown) was similar to that of the WKY rats shown in Figure 3.9. Importantly, ISO had no effect on LV E in WKY rats (data not shown).

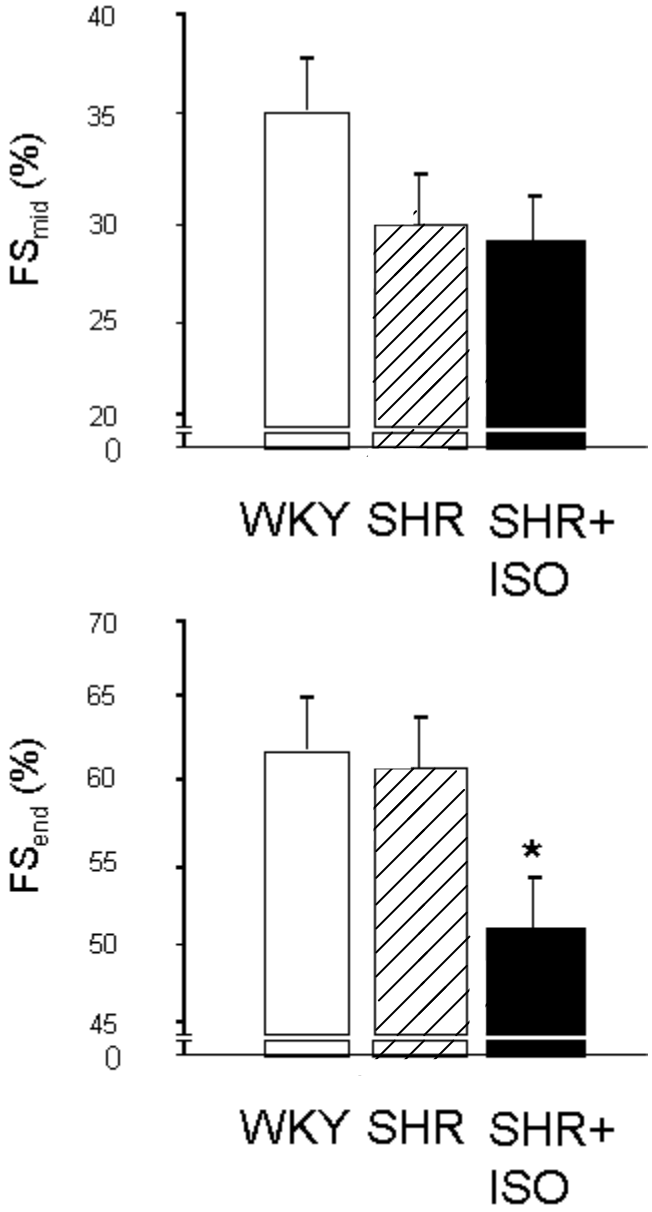

Figure 3.8. Effects of chronic isoproterenol administration (ISO) on left ventricular systolic chamber (lower panel) and myocardial (upper panel) function as assessed *in vivo* (echocardiography) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol; FS_{end}, left ventricular endocardial fractional shortening; FS_{mid}, left ventricular midwall fractional shortening. * $p<0.01$ versus other groups. See Table 3.1 for sample sizes.

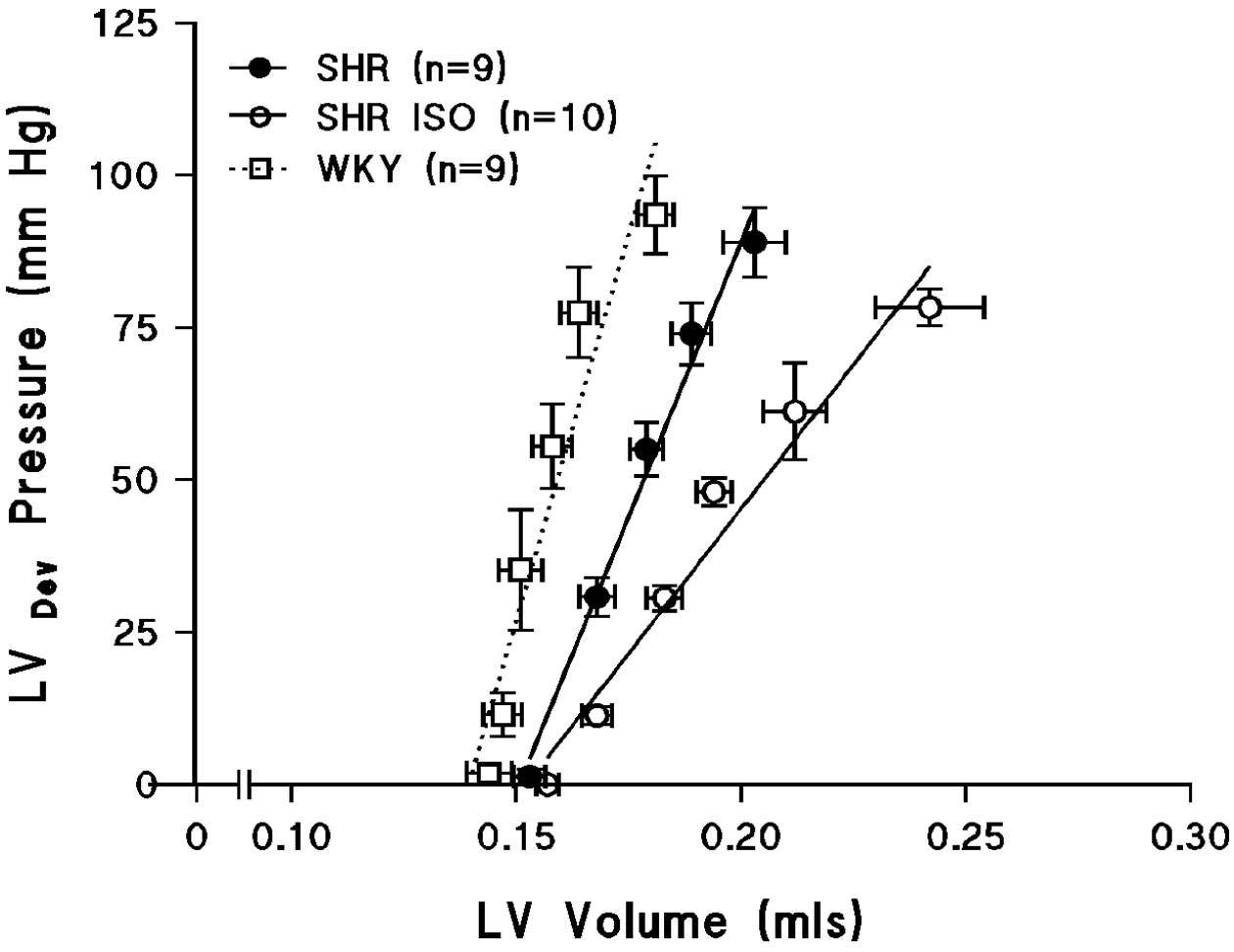

Figure 3.9 (see continuation of figure over the page). Effects of chronic isoproterenol administration (ISO) on left ventricular developed pressure (LV_{dev}Pressure) –volume relations in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. The mean slope of the relations (systolic elastance or E), which represents a load-independent measure of systolic chamber function, is compared statistically over the page.

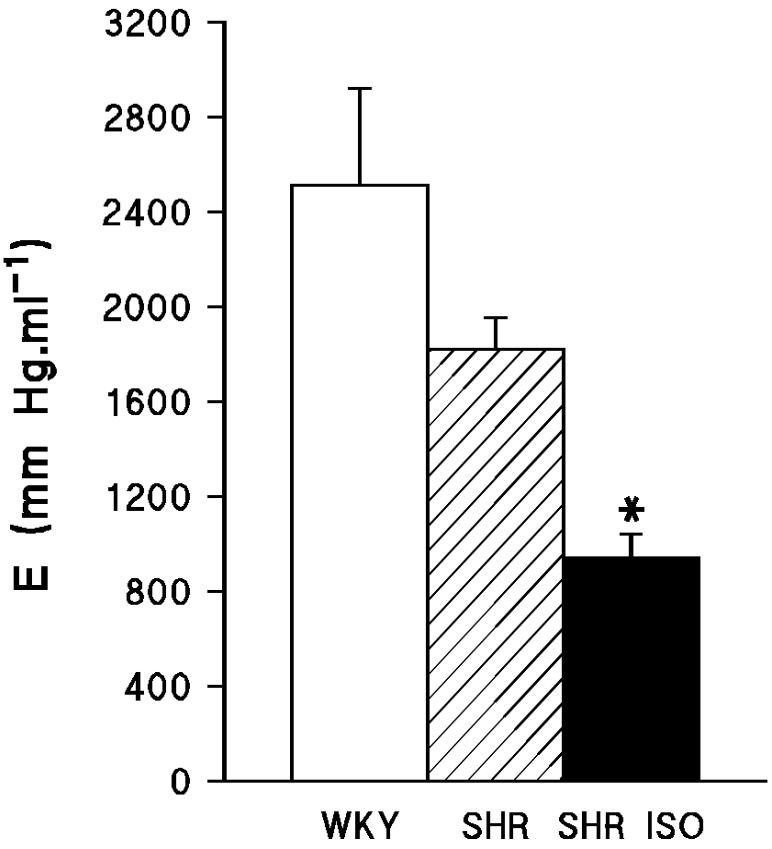

Figure 3.9 continued. Effects of chronic isoproterenol administration (ISO) on the mean slope (systolic elastance or E) of the left ventricular developed pressure (LV_{dev}Pressure) - volume relations (shown on the previous page) in spontaneously hypertensive rats (SHR). E represents a load-independent measure of systolic chamber function. Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol.* $p<0.01$ versus other groups.

3.9 Systolic myocardial function

Figures 3.8 (upper panel) and 3.10 show the effect of chronic ISO administration to SHR on a load-dependent measure of systolic myocardial function measured *in vivo* (Figure 3.8, upper panel) and a load-independent measure of systolic myocardial function measured *ex vivo* (Figure 3.10). Myocardial systolic function assessed *in vivo* (LV FS_{mid}) was maintained in both SHR and in SHR receiving ISO as compared to WKY controls. (Figure 3.8, upper panel). Similarly, as assessed *ex vivo*, intrinsic myocardial systolic function (En in the absence of an adrenergic stimulus is shown) was unchanged in SHR at 19 months of age irrespective of whether ISO had been administered chronically or not (data obtained in the absence of an adrenergic stimulus are indicated in Figure 3.10).

Left ventricular intrinsic myocardial function (En) in 11-14 month old WKY rats receiving either ISO or the vehicle of ISO for 7 months (data not shown) was similar to that of the WKY rats shown in Figure 3.9. Importantly, ISO had no effect on LV En in WKY rats (data not shown).

3.10 Pathological score and myocardial collagen content.

Figure 3.11 shows the effects of chronic ISO administration on cardiac myocyte necrosis as assessed from a pathological score of fibrotic areas (lower panel) and myocardial collagen content as assessed from hydroxyproline concentrations (upper panel) in spontaneously hypertensive rats. Myocardial fibrosis was noted to occur in untreated SHR as compared to WKY controls and ISO administration tended to further

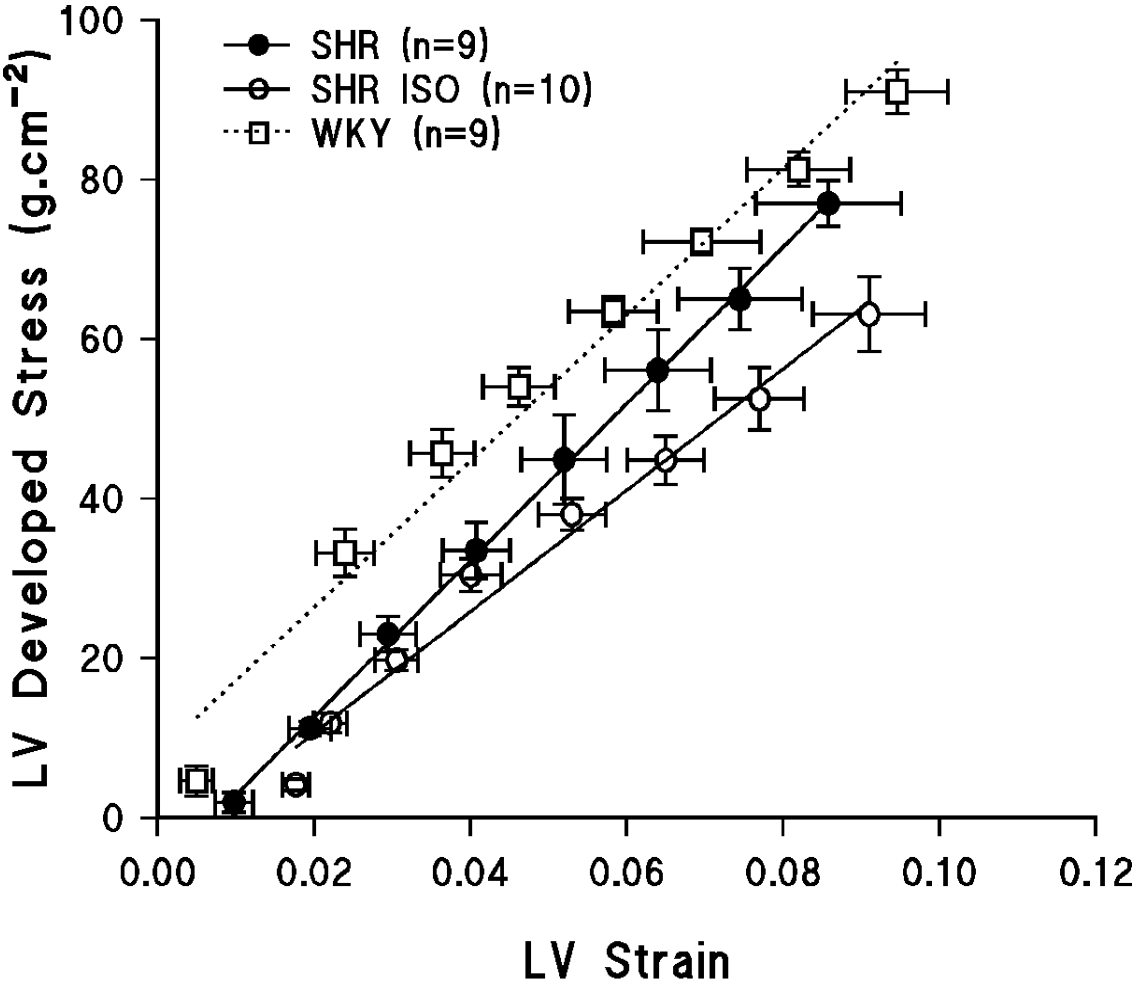

Figure 3.10 (see continuation of figure over the page). Effects of chronic isoproterenol administration (ISO) on left ventricular (LV) developed stress –strain relations in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. The mean slope of the relations (systolic myocardial elastance or En), which represents a load-independent measure of systolic myocardial function are compared over the page.

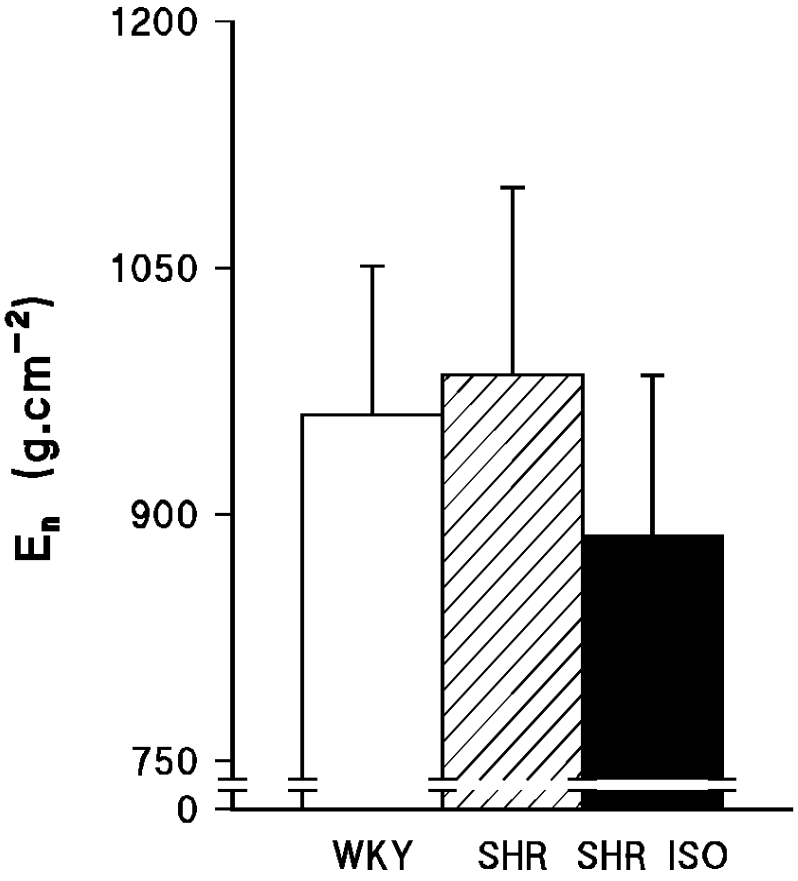

Figure 3.10 continued. Effects of chronic isoproterenol administration (ISO) on the mean slope (systolic myocardial elastance or En) of the left ventricular developed stress –strain relations (shown on the previous page) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. No differences between the groups were noted.

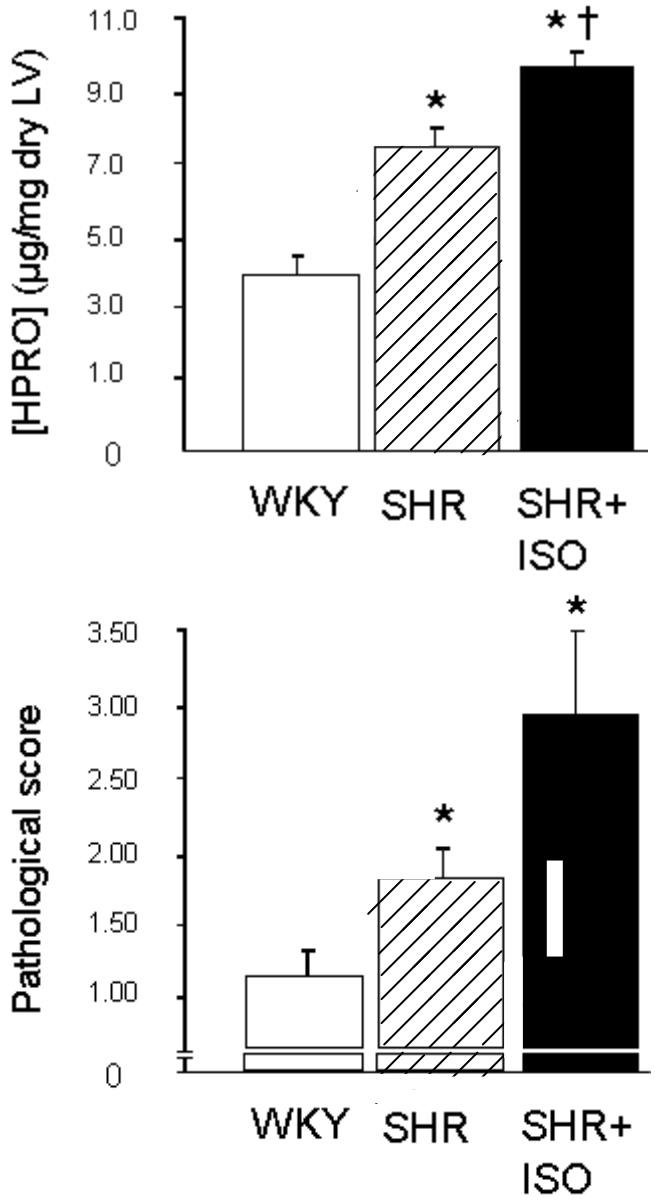

Figure 3.11. Effects of chronic isoproterenol administration (ISO) on cardiac myocyte necrosis as assessed from a pathological score of fibrotic areas (lower panel) and myocardial collagen content as assessed from hydroxyproline concentrations [HPRO] (upper panel) in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol.* $p<0.05$ versus WKY, † $p<0.01$ versus SHR. See Table 3.1 for sample sizes.

increase the degree of fibrosis, an effect that however failed to achieve statistical significance (Figure 3.11). Nevertheless, an increase in myocardial hydroxyproline concentration occurred in untreated SHR and ISO administered to SHR resulted in a further increment in myocardial hydroxyproline concentrations (Figure 3.11).

3.11 Cross-linked characteristics of myocardial collagen

Figure 3.12 shows the effect of chronic ISO administration on the cross-linked characteristics of myocardial collagen in SHR. The solubility of myocardial collagen to cyanogen bromide (CNBr) digestion was markedly reduced in SHR (Figure 3.12, lower panel) indicating an increase in the cross-linked properties of myocardial collagen. Untreated SHR therefore had a marked increase in insoluble (cross-linked collagen), but no change in soluble (non-cross-linked) myocardial collagen concentrations (Figure 3.12). However, the administration of ISO to SHR returned the percentage solubility of myocardial collagen to WKY values (Figure 3.12). Consequently, the increase in myocardial [HPRO] in SHR receiving ISO was of both the cross-linked and the non-cross-linked phenotype (Figure 3.12).

3.12 Phenotypic characteristics of myocardial collagen

Figure 3.13 shows the effects of chronic ISO administration on the phenotypic characteristics of myocardial collagen in SHR. Compared to age-matched WKY controls, SHR at 19 months of age had no change in the ratio of type I-to-III myocardial collagen

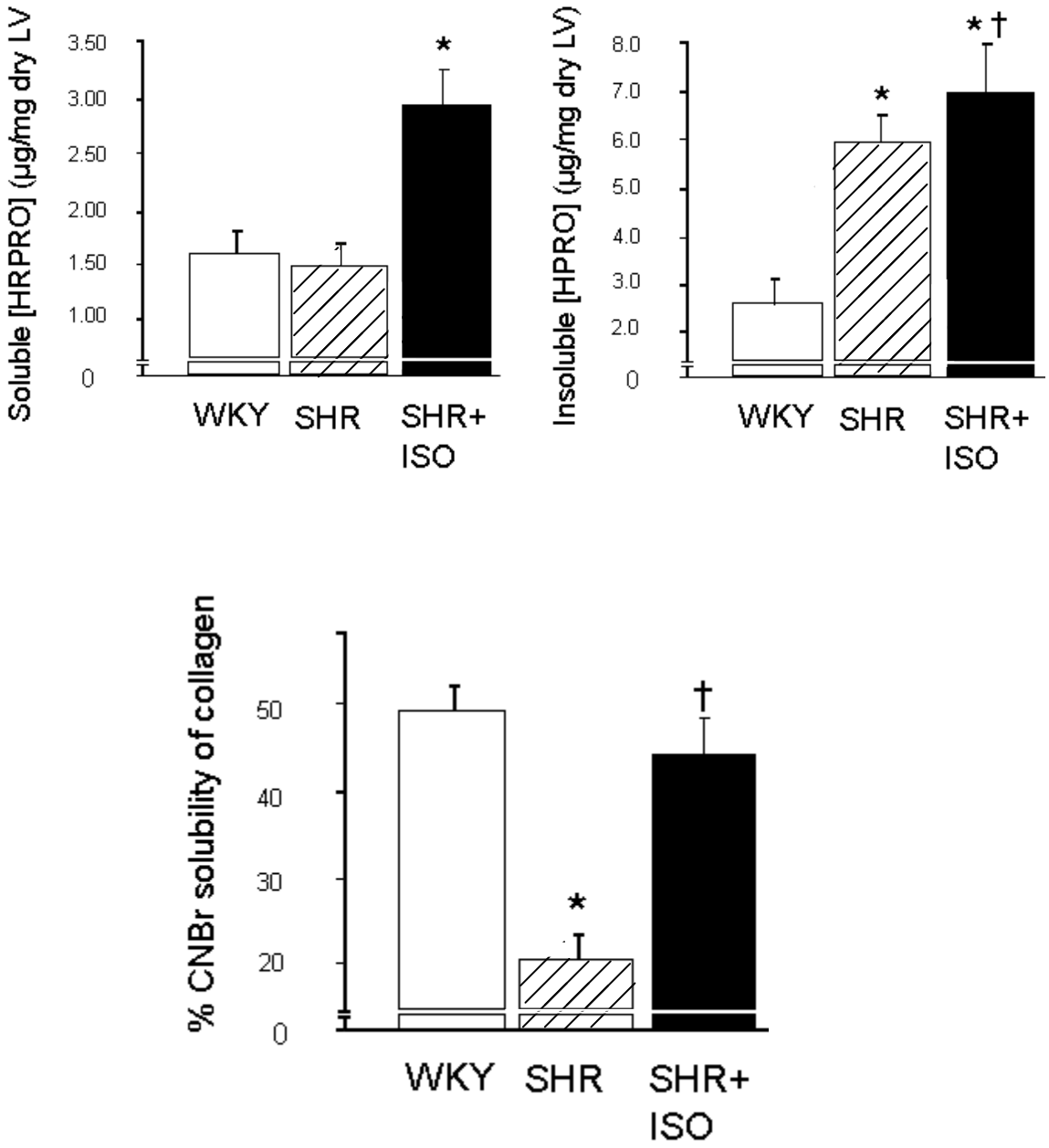

Figure 3.12. Effects of chronic isoproterenol administration (ISO) on the cross-linked properties of myocardial collagen in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol; CNBr, cyanogen bromide. * $p<0.05$ versus WKY, † $p<0.01$ versus SHR. See Table 1 for sample sizes.

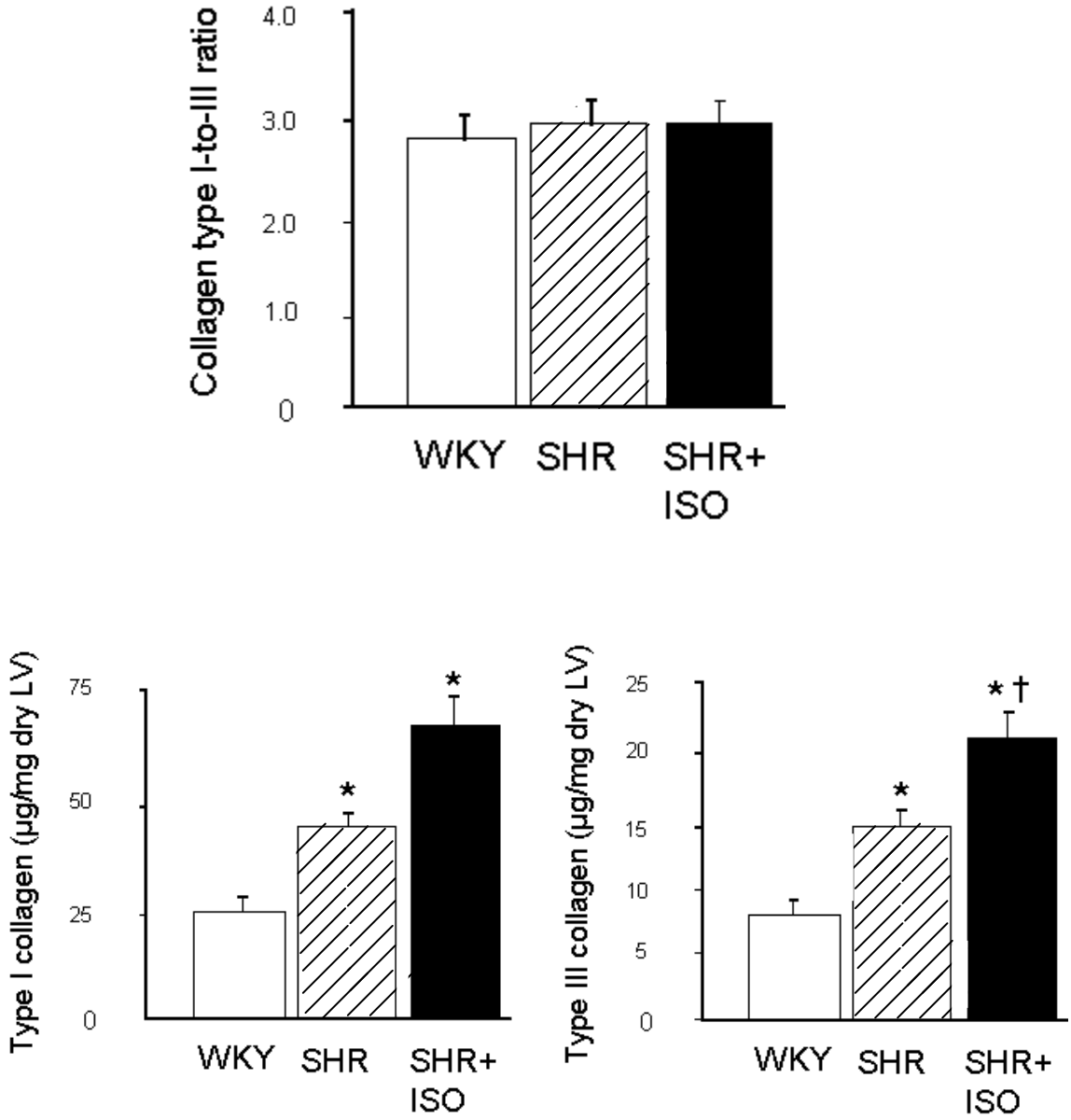

Figure 3.13. Effects of chronic isoproterenol administration (ISO) on the phenotypic characteristics (type I and type III) of myocardial collagen in spontaneously hypertensive rats (SHR). Data are compared to Wistar Kyoto control rats receiving the saline vehicle (WKY). SHR, SHR receiving the saline vehicle; SHR+ISO, SHR receiving isoproterenol. * $p<0.05$ versus WKY, † $p<0.01$ versus SHR. See Table 1 for ample sizes.

(Figure 3.13). Consequently, both type I and type III myocardial collagen concentrations were increased in SHR (Figure 3.13). Moreover, ISO failed to modify type I-to-III myocardial collagen ratios in SHR (Figure 3.13) and hence proportionately similar increases in type I and type III myocardial collagen concentrations occurred as compared to both WKY and untreated SHR (Figure 3.13).

Chapter 4

Discussion

4.1 Summary of main findings

The main findings of the present study are as follows. As previously demonstrated (Badenhorst et al 2003a), chronic administration of a β-adrenoreceptor agonist to SHR promoted an increase in left ventricular cavity dimensions as assessed *in vivo* in association with a right shift in left ventricular diastolic P-V relations as assessed *ex vivo* under controlled conditions. Although as also demonstrated by Badenhorst et al (2003a), chronic β-adrenoreceptor agonist administration to SHR resulted in the accumulation of myocardial collagen with a low tensile strength (non-cross-linked myocardial collagen concentrations), the novel finding of the present study is that this collagen change failed to modify diastolic myocardial material properties (myocardial stiffness). The increases in left ventricular diastolic cavity dimensions noted to occur after chronic β-adrenoreceptor activation were therefore attributed to adverse chamber remodeling, as confirmed by increases in the volume intercept of the left ventricular diastolic P-V relation and reductions in relative wall thickness in SHR receiving the β-adrenoreceptor agonist.

Moreover, chronic administration of a β-adrenoreceptor agonist to SHR induced pump dysfunction as determined from an afterload and heart rate-dependent, but preload independent assessment of systolic function *in vivo* (endocardial fractional shortening), as well as an afterload, preload and heart rate-independent assessment of systolic function *ex vivo* (systolic elastance-E). As previously demonstrated (Badenhorst et al 2003a), chronic administration of a β-adrenoreceptor agonist to SHR did not however result in a decrease in myocardial systolic function as assessed from an afterload and heart rate-

dependent, but preload independent assessment of myocardial systolic function *in vivo* (midwall fractional shortening), as well as an afterload, preload and heart rate-independent assessment of intrinsic myocardial systolic function *ex vivo* (systolic myocardial elastance-En). Consequently, the reduction in pump function could not be attributed to decreases in myocardial function. Rather, the effect of chronic β-adrenoreceptor activation on pump function could only be attributed to adverse structural remodelling (cardiac dilatation).

4.2 Comparison with previous studies

4.2.1 Myocardial material properties and cardiac dilatation

The present data are largely consistent with data published following the use of a left ventricular assist device in patients with idiopathic dilated cardiomyopathy (Barbone et al 2001). Whereas patients with idiopathic dilated cardiomyopathy have a right shift in left ventricular diastolic P-V relations, implantation of a left ventricular assist device produces a marked left shift in left ventricular diastolic P-V relations to control values, an alteration that is attributed to reverse remodelling and not to alterations in myocardial material properties (Barbone et al 2001). Left ventricular assist device-induced reverse remodelling therefore suggests that the right shift in left ventricular diastolic P-V relations noted to occur in dilated cardiomyopathy could be attributed to alterations in remodelling rather than to passive myocardial material properties. Importantly, however, reverse remodelling following the use of a left ventricular assist device does not modify

the markedly reduced wall thickness-to-radius ratio noted in patients with idiopathic dilated cardiomyopathy (Barbone et al 2001). It is therefore questionable whether reductions in cavity size following the use of a left ventricular assist device truly represent the 'reverse' of the mechanisms involved in producing the initial increments in cavity size noted to occur during the development of a dilated cardiomyopathy. Nevertheless, data obtained in the present study provide further evidence to indicate that the evolution of increases in cavity volume in cardiac disease involve adverse remodelling and not modifications in myocardial material properties. In the present study, what was particularly noteworthy was the fact that the right shifts in left ventricular diastolic P-V relations occurred in association with wall thinning, and thus are representative of adverse chamber remodelling. The model of increased cavity volumes studied by our group is particularly relevant as it is well recognized that chronic β-adrenoreceptor activation occurs in chronic heart failure (see section 1.2.2.1 of the introduction) and contributes to increases in cavity volumes (see section 1.2.2.3.3 of the introduction), and that β-adrenoreceptor blocking agents reduce cardiac cavity volumes in chronic heart failure (see section 1.2.2.3.3 of the introduction).

4.2.2 Collagen cross-linking and myocardial material properties

In the present study, SHR had marked increases in myocardial collagen concentrations, a change that was augmented by chronic β-adrenoreceptor activation. Increases in myocardial collagen concentrations are well recognized as being a major determinant of myocardial stiffness (Brooks et al 1997, Brilla et al 1996, Conrad et al

1995, Weber et al 1990). In this regard, it is therefore not surprising that SHR had increases in myocardial stiffness as compared to WKY. However, what does appear surprising at first glance is that chronic β-adrenoreceptor activation in SHR did not further significantly augment myocardial stiffness, despite additional increases in myocardial collagen concentrations. However, this apparent anomaly is consistent with other apparent discrepancies regarding the relationship between myocardial collagen concentrations on myocardial stiffness. Indeed, a normal myocardial stiffness (Thiedermann et al 1983) and only modest increases in myocardial stiffness (Norton et al 2002) are both associated with increments in myocardial collagen concentrations. Moreover, pharmacological-induced improvements in stiffness may occur without decreases in collagen concentrations (Norton et al 1997); and decrements in collagen concentrations do not necessarily reduce stiffness (Motz and Strauer 1989). Thus, alternative mechanisms have been sought to explain changes in the material properties of the myocardium. In this regard, our group have provided substantial evidence to indicate that the impact of myocardial collagen on myocardial material properties and stiffness is determined by the cross-linked properties of myocardial collagen (Norton et al 1997, Norton et al 1996).

Are the myocardial cross-linked collagen changes noted in the present study consistent with the hypothesis that these changes are causally related to alterations in myocardial stiffness in cardiac pathology? An enhanced myocardial collagen cross-linking is associated with an increased passive stiffness of the myocardium (Norton et al 1997). The finding in the present study that a reduced myocardial collagen solubility, which implies an increased myocardial collagen cross-linking, occurred together with an

increased diastolic myocardial stiffness in SHR, supports this notion. These data are consistent with the fact that in older SHR, although a rightward shift in left ventricular diastolic P-V relations is noted, myocardial stiffness is maintained at a considerably higher level than normotensive controls, despite the accumulation of myocardial collagen in the non-cross-linked form (Badenhorst et al 2003b, Tsotetsi et al 2001). Our group have previously attributed the increased myocardial stiffness in SHR to an initial increase in myocardial collagen of the cross-linked form, a change which occurs well before the onset of heart failure (Badenhorst et al 2003b, Norton et al 1997), but which contributes toward diastolic dysfunction even as SHR progress to heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001). Similarly, in the present study, SHR not receiving the β-adrenoreceptor agonist had a marked increase in myocardial collagen concentrations of the cross-linked form. Moreover, in SHR receiving the β-adrenoreceptor agonist, although further increases in myocardial collagen concentrations of the non-cross-linked form occurred, the increases in cross-linked myocardial collagen concentrations that characterize SHR of this age were sustained. Hence, not surprisingly myocardial stiffness was maintained in SHR receiving the β-adrenoreceptor agonist as the amplified quantity of cross-linked collagen that purportedly mediates an increased myocardial stiffness was preserved. However, SHR receiving the β-adrenoreceptor agonist did not have a further increase in myocardial stiffness as compared to untreated SHR, as the β-adrenoreceptor agonist promoted further increases in non-cross-linked, rather than cross-linked myocardial collagen.

4.2.3 Myocardial collagen phenotypes and myocardial stiffness

In the present study, alternative changes in the qualitative characteristics of myocardial collagen mediated by chronic β-adrenoreceptor agonist administration could have offset the impact of an accumulation of myocardial collagen in the non-cross-linked form on myocardial stiffness. In this regard, myocardial collagen type I-to-III ratios have previously been suggested to influence myocardial stiffness in SHR (Mukherjee and Sen 1993). However, in the present study myocardial collagen type I-to-III ratios were unchanged in SHR. Moreover, chronic β-adrenoreceptor agonist administration to SHR failed to modify the markedly greater quantity of the stiffer collagen phenotype, namely type I collagen, a change that characterizes SHR. The unchanged myocardial collagen type I-to-III ratio in 19 month old SHR in the present study is not contrary to what has previously been demonstrated (Mukherjee and Sen 1993). Indeed, both our group (Tsotetsi et al 2001) and others (Mukherjee and Sen 1993) have shown that the increased type I-to-III myocardial collagen ratios are attenuated in the aging SHR.

4.2.4 Intrinsic myocardial systolic function as a determinant of pump function.

In the present study, although intrinsic myocardial systolic function of the left ventricle as determined both *in vivo* (FS_{mid})(load-dependent) and *ex vivo* (E_n)(load-independent) was maintained, left ventricular pump dysfunction was noted both *in vivo* (FS_{end})(load-dependent) and *ex vivo* (E)(load-independent) in SHR exposed to chronic β-adrenoreceptor activation. Thus, as indicated above, the reduction in left ventricular

systolic (pump) function was attributed to adverse chamber remodelling rather than to decreases in intrinsic myocardial function. These data support the concept originally proposed by Cohn (1995), and subsequently substantiated by data obtained in human (Vasan et al 1997) and animal (Norton et al 2002, Badenhorst et al 2003a) studies, that pump dysfunction can occur mainly as a consequence of cardiac dilatation rather myocardial contractile disturbances. The present study also supports the notion that chronic β-adrenoreceptor activation in hypertensive LVH can mediate cardiac dilatation primarily through direct effects rather than effects secondary to alterations in intrinsic myocardial dysfunction and subsequent increases in left ventricular preloads (Badenhorst et al 2003a). Although previous data have indicated that non-necrotic doses of a β-adrenoreceptor agonist given to normotensive rats are able to produce left ventricular dilatation and pump dysfunction (Woodiwiss et al 2001), no distinction was made in this study between myocardial and geometric effects on pump abnormalities. Hence, the present study together with a previous study conducted in younger SHR (Badenhorst et al 2003a), provide the first evidence to indicate that pump dysfunction following chronic β-adrenoreceptor activation occurs principally through adverse chamber remodelling rather than abnormalities in intrinsic myocardial function. Moreover, recent data obtained by members of our group, where the impact of chronic β-adrenoreceptor activation on left ventricular structure and function in normotensive Sprague Dawley rats was studied, essentially confirm the notion that pump dysfunction can occur as a consequence of dilatation and not the abnormalities of intrinsic myocardial contractile disturbances (Osadchii et al 2007).

The factors that maintain intrinsic myocardial contractile function following chronic β-adrenoreceptor activation, despite the well recognized sympathetic-induced decreased β-adrenoreceptor contractile responsiveness and cardiomyocyte apoptosis, have recently been demonstrated by our group (Osadchii et al 2007). In this regard, members of our groups have demonstrated that contractile function could be maintained under these circumstances largely because of increases in myocardial norepinephrine release and α-AR-mediated inotropic up-regulation counter-balancing the negative inotropic actions of apoptosis and β-AR down-regulation (Osadchii et al 2007).

4.2.5 Relevance of the present model studied to the transition to heart failure in pressure overload hypertrophy.

As the induction of pump dysfunction in SHR in this study was produced by chronic exogenous administration of a β-adrenoreceptor agonist, the question arises as to whether this has relevance to the transition from compensated LVH to pump dysfunction in hypertensive hypertrophy? In this regard there is now substantial evidence to indicate that chronic β-adrenoreceptor activation promotes the transition to heart failure. This evidence has been reviewed in chapter 1, but is worth summarising the main evidence at this point. First, increased myocardial noradrenaline concentrations occur in the coronary effluent in hypertensive LVH prior to the development of heart failure (Agabiti-Rosei et al 1987, Kelm et al 1996, Schlaich et al 2003, Veliotes et al 2005). Second, transgenic animal models with decreased adrenergic activation are protected against the development of cardiac dilatation, pump dysfunction and heart failure when the left ventricle is exposed

to a pressure-overload (Esposito et al 2002). Third, blockade of β-adrenoreceptors prevents the transition from cardiac hypertrophy to heart failure in SHR independent of blood pressure effects (Chan et al 2004). Thus, it is entirely feasible that chronic β-adrenoreceptor activation promotes the transition to pump dysfunction in pressure overload hypertrophy. Consequently, it is not unreasonable to assume that the present model is an approximation of a major pathophysiological mechanism responsible for the transition to heart failure in pressure overload hypertrophy, namely β-adrenoreceptor over-activation.

With respect to the relevance of the present model studied, it is also important to consider whether the impact of chronic β-adrenoreceptor activation in SHR produces similar cardiac and myocardial collagen changes as those noted in SHR in heart failure. In this regard our group have recently characterised the cardiac diastolic functional and myocardial collagen changes in SHR as these animals progress to heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001). Our group have shown that the cardiac diastolic functional changes and myocardial collagen changes noted after chronic β-adrenoreceptor activation in SHR are remarkably similar to the cardiac and myocardial collagen changes noted in ~26 month old SHR in heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001). Importantly, the myocardial collagen changes following chronic β-adrenoreceptor activation described in the present dissertation are also reminiscent of those noted in SHR in heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001). That is, SHR in heart failure develop a dilated left ventricle with wall thinning, a preferential accumulation of myocardial collagen of the non-cross-linked subtype (despite

maintaining increases in collagen of the cross-linked subtype), and an accumulation of both type I and type III myocardial collagen (Badenhorst et al 2003b, Tsotetsi et al 2001).

Although the myocardial collagen changes and the chamber remodelling changes produced by chronic β-adrenoreceptor activation in SHR are very similar to those changes noted in SHR when they develop heart failure (Badenhorst et al 2003b, Tsotetsi et al 2001), the impact of chronic β-adrenoreceptor activation and heart failure in SHR on intrinsic contractile function are different. In contrast to the lack of change in contractile function noted in SHR following chronic β-adrenoreceptor activation in the present and a previous study (Badenhorst et al 2003a), SHR in heart failure have a reduced contractile function as determined from isolated papillary muscle strips (Brooks et al 1997, Bing et al 1995). Whether the apparent discrepancies in these data (Brooks et al 1997, Bing et al 1995) and the present studies data are because chronic β-adrenoreceptor activation does not contribute toward contractile dysfunction in SHR, or because of the differences in measurement techniques used in the present and a previous (Brooks et al 1997, Bing et al 1995) study, requires further investigation. Importantly, as SHR develop cardiac dilatation, a reduced papillary muscle contractile function (Brooks et al 1997, Bing et al 1995) could occur through abnormal loads placed on the mitral valve in a dilated heart rather than through hypertensive effects.

4.3 The potential clinical importance of the novel findings of the present study.

What is the clinical relevance of discovering that chronic β-adrenoreceptor activation in hypertensive LVH promotes right shifts in left ventricular diastolic P-V

relations principally through adverse remodelling rather than through changes in myocardial material properties? The obvious clinical relevance is that therapeutically targeting myocardial material properties is unlikely to benefit patients with adrenergic-induced cardiac dilatation and pump dysfunction. However, there are other less obvious clinical implications that should be considered.

Importantly, heart failure in hypertension may be the outcome of a leftward shift in left ventricular diastolic P-V relations following both concentric left ventricular remodelling and a stiffer myocardium (Frohlich et al 1992). In the presence of a preserved left ventricular pump function this is considered to be a cause of diastolic heart failure (see chapter 1). Thus, it may be argued that any rightward shift in the left ventricular diastolic P-V relationship in a hypertensive heart could be beneficial at least to a patient with diastolic heart failure. However, as clearly demonstrated in the present study, pump function is reduced following chronic β-adrenoreceptor activation, an effect attributed to adverse chamber remodelling (indexed by a rightward shift in the diastolic P-V relation) and not to intrinsic myocardial dysfunction. Therefore, in therapeutically targeting the rightward shift in the left ventricular diastolic P-V relationship one has to weigh up the consequences of improving pump function against that of promoting diastolic dysfunction and potentially diastolic heart failure.

The fact that I have been unable to demonstrate a key role for myocardial material properties in contributing toward cardiac dilatation following chronic β-adrenoreceptor activation in SHR, does not exclude a role for decreases in myocardial collagen cross-linking as a principal determinant of adverse structural remodelling. Myocardial collagen cross-linking produces effects on myocardial stiffness and cardiac dilatation through

separate mechanisms. Indeed, an increase in cross-linking increases myocardial stiffness by changing the tensile strength of myocardial collagen (Norton et al 1997, Norton et al 1996). However, a decrease in collagen cross-linking increases the susceptibility of myocardial collagen to MMP degradation, a change that will promote cardiomyocyte side-to-side slippage and hence cardiac dilatation. Further work is required to test the hypothesis that modifications in myocardial cross-linking determine the diastolic properties of the left ventricle. In this regard targeting genes that influence myocardial collagen cross-linking may be a method of testing this hypothesis, but this approach is only likely to be effective if gene activation or inhibition can be induced in adult rats and under controlled conditions.

4.4 Strengths and limitations of the present study

As with any study a number of strengths and weaknesses of the present study need to be underscored.

4.4.1 Measurement considerations

In the present study LV remodelling and function were assessed using two approaches each with its own strengths and limitations. Cardiac dilatation and pump dysfunction were detected using echocardiography in anaesthetised rats and from measurements made in isolated, perfused heart preparations. The value of echocardiography is that dimensions and function can be determined in an emptying and

filling ventricle, in the presence of intact neurohumoral systems, and in blood perfused hearts. The limitations of the use of echocardiography are however numerous and include the fact that an anaesthetic is required, that afterload, heart rate and coronary flow are not controlled for, and that measurements are made in a single axis of the heart. The value of isolated, perfused heart measurements are that heart rate, afterload, preload and coronary flow are controlled for, left ventricular volume measurements are made using direct techniques, and the effect of anaesthesia is eliminated. The limitations of the use of isolated, perfused heart preparations are however numerous and include the fact that measurements are made under unphysiological conditions in a preparation that is not filling or emptying. Despite the varying strengths and weaknesses of each approach, the fact that the same outcomes were reproduced using two techniques each with separate strengths and weaknesses provides substantial support for the notion that the data are indeed reliable.

4.4.2 Considerations of study design

Although in the present study, for logistic rather than academic reasons, I could not include a perfectly age-matched (12 months of age) WKY group receiving isoproterenol in the formal study, I nevertheless performed a parallel study in WKY rats of a similar age range (11-14 months), where I assessed the effect of isoproterenol. In this parallel study, as previously reported on in younger WKY rats (Badenhorst et al 2003a), I demonstrated that chronic adrenergic activation failed to promote cardiac dilatation in older WKY rats. Moreover, as with SHR in the present study, I also showed that chronic

adrenergic activation was unable to induce alterations in myocardial material properties (myocardial *k*) in WKY rats. Thus, the effect of chronic adrenergic activation on LV diastolic properties in SHR, appears to be due to an increased sensitivity of the myocardium of SHR to the adverse effects of chronic adrenergic activation.

A potential limitation of the present study is that I did not assess myocardial stiffness in SHR at various time intervals during the administration of the β-adrenoreceptor agonist. It may be argued that myocardial stiffness changes are the early modifications that mediate right shifts in left ventricular diastolic P-V relations following chronic β-adrenoreceptor activation, and that these alterations ultimately translate into adverse geometric remodeling. However, there are no data to support this supposition. Furthermore, the myocardial collagen changes that could have mediated a reduced myocardial stiffness (increased myocardial collagen of the non-cross-linked form) following chronic β-adrenoreceptor activation were clearly evident in the present study. Consequently in the present study it is reasonable to have expected to identify material property changes at the time that these were measured.

4.4.3 Considerations of data interpretation

In the present study, as with other studies that we have conducted (Osadchii et al 2007, Badenhorst et al 2003a), the left ventricular diastolic P-V relationship was largely linear, as opposed to exponential relations normally reported on in blood perfused hearts (Norton et al 1997). Linear diastolic P-V relations may occur in ischaemic/hypoxic hearts or over-perfused hearts when oedema occurs. Although I cannot exclude a degree of

hypoxia mediated by the use of a crystalloid rather than a blood perfusate, or the possibility that demand-induced ischaemia is not present at higher than normal filling volumes, I can certainly exclude flow-induced ischaemia, demand-induced ischaemia mediated by high pacing rates and the presence of oedema. The following outlines these arguments.

In our laboratory we have established the flow rate at which the myocardium achieves maximal left ventricular developed pressures. Increasing flow rates above that used in the present study has no impact on left ventricular developed pressures. Hence, flow-induced ischaemia is an unlikely explanation for the linear diastolic P-V relations. With respect to demand-induced ischaemia I have already outlined the approach adopted by members of our laboratory to avoid demand-induced ischaemia with respect to pacing rates (see page 50). However, I cannot exclude the possibility that at high filling volumes, where wall tension will be high, demand-induced ischaemia isn't a possibility. Lastly, no differences in the ratio of dry-to-wet left ventricular weights were noted between hearts studied in this dissertation and those ratios observed in hearts which have not been perfused with a crystalloid solution (Norton et al 1997). Hence oedema is an unlikely explanation for the linear P-V relationship.

What then could explain the linear P-V relationship shown in this dissertation? Although the characteristics of the cardiac balloon used in these studies was appropriate in that pressures only started increasing in the balloon well beyond maximal filling volumes of any single heart tested, I cannot exclude a possibility that interactions between the balloon and the myocardial wall contributed toward the diastolic P-V relations. Hence, it is possible that the characteristics of the balloon possibly contributed

toward the linear diastolic P-V relations. Second, in isovolumic heart preparations, to avoid the impact of active properties of the heart influencing diastolic P-V relations, these relations are normally constructed in K^+ arrested hearts. However, this would not have allowed me to subsequently assess left ventricular systolic function in the presence of an adrenergic stimulus. Consequently, I could not assess left ventricular diastolic P-V relations in arrested hearts. Moreover, our group have shown that left ventricular diastolic P-V relations in K^+ arrested hearts are very different from end diastolic P-V relations obtained in filling and emptying hearts (Norton and Woodiwiss, personal communications). Hence, the value of diastolic P-V relations obtained in K^+ arrested hearts is questionable.

4.5 Conclusions

In conclusion, the data provided in the present study indicate that despite inducing the accumulation of myocardial collagen with a low tensile strength (non-cross-linked collagen), chronic β-adrenoreceptor activation mediates right shifts in left ventricular diastolic P-V relations with subsequent pump dysfunction, through adverse chamber remodeling rather than through alterations in myocardial material properties. Consequently, although these data do not exclude a role for non-cross-linked collagen in contributing toward β-adrenoreceptor-mediated cardiac dilatation and pump dysfunction, these results indicate that therapeutically targeting myocardial material properties is unlikely to benefit patients with adrenergic-induced cardiac dilatation and pump dysfunction.

REFERENCES

Agabiti-Rosei E, Muiesan ML, Romanelli G, Castellano M, Beschi M, Alicandri C and Muiesan G. Cardiac function as related to adrenergic activity in hypertensive left ventricular hypertrophy. *J Clin Hypertens.* 1987; 3: 203-210

Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G and Cohn JN. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). *Circulation*. 2003; 107: 1278-1283.

Anand IS, Liu D, Chugh SS, Prahash AJ, Gupta S, John R, Popescu F and Chandrashekhar Y. Isolated myocyte contractile function is normal in postinfarct remodeled rat heart with systolic dysfunction. *Circulation*. 1997; 96: 3974-3984.

Badenhorst D, Veliotes D, Maseko M., Tsotetsi O.J., Brooksbank R., Naidoo A., Woodiwiss A.J., Norton G.R. β-adrenergic activation initiates chamber dilatation in concentric hypertrophy. *Hypertension*. 2003a; 41: 499-504.

Badenhorst D, Maseko M, Tsotetsi OJ, Naidoo A, Brooksbank R, Norton GR, Woodiwiss AJ. Cross-linking influences the impact of quantitative changes in myocardial collagen on cardiac stiffness and remodelling in hypertension in rats. *Cardiovascular Research*. 2003b; 57: 632-641.

Barbone A, Oz MC, Burkhoff D, Holmes JW. Normalized diastolic properties after left ventricular assist result from reverse remodeling of chamber geometry. *Circulation*. 2001; 104 [suppl I]: I 229-I 232.

Beltrami CA, Finato N, Rocco M, Feruglio GA, Puricelli C, Cigola E, Sonnenblick EH, Olivetti G and Anversa P. The cellular basis of dilated cardiomyopathy in humans. *J Mol Cell Cardiol*. 1995; 27: 291-305.

Bing OH, Brooks WW, Robinson KG, Slawsky MT, Hayes JA, Litwin SE, Sen S, Conrad CH. The spontaneously hypertensive rat as a model of the transition from compensated left ventricular hypertrophy to failure. *J Mol Cell Cardiol*. 1995; 27: 383-396.

Böhm M, Gräbel C, Knorr A and Erdmann E. Treatment in hypertensive cardiac hypertrophy, I: Neuropeptide Y and ß-adrenoceptors. *Hypertension.* 1995; 25: 954-961.

Böhm M, Castellano M, Paul M and Erdmann E. Cardiac norepinephrine, ß-adrenoceptors, and Giα-proteins in prehypertensive and hypertensive spontaneously hypertensive rats. *J Cardiovasc Pharmacol.* 1994; 23: 980-987.

Böhm M, Gierschik P, Jakobs KH, Pieske B, Schnabel P, Ungerer M and Erdmann E. Increase of G_{ia} in human hearts with dilated but not ischemic cardiomyopathy. *Circulation*. 1990; 82: 1249-1265.

Bongers FJ, Schellevis FG, Bakx C,van den Bosch WJ, van der Zee J. Treatment of heart failure in Dutch general practice. *BMC Family Practice.* 2006;7:40

Brilla CG, Matsubara L, Weber KT. Advanced hypertensive heart disease in spontaneously hypertensive rats. Lisinopril-mediated regression of myocardial fibrosis. *Hypertension.* 1996; 28: 269-275.

Bristow MR. Beta-adrenergic receptor blockade in chronic heart failure. *Circulation.* 2000; 101: 558-569.

Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, Zera P, Menlove R, Shah P and Jamieson S. ß$_1$- and ß$_2$-adrenergic receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective ß$_1$-receptor down-regulation in heart failure. Circ Res. 1986; 59: 297-309.

Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K, Billingham ME, Harrison DC and Stinson EB. Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. *N Engl J Med*. 1982; 307: 205-211.

Brodde OE. ß$_1$- and ß$_2$-adrenoceptors in the human heart: Properties, function and alterations in chronic heart failure. *Pharmacol Rev*. 1991; 43: 203-242.

Brodde OE, Zerkkowski HR, Gorst HG, Maier W and Michel MC. Drug- and diseased-induced changes of human cardiac ß$_1$- and ß$_2$-adrenoceptors. *Eur Heart J*. 1989; 10: 38-44.

Brodde OE, Kretsch R, Ikezono K, Zerkowski HR and Reidemeister JC. Human ß-adrenoceptors: Relation of myocardial and lymphocyte ß-adrenoceptor density. *Science*. 1986; 231: 1584-1585.

Brooks WW, Bing OHL, Robinson KG, Slawsky MT, Chaletsky DM, Conrad CH. Effect of angiotensin-converting enzyme inhibition on myocardial fibrosis and function in hypertrophied and failing myocardium from the spontaneously hypertensive rat. *Circulation*. 1997; 96: 4002-4010.

Capasso JM, Robinson TF and Anversa P. Alterations in collagen cross-linking impair myocardial contractility in the mouse heart. *Circ Res.* 1989; 65: 1657-1664.

Casale PN, Devereux RB, Milner M, Zullo G, Harshfield GA, Pickering TG, Laragh JH. Value of echocardiographic measurement of left ventricular mass in predicting cardiovascular morbid events in hypertensive men. *Ann Intern Med.* 1986;105:173-178.

Castellano M, Beschi M, Rizzoni D, Paul M, Böhm M, Mantero G, Bettoni G, Porteri E, Albertini A and Agabiti-Rosei E. Gene expression of cardiac beta$_1$-adrenergic receptors during the development of hypertension in spontaneously hypertensive rats. *J Hypertens.* 1993; 11: 787-791.

Chan V, Wilson K, Burstow D, Hoey A and Brown L. Chronic ß-adrenoceptor blockade prevents progression of heart failure in ageing SHR. *J Mol Cell Cardiol*. 2004; 37: 321. Abstract

Chung ES, Perlini S, Aurigemma GP, Fenton RA, Dobson JG, Meyer TE. Effects of chronic adenosine uptake blockade on adrenergic responsiveness and left ventricular chamber function in pressure overload hypertrophy in the rat. *J Hypertens*. 1998; 16: 1813-1822.

CIBIS-II. Investigators and Committees. The cardiac insufficiency bisoprolol study II (CIBIS-II): a randomized trial. *Lancet.* 1999; 353: 9-13

Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol*. 2000; 35: 569-582.

Cohn JN, Levine TB, Olivari MT, Carberg V Lara D, Francis CS, Simon AB & Rector T. Plasma norepinephrine as a guide to prognosis in patients with chronic congestive heart failure. *N Engl J Med*.1984; 311: 819-823.

Cohn JN. Structural basis for heart failure: Ventricular remodeling and its pharmacological inhibition. *Circulation*. 1995; 91: 2504-2507.

Coker ML, Jolly JR, Joffs C, Etoh T, Holder JR, Bond BR and Spinale FG. Matrix metalloproteinase expression and activity in isolated myocytes after neurohormonal stimulation. *Am J Physiol*. 2001; 281: H543-H551

Colucci WS, Sawyer DB, Singh K and Communal C. Adrenergic overload and apoptosis in heart failure: implications for therapy. *J Card Fail.* 2000; 6: 1-7.

Communal C, Singh K, Sawyer DB and Colucci WS. Opposing effects of beta(1) and beta(2)-adrenergic receptors on cardiac myocyte apoptosis: role of a pertussis-toxin sensitive G protein. *Circulation*. 1999; 100: 2210-2212.

Communal C, Singh K, Pimentel DR, Colucci WS. Norepinephrine stimulates apoptosis in adult rat ventricular myocytes by activation of β-adrenergic pathway. *Circulation*. 1998; 98: 1329-1334.

Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation*. 1995; 91: 161-170.

Davila DF, Donis JH, Bellabarba G, Torres A, Casado J and Mazzei de Davila C. Cardiac afferents and neurohormonal activation in congestive heart failure. *Med Hypotheses.* 2000; 54: 242-253.

de Kam PJ, Nicolosi GL, Voors AA, van den Berg MP, Brouwer J, van Veldhuisen DJ, Barlera S, Maggioni AP, Giannuzzi P, Temporelli PL, Latini R and van Gilst WH. Prediction of 6 months left ventricular dilatation after myocardial infarction in relation to

cardiac morbidity and mortality. Application of a new dilatation model to GISSI-3 data. *Eur Heart J*. 2002; 23: 536-542.

Devereux RB, Wachtell K, Gerdts E, Boman K, Nieminen MS, Papademetriou V, Rokkedal J, Harris K, Aurup P, Dahlof B. Prognostic significance of left ventricular mass change during treatment of hypertension. *JAMA.* 2004;19:2350-2356.

Doughty RN, Whalley GA, Gamble G, MacMahon S and Sharpe N. Left ventricular remodeling with carvedilol in patients with congestive heart failure due to ischemic heart disease. Australia-New Zealand Heart Failure Research Collaborative Group. *J Am Coll Cardiol*. 1997; 29: 1060-1066

Douglas PS, Morrow R, Loli A, Reichek N. Left ventricular shape, afterload and survival in idiopathic dilated cardiomyopathy. *J Am Coll Cardiol*. 1989;13:311-315.

Drazner MH, Rame JE, Marino EK, Gottdiener JS, Kitzman DW, Gardin JM, Manolio TA, Dries DL, Siscovick DS. Increased left ventricular mass is a risk factor for the development of a depressed left ventricular ejection fraction within five years. *Hypertension*. 2004 ;43(12):2207-15.

Ertl G, Gaudron P, Eilles C, Schorb W and Kochsiek K. Compensatory mechanisms for cardiac dysfunction in myocardial infarction. *Basic Res Cardiol.* 1991; 86: 159-165.

Eschenhagen T, Mende U, Nose M, Schmitz W, Scholz H, Haverich A, Hirt S, Doring V, Kalmar P, Hoppner W and Seitz HJ. Increased messenger RNA level of the inhibitory G-protein alpha-subunit G_{ia2} in human end-stage heart failure. *Circ Res*. 1992; 70: 688-696.

Esler M, Kaye D, Lambert G, Esler D and Jennings G. Adrenergic nervous system in heart failure. *Am J Cardiol*. 1997; 80: 7L-14L.

Esposito G, Rapacciuolo A, Naga Prasad SV, Takaoka H, Thomas SA, Koch WJ and Rockman HA. Genetic alterations that inhibit in vivo pressure overload hypertrophy prevent cardiac dysfunction despite increased wall stress. *Circulation.* 2002; 105: 85-92.

Feldman AM, Cates AE, Veazey WB, Hershberger RE, Bristow MR, Baughman KL, Baumgartner WA and van Dop C. Increase of the 40,000-mol wt pertussis toxin substrate (G protein) in the failing human heart. *J Clin Invest*. 1988; 82: 189-197.

Fielitz J, Hein S, Mitrovic V, Pregla R, Zurbrugg HR, Warnecke C, Schaper J, Fleck E, Regitz-Zagrosek V. Activity of the cardiac renin-angiotensin system and increasing myocardial collagen expression in human aortic valve disease. *J Am Coll Cardiol*. 2001;37:1443-1449

Flather MD, Shibata MC, Coats AJ, van Veldhuisen DJ, Parkhomenko A, Borbola J, Cohen-Solal A, Dumitrascu D, Ferrari R, Lechat P, Soler-Soler J, Tavazzi L, Spinarova L, Toman J, Bohm M, Anker SD, Thompson SG and Pool-Wilson PA. Randomized trial to determine the effect of nebivolol on mortality and cardiovascular hospital admission in elderly patients with heart failure (SENIORS). *Eur Heart J*. 2005; 26: 215-225

Foley RN, Parfrey PS, Harnett JD, Kent GM, Murray DC and Barre PE. The prognostic importance of left ventricular geometry in uremic cardiomyopathy. *J Am Soc Nephrol*. 1995; 5: 2024-2031

Francis GS, Cohn JN, Johnson G, Rector TS, Goldman S and Simon A. Plasma norepinephrine, plasma renin activity and congestive heart failure. Relations to survival and the effects of therapy in V-HeFT II. *Circulation*. 1993; 87: S40-S48.

Frohlich ED, Apstein C, Chobanian AV, Devereux RB, Dustan HP, Dzau V, Fauad-Tarazi F, Horan MJ, Marcus M, Massie B, Pfeffer MA, Re RN, Roccella EJ, Savage D, Shub C. The heart in hypertension. *N Engl J Med*. 1992; 327: 998-1006.

Gadsboll N, Hoilund-Carlsen PF, Badsberg JH, Marving J, Lonborg-Jensen H and Jensen B. Left ventricular volumes in the recovery phase after myocardial infarction: relation to infarct location, left ventricular function and one-year cardiac mortality. *Eur Heart J*. 1990; 11: 791-799

Gardin JM, McClelland R, Kitzman D, Lima JAC, Bommer W, Klopfenstein HS, Wong ND, Smith VE, Gottdiener J. M-mode echocardiographic predictors of six-to seven-year incidence of coronary heart disease, stroke, congestive heart failure, and mortality in an elderly cohort (The Cardiovascular Health Study). *Am J Cardiol*.2001;87:1051-1057.

Gaudron P, Eilles C, Kugler I and Ertl G. Progressive left ventricular dysfunction and remodeling after myocardial infarction. Potential mechanisms and early predictors. *Circulation*. 1993; 87: 755-763.

Gerdes AM and Capasso JM. Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure. *J Mol Cell Cardiol*. 1995; 27: 849-856.

Gerdes AM, Kellerman SE, Moore JA, Muffly KE, Clark LC, Reaves PY, Malec KB, McKeown PP and Schocken DD. Structural remodeling of cardiac myocytes in patients with ischemic cardiomyopathy. *Circulation*. 1992; 86: 426-430.

Gerson MC, Craft LL, McGuire N, Suresh DP, Abraham WT and Wagoner LE. Carvedilol improves left ventricular function in heart failure patients with idiopathic dilated cardiomyopathy and a wide range of sympathetic nervous system function as measured by iodine 123 metaiodobenzylguanidine. *J Nucl Cardiol.* 2002; 9: 608-615.

Ghali JK, Liao Y, Cooper RS. Influence of left ventricular geometric patterns on prognosis in patients with or without coronary artery disease. *J Am Coll Cardiol*. 1998;31:1635-1640.

Gilbert JC, Glantz SA. Determinants of left ventricular filling and the diastolic pressure-volume relation. *Circ Res*. 1989; 64: 827-852.

Grossman W, Jones D, McLaurin LP. Wall stress and patterns of hypertrophy in the human left ventricle. *J Clin Inves*. 1976; 56: 56-64.

Gunasinghe S, Ikonomidis J and Spinale F. Contributory role of matrix metalloproteinases in cardiovascular remodeling. *Curr Drug Targets Cardiovasc Haematol Disorders*. 2001; 1: 75-91.

Gunja-Smith Z, Morales AR, Romanelli R and Woessner JF, Jr. Remodeling of human myocardial collagen in idiopathic dilated cardiomyopathy. Role of metalloproteinases and pyridinoline cross-links. *Am J Pathology*. 1996; 148: 1639-1648.

Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA and Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: evidence of increased overall and cardiorenal sympathethic nervous activity. *Circulation*. 1986; 73: 615-621

Hein S, Arnon E, Kostin S, Schonburb M, Elsasser A, Polyakova V, Bauer EP, Klovekorn WP, Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart : structural deterioration and compensatory mechanisms. *Circulation.* 2003;107:984-991

Hobbs RE. Guidelines for the diagnosis and management of heart failure. *Am J Therapeutics*. 2004; 11: 467-472.

Hunt SA, Baker DW, Chin MH, Cinquegrani MP, Feldman AM, Francis GS, et al. ACC/AHA guidelines for the evaluation and management of chronic heart failure in the adult: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1995 Guidelines for the Evaluation and Management of Heart Failure. *Circulation*. 2001; 104: 2996-3007.

Iomoto DS, Covell JW, Harper E. Increase in cross-linking of type I and III collagens associated with volume-overload hypertrophy. *Circ Res*. 1988; 63:399-408.

Kaye DM, Lefkovits J, Jennings GL, Bergin P, Broughton A and Esler MD. Adverse consequences of high sympathetic nervous activity in the failing human heart. *J Am Coll Cardiol*. 1995; 26: 1257-1263.

Kelm M, Schafer S, Mingers S, Heydthausen M, Vogt M, Motz W and Strauer BE. Left ventricular mass is linked to cardiac noradrenaline in normotensive and hypertensive patients. *J Hypertens.* 1996; 14: 1365-1367.

King MK, Coker ML, Goldberg A, McElmurray JH, 3rd, Gunasinghe HR, Mukherjee R, Zile MR, O'Neill TP and Spinale FG. Selective matrix metalloproteinase inhibition with developing heart failure: effects on left ventricular function and structure. *Circ Res*. 2003; 92: 177-185.

Kluger J, Cody RJ and Laragh JH. The contributions of sympathetic tone and the renin-angiotensin system to severe chronic congestive heart failure: response to specific inhibitors (prazosin and captopril). *Am J Cardiol*. 1982; 49: 1667-1674.

Koren MJ, Devereux RB, Casale PN, Savage DD, Laragh JH. Relation of left ventricular mass and geometry to morbidity and mortality in men and women with essential hypertension. *Ann Intern Med*. 1991;114:345-352.

Laskey WK, St. John Sutton M, Zeevi G, Hirshfield JW, Reichek N. Left ventricular mechanics in dilated cardiomyopathy. *Am J Cardiol.* 1984;54:620-625.

Laurent GJ, Cockerill P, McAnulty RJ and Hastings JRB. A simplified method for quantification of the relative amounts of type I and III collagen in small tissue samples. *Anal Biochem.* 1981; 113: 300-312.

Lechat P, Escolano S, Golmard JL, Lardoux H, Witchitz S, Henneman JA, Maisch B, Hetzel M, Jaillon P, Boissel JP, Mallet A. Prognostic value of bisprolol-induced hemodynamic effects in heart failure during the Cardiac Insufficiency Bisoprolol Study (CIBIS). *Circulation.* 1997;96:2197-2205.

Lee TH, Hamilston MA, Stevenson LW, Moriguchi JD, Fonarow GC, Child JS, Laks H, Walden JA. Impact of left ventricular cavity size on survival in advanced heart failure. *Am J Cardiol*. 1993;72:672-676.

Lenfant C. Report of the Task Force on Research in Heart Failure. *Circulation*. 1994; 90: 1118-1123.

Levine TB, Levine AB, Bolenbaugh J, Stomel RJ. Impact of left ventricular size on pharmacological reverse remodelling in heart failure. *Clin Cardiol*. 2000;23:355-358.

Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med*. 1990;322:1561-1566.

Levy D, Salomon M, Agostino RB, Belanger AJ, Kannel WB. Prognostic implications of baseline electrocardiographic features and their serial changes in subjects with left ventricular hypertrophy. *Circulation*. 1994;90:1786-1793.

Li YY, Feldman AM, Sun Y and McTiernan CF. Differential expression of tissue inhibitors of metalloproteinases in the failing human heart. *Circulation*. 1998; 98: 1728-1734.

Li YY, Feng Y, McTieran F, Pei W, Moravec CS, Wang P, Rosenblum W, Kormos RL, Feldman AM. Downregulation of matrix metalloproteinases and reduction in collagen damage in the failing human heart after support with left ventricular assist devices. *Circulation*. 2001; 104: 1147-1152.

Liggett SB, Tepe NM, Lorenz JN, Canning AM, Jantz TD, Mitarai S, Yatani A and Dorn GW, 2nd. Early and delayed consequences of beta(2)-adrenergic receptor overexpression in mouse hearts: critical role for expression level. *Circulation*. 2000; 101: 1707-1714.

Limas C and Limas CJ. Reduced number of ß-adrenergic receptors in the myocardium of spontaneously hypertensive rats. *Biochemical Biophys Res Comm.* 1978; 83: 710-714.

Lindsey ML, Yoshioka J, MacGillivray C, Muangman S, Gannon J, Verghese A, Aikawa M, Libby P, Krane SM and Lee RT. Effect of a cleavage-resistant collagen mutation on left ventricular remodeling. *Circ Res*. 2003; 93: 238-245.

Linzbach AJ. Heart failure from the point of view of quantitative anatomy. *Am J Cardiol*. 1960; 5: 370-382.

Lombardi R, Betocchi S, Losi MA, Tocchetti CG, Aversa M, Miranda M, D'Alessandro G, Cacace A, Ciampi Q and Chiariello M. Myocardial collagen turnover in hypertrophic cardiomyopathy. *Circulation*. 2003; 108: 1455-1460.

Madigan JD, Barbone A, Choudhri AF, Morales DL, Cai B, Oz MC and Burkhoff D. Time course of reverse remodeling of the left ventricle during support with a left ventricular assist device. *J Thoracic Surg*. 2001; 121: 902-908.

Mann DL. Mechanisms and models in heart failure: A combinational approach. *Circulation*. 1999; 100: 999-1008.

Mann DL, Kent RL, Parsons B and Cooper G, 4th. Adrenergic effects on the biology of the adult mammalian cardiocyte. *Circulation*. 1992; 85: 790-804

Massie BM, Shah NB. Evolving trends in the epidemiologic factors of heart failure: Rationale for preventative strategies and comprehensive disease management. *Am Heart J*. 1997; 133: 703-712.

Mathew J, Sleight P, Lonn F, Johnstone D, Pogue J, Yi Q, Bosh J, Sussex B, Probstfield J, Yusuf S. Heart Outcomes Prevention Evaluation (HOPE) Investigators. Reduction of cardiovascular risk by regression of electrocardiograph markers of left ventricular hypertrophy by the angiotensin-converting enzyme inhibitor, ramipril. *Circulation.* 2001;104:1615-1621.

McCarthy PM, Nakatani S, Vargo R, Kottke-Marchant K, Harasaki H, James KB, Savage RM and Thomas JD. Structural and left ventricular histologic changes after implantable LVAD insertion. *Ann Thorac Surg.* 1995; 59: 609-613.

Menon B, Singh M and Singh K. Matrix metalloproteinases mediate beta-adrenergic receptor-stimulated apoptosis in adult rat ventricular myocytes. *Am J Physiol*. 2005; 289: C168-C176.

MERIT-HF. Study Group. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomized Intervention Trial in congestive Heart Failure (MERIT-HF). *Lancet*. 1999; 353: 2001-2007.

Molenaar P and Parsonage WA. Fundamental considerations of beta-adrenoceptor subtypes in human heart failure. *Trends Pharmacol Sci*. 2005; 26: 368-375.

Motz W and Strauer BE. Left ventricular function and collagen content after regression of hypertensive hypertrophy. *Hypertension*. 1989; 13: 43-50.

Mujumdar VS and Tyagi SC. Temporal regulation of extracellular matrix components in transition from compensatory hypertrophy to decompensatory heart failure. *J Hypertens.* 1999; 17: 261-270.

Mukherjee D, Sen S. Alteration of collagen phenotypes in hypertensive hypertrophy: role of blood pressure. *J Mol Cell Cardiol*. 1993; 25:185-196.

Mukherjee R, Brinsa TA, Dowdy KB, Scott AA, Baskin JM, Deschamps AM, Lowry AS, Escobar GP, Lucas DG, Yarbrough WM, Zile MR and Spinale FG. Myocardial infarct expansion and matrix metalloproteinase inhibition. *Circulation*. 2003; 107: 618-625.

Murphy NF, Simpson CR, McAlister FA, Stewart S, MacIntyre K, Kirkpatrick M, Chalmers J, Redpath A, Copewell S, McMurray JJ. National survey of the prevalence, incidence, primary care burden and treatment of heart failure in Scotland. *Heart* 2004;90:1129-1136.

Narula J, Haider N, Virmani R, DiSalvo TG, Kolodgie FD, Hajjar RJ, Schmidt U, Semigran MJ, Dec GW and Khaw BA. Apoptosis in myocytes in end-stage heart failure. *N Engl J Med.* 1996; 335: 1182-1189.

Nestico PF, Hakki AH and Iskandrian AS. Left ventricular dilatation. Prognostic value in severe left ventricular dysfunction secondary to coronary artery disease. *Chest*. 1985; 88: 215-220.

Neumann J, Schmitz W, Scholz H, van Meyerinck L, Doring V and Kalmar P. Increase of myocardial Gi-proteins in human heart failure. *Lancet*. 1988; 22: 936-937.

Norton GR, Woodiwiss AJ, Gaasch WH, Mela T, Chung ES, Aurigemma GP, Meyer TE. Heart failure in pressure overload hypertrophy: the relative roles of ventricular remodeling and myocardial dysfunction. *J Am Coll Cardiol*. 2002; 39: 664-671.

Norton GR, Tsotetsi J, Trifunovic B, Hartford C, Candy GP, Woodiwiss AJ. Myocardial stiffness is attributed to alterations in cross-linked collagen rather than total collagen or phenotypes in spontaneously hypertensive rats. *Circulation*. 1997; 96: 1991-1998.

Norton GR, Candy G, Woodiwiss AJ. Aminoguanidine prevents the decreased myocardial compliance produced by streptozotocin-induced diabetes mellitus in rats. *Circulation*. 1996; 93:1905-1912.

Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, Snappinn S, Harris KE, Aurup P, Edelman JM, Wedel H, Lindholm LH, Dahlof B. Regression of electrocardiographic left ventricular hypertrophy during antihypertensive treatment and the prediction of major cardiovascular events. *JAMA.* 2004;292:2343-2349.

Olivetti G, Capasso JM, Sonnenblick EH and Anversa P. Side-to-side slippage of myocytes participates in ventricular wall remodeling acutely after myocardial infarction in rats. *Circ Res*. 1990; 67: 23-34.

Osadchii OE, Norton GR, McKechnie R, Deftereos D and Woodiwiss AJ. Cardiac dilatation and pump dysfunction without intrinsic myocardial systolic failure following chronic beta-adrenoreceptor activation. *Am J Physiol*. 2007:292; H246-253.

Osadchii O, Woodiwiss AJ, Alves N and Norton GR. Mechanisms of preserved baseline cardiac systolic function in rats with adrenergic inotropic downregulation. *Life Sci.* 2005; 78: 366-375.

Packer M, Coats AJ, Fowler MB, Katus HA, Krum H, Mohacsi P, Rouleau JL, Tendera M, Castaigne A, Roecker EB, Schultz MK and DeMets DL. Effect of carvedilol on survival in severe chronic heart failure. *N Engl J Med.* 2001; 344: 1651-1658.

Packer M, Bristow MR, Cohn JN, Colucci WS, Fowler MB, Gilbert EM and Shusterman NH. The effect of Carvedilol on morbidity and mortality in patients with chronic heart failure. *N Engl J Med*. 1996; 334: 1349-1355.

Patten RD, Udelson JE and Konstam MA. Ventricular remodeling and its prevention in the treatment of heart failure. *Curr Opin Cardiol.* 1998; 13: 162-167

Patterson JH and Adams KF, Jr. Pathophysiology of heart failure: changing perceptions. *Pharmacotherapy*. 1996; 16: 27S-36S.

Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, Bocan TMA, Coker ML, Etoh T and Spinale FG. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circulation.* 2001; 103: 2303-2309.

Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ Jr, Geltman EM, Goldman S, Cuddy TE, Davis BR, Flaker GC. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the

survival and ventricular enlargement trial. The SAVE investigators. *N Engl J Med*. 1992; 327: 669-677.

Polyakova V, Hein S, Kostin S, Ziegelhoeffer T and Schaper J. Matrix metalloproteinases and their tissue inhibitors in pressure-overloaded human myocardium during heart failure progression. *J Am Coll Cardiol*. 2004; 44: 1609-1618.

Pont LG, Sturkenboom MC, van Gilst WH, Denig P, Haaijer-Ruskamp FM. Trends in prescribing for heart failure in Dutch primary care from 1996 to 2000. *Pharmacoepidemiol Drug Saf*. 2003;12:327-334

Poole-Wilson PA, Swedberg K, Cleland JG, Di Lenarda A, Hanrath P, Komajda M, Lubsen J, Lutiger B, Metra M, Remme WJ, Torp-Pederson C, Scherhag A and Skene A. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. *Lancet*. 2003; 362: 7-13.

Qin W, Rudolph AE, Bond BR, Rocha R, Blomme EAG, Goellner JJ, Funder JW, McMahon EG. Transgenic model of aldosterone-driven cardiac hypertrophy and heart failure..*Circ Res*. 2003; 93: 69-76.

Reddy HK, Tjahja IE, Campbell SE, Janicki JS, Hayden MR and Tyagi SC. Expression of matrix metalloproteinase activity in idiopathic dilated cardiomyopathy: a marker of cardiac dilatation. *Mol Cell Biochem.* 2004; 264: 183-191.

Remondino A, Kwon SH, Communal C, Pimentel DR, Sawyer DB, Singh K and Colucci WS. Beta-adrenergic receptor-stimulated apoptosis in cardiac myocytes is mediated by reactive oxygen species/c-Jun NH_2-terminal kinase-dependent activation of the mitochondrial pathway. *Circ Res.* 2003; 92: 136-138.

Rohde LE, Ducharme A, Arroyo LH, Aikawa M, Sukhova GH, Lopez-Anaya A, McClure KF, Mitchell PG, Libby P and Lee RT. Matrix metalloproteinase inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation*. 1999; 99: 3063-3070

Roten L, Nemoto S, Simsic J, Coker ML, Rao V, Baicu S, Defreyte G, Soloway PJ, Zile MR and Spinale FG. Effects of gene deletion of the tissue inhibitor of the matrix metalloproteinase-type 1 (TIMP-1) on left ventricular geometry and function in mice. *J Mol Cell Cardiol*. 2000; 32: 109-120.

Rumantir MS, Kaye DM, Jennings GL, Vaz M, Hastings JA and Esler MD. Phenotypic evidence of faulty neuronal norepinephrine reuptake in essential hypertension. *Hypertension.* 2000; 36: 824-829.

Rutten FH, Grobbee DE, Hoes AW. Diagnosis and management of heart failure: a questionnaire among general practitioners and cardiologists. *Eur J Heart Fail*. 2003;5:345-348

Sabbah HN. Apoptotic cell death in heart failure. *Cardiovasc Res*. 2000; 45: 704-712.

Sahn DJ, De Maria A, Kisslo J and Weyman A. Recommendations regarding quantitation of M-mode echocardiography: results of a survey of echocardiographic measurements. *Circulation*. 1978; 58:1072-1083.

Sakata Y, Yamamoto K, Mano T, Nishikawa N, Yoshida J, Hori M, Miwa T and Masuyama T. Activation of matrix metalloproteinases precedes left ventricular remodeling in hypertensive heart failure rats: its inhibition as a primary effect of angiotensin-converting enzyme inhibitor. *Circulation*. 2004; 109: 2143-2149.

Satoshi K, Spinale FG, Tanaka R, Johnson W, Cooper G IV, Zile MR. Inhibition of collagen cross-linking: effects of fibrillar collagen and ventricular diastolic function. *Am J Physiol*. 1995; 269:H863-H868

Scheinin SA, Capek P, Radovancevic B, Duncan JM, McAllister HA and Frazier OH. The effect of prolonged left ventricular support on myocardial histopathology in patients with end-stage cardiomyopahty. *ASAIO Journal*. 1992; 38: M271-M274.

Schlaich MP, Kaye DM, Lambert E, Sommerville M, Socratous F and Esler MD. Relation between cardiac sympathetic activity and hypertensive left ventricular hypertrophy. *Circulation.* 2003; 108: 560-565.

Schotten U, Filzmaier K, Borghardt B, Kulka S, Schoendube F, Schumacher C and Hanrath P. Changes of beta-adrenergic signaling in compensated human cardiac hypertrophy depend on the underlying disease. *Am J Physiol.* 2000; 278: H2076-H2083.

Sharpe N and Doughty RN. Left ventricular remodelling and improved long-term outcomes in chronic heart failure. *Eur Heart J*. 1998; 19: B36-B39

Shirani J, Pick R, Roberts WC, Maron BJ. Morphology and significance of the left ventricular collagen network in young patients with hypetrophic cardiomyopathy and sudden cardiac death. *J Am Coll Cardiol.* 2000;35:36-44

Sigurdsson A, Amtorp O, Gundersen T, Nilsson B, Remes J and Swedberg K. Neurohormonal activation in patients with mild or moderately severe congestive heart failure and effects of ramipril. The Ramipril Trial Study Group. *Br Heart J*. 1994; 72: 422-427

Simpson PC, Kariya K, Karns LR, Long CS and Karliner JS. Adrenergic hormones and control of cardiac myocyte growth. *Mol Cell Biochem.* 1991; 75: 977-984.

Singh K, Xiao L, Remondino A, Sawyer DB, Colucci WS. Adrenergic regulation of cardiac myocyte apoptosis. *J Cell Physiol*. 2001; 189: 257-265.

Spinale FG. Matrix metalloproteinases: regulation and dysregulation in the failing heart. *Circ Res.* 2002; 90: 520-530.

Spinale FG, Coker ML, Heung LJ, Bond BR, Gunasinghe HR, Etoh T, Goldberg AT, Zellner JL and Crumbley AJ. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. *Circulation*. 2000; 102: 1944-1949.

Spinale FG, Coker ML, Krombach SR, Mukherjee R, Hallak H, Houck WV, Clair MJ, Kribbs SB, Johnson LL, Peterson JT and Zile MR. Matrix metalloproteinase inhibition during the development of congestive heart failure: effects on left ventricular dimensions and function. *Circ Res*. 1999; 85: 364-376.

Spinale FG, Coker ML, Thomas CV, Walker JD, Mukherjee R, Hebbar L. Time-dependent changes in matrix metalloproteinase activity and expression during the progression of congestive heart failure: relation to ventricular and myocyte function. *Circ Res*. 1998; 82: 482-495.

Spinale FG, Zellner JL, Johnson WS, Eble DM and Munyer PD. Cellular and extracellular remodeling with the development and recovery from tachycardia-induced cardiomyopathy: changes in fibrillar collagen, myocyte adhesion capacity and proteoglycans. *J Mol Cell Cardiol.* 1996; 28: 1591-1608.

Spinale FG, Tomita M, Zellner JL, Cook JC, Crawford FA, Zile MR. Collagen remodeling and changes in LV function during the development and recovery from supraventricular tachycardia. *Am J Physiol*. 1991a; 261: H308-H318.

Spinale FG, Crawford FA, Jr, Hewett KW and Carabello BA. Ventricular failure and cellular remodeling with chronic supraventricular tachycardia. *J Thorac Cardiovasc Surg*. 1991b; 102: 874-882.

Stegemann H and Stalder K. Determination of hydroxyproline. *Clin Chem Acta*. 1967; 18: 267-273.

Steinfath M, Geertz B, Schmitz W, Scholz H, Haveerich A, Greil I, Hanrath P, Reupcke C, Sigmund M and Lo HB. Distinct down-regulation of cardiac ß$_1$- and ß$_2$-adrenoceptors in different human heart disease. *Arch Pharmacol*. 1991; 343: 217-220.

Stewart S, MacIntyre K, Hole DJ, Capewell S and McMurray JJ. More 'malignant' than cancer? Five-year survival following a first admission for heart failure. *Eur J Heart Fail.* 2001; 3: 315-322.

Sturm HB, van Gilst WH, Veeger N, Haajer-Ruskamp FM. Prescribing for chronic heart failure in Europe: does the country make the difference? A European Survey. *Pharmacoepidemiol Drug Saf.* 2007;16:96-103.

Sugawa M, Maughan L, Suga H, Sunugawa K. Physiological determinants of the left ventricular pressure-volume relationship. In: Sagawa K, Maughan L, Suga H, Sunagawa K, editors. Cardiac contraction and the pressure-volume relationship. New York, NY:Oxford University Press, 1988:114-119.

Swedberg K, Eneroth P, Kjekshus J and Wilhelmsen L. Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. *Circulation*. 1990; 82: 1730-1736.

Tevaearai HT and Koch WJ. Molecular restoration of beta-adrenergic receptor signaling improves contractile function of failing hearts. *Trends Cardiovasc Med*. 2004; 14: 252-256.

Tamura T, Onodera T, Said S and Gerdes AM. Correlation of myocyte lengthening to chamber dilation in the spontaneously hypertensive heart failure (SHHF) rat. *J Mol Cell Cardiol*. 1998; 30: 2175-2181.

Teerlink JR, Pfeffer JM and Pfeffer MA. Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats. *Circ Res.* 1994; 75: 105-113.

Thiedermann KU, Holubarsch C, Medugorac I and Jacob R. Connective tissue content and myocardial stiffness in pressure overload hypertrophy. *Basic Res Cardiol*. 1983; 78: 140-155

Toyama T, Hoshizaki H, Seki R, Isobe N, Adachi H, Naito S, Oshima S and Taniguchi K. Efficacy of carvedilol treatment on cardiac function and cardiac sympathetic nerve activity in patients with dilated cardiomyopathy: comparison with metoprolol therapy. *J Nucl Med*. 2003; 44: 1604-1611.

Tsotetsi OJ, Woodiwiss AJ, Netjhardt M, Qubu, D., Brooksbank, R., Norton, G.R. Attenuation of cardiac failure, dilatation, damage, and detrimental interstitial remodeling without regression of hypertrophy in hypertensive rats. *Hypertension*. 2001; 38:846-851.

Umeda H, Iwase M, Izawa H, Nishizama T, Nonokawa M, Isobe S, Noda A, Nagat K, Ishihara H, Yokota M. Biphasic relaxation-frequency relations in patients with effort angina pectoris: a new marker of myocardial demand ischemia. *Am Heart J*. 2003;146:75-83

Vasan RS, Larson MG, Benjamin EJ, Evans JC, Reiss CK, Levy D. Congestive heart failure in subjects with normal versus reduced left ventricular ejection fraction. *J Am Coll Cardiol*. 1999; 33: 1948-1955.

Vasan RS, Larson MG, Benjamin EJ, Evans JC Levy D. Left ventricular dilatation and the risk of congestive heart failure in people without myocardial infarction. *N Engl J Med*. 1997; 336: 1350-1355.

Veliotes DGA, Woodiwiss AJ, Deftereos DAJ, Gray D, Osadchii O and Norton GR. Aldosterone receptor blockade prevents the transition to cardiac pump dysfunction induced by beta-adrenoreceptor activation. *Hypertension*. 2005; 45: 914-920.

Verdecchia P, Carini G, Circo A, Dovellini E, Giovannini E, Lombardo M, Solinas P, Gorini M, Maggioni AP; MAVI (Masa Ventricolare sinistra nell'Ipertensione) Study Group. Left ventricular mass and cardiovascular morbidity in essential hypertension: the MAVI study J *Am Coll Cardiol*. 2001;38:1829-35.

Verdecchia P, Schillaci G, Borgioni C, Ciucci A, Gattobigio R, Zampi I, Reboldi G, Porcellati C. Prognostic significance of serial changes in left ventricular mass in essential hypertension. *Circulation*. 1998;97:48-54.

Waagstein F, Bristow MR, Swedberg K, Camerini F, Fowler MB, Silver MA, Gilbert EM, Johnson MR, Gross FG and Hjalmarson A. Beneficial effects of metoprolol in idiopathic dilated cardiomyopathy. *Lancet*. 1993; 342: 1441-1446.

Waagstein F, Stromblad O, Andersson B, Bohm M, Darius M, Delius W, Goss F, Osterziel KJ, Sigmund M, Trenkwalder SP and Wahlqvist I. Increased exercise ejection fraction and reversed remodeling after long-term treatment with metoprolol in congestive heart failure: a randomized, stratified, double-blind, placebo-controlled trial in mild to

moderate heart failure due to ischemic or idiopathic dilated cardiomyopathy. *Eur J Heart Fail*. 2003; 5: 679-691.

Weber KT. Aldosterone and spironolactone in heart failure. *N Engl J Med*. 1999; 341: 753-755.

Weber KT, Brilla CG. Pathological hypertrophy and cardiac interstitium: fibrosis and renin-angiotensin-aldosterone system. *Circulation*. 1991; 83: 1849-1865.

Weber KT, Brilla CG, Janicki JS. Structural remodeling of myocardial collagen in systemic hypertension: Functional consequences and potential therapy. *Heart Failure*. 1990; 6: 129-137.

Weber KT, Janicki J, Schroff SG, Pick R, Chen RM, Abrahams G, Bashey RI. Collagen remodeling of the pressure-overloaded hypertrophied non-human primate myocardium. *Circ Res*. 1988a; 62:757-765.

Weber KT, Pick R, Janicki JS, Gadodia G, Lakier JB. Inadequate collagen tethers in dilated cardiomyopathy. *Am Heart J*. 1988b; 116: 1641-1646.

Woodiwiss AJ, Tsotetsi OJ, Sprott S, Lancaster EJ, Mela, T, Chung ES, Meyer TE, Norton GR. Reduction in myocardial collagen cross-linking parallels left ventricular dilatation in rat models of systolic chamber dysfunction. *Circulation*. 2001; 103: 155-160.

Woodiwiss AJ, Norton GR. Exercise-induced cardiac hypertrophy is associated with an increased myocardial compliance. *J Appl Physiol*. 1995; 78: 1303-1311.

Zile MR, Baicu CF and Gaasch WH. Diastolic heart failure - abnormalities in active relaxation and passive stiffness of the left ventricle. *N Engl J Med*. 2004; 350: 1953-1959.

Zimmer HG, Gerdes AM, Lortet S and Mall G. Changes in heart function and cardiac cell size in rats with chronic myocardial infarction. *J Mol Cell Cardiol.* 1990; 22: 1231-1243