# STUDIES OF VASCULAR FUNCTION IN PATIENTS WITH HEART FAILURE AND EITHER PRESERVED OR REDUCED LEFT VENTRICULAR SYSTOLIC FUNCTION

**Dr Sean Balmain**

**British Heart Foundation Glasgow Cardiovascular Research Centre**

**University of Glasgow**

**Glasgow**

**G12 8TA**

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**University of Glasgow**

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## Declaration

The experimental design of the work presented in this thesis was that of the author and his supervisors, Dr Neal Padmanabhan and Professor John JV McMurray. The author performed all experimental work. Assays for NT-proBNP were performed by Dr JJ Morton.

Dr Sean Balmain 10th June 2008

## Presentations and Publications arising from this thesis

The results from chapters 3, 5 and 6 have been presented and published as follows:

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Sean Balmain, Neal Padmanabhan, William R Ferrell, John J Morton, John JV McMurray. "Differences in arterial compliance, microvascular function and venous capacitance between patients with heart failure and either preserved or reduced left ventricular systolic function." *Eur J Heart Fail. 2007 Sep;9(9):865-71.*

## Summary

Up to 50% of patients with the clinical syndrome of heart failure have preserved left ventricular systolic function (HF-PSF). Invasive studies utilising cardiac catheterisation have demonstrated that patients with HF-PSF have abnormalities of left ventricular (LV) relaxation and filling, or LV diastolic dysfunction. As a result, it has been proposed that LV diastolic dysfunction is the primary pathophysiological process in HF-PSF. However, population-based studies have shown that there is poor correlation between the presence of LV diastolic dysfunction and the presence of heart failure. This controversy has led to a search for alternative pathophysiological processes which could potentially cause HF-PSF. There are some data to suggest that patients with HF-PSF have a combination of LV diastolic dysfunction, or 'LV stiffness', and large artery stiffness, when compared with normal subjects and patients with systemic hypertension. This implies that the interaction between the left ventricle and the vasculature is dysfunctional and a potential cause of HF-PSF. Although there are limited data on arterial stiffness in HF-PSF, there have been no studies examining other parameters of vascular function in HF-PSF and vascular function has never been formally compared in cohorts of patients with HF-PSF and heart failure due to reduced LV systolic function (HF-RSF).

The studies presented in this thesis were designed to further characterise vascular function in HF-PSF and to compare vascular function between patients with HF-PSF, patients with HF-RSF and control subjects. I used non-invasive techniques to assess parameters of arterial function, such as arterial stiffness and arterial endothelial function. I also evaluated parameters of venous function, namely venous capacitance and venous endothelial function.

Arterial stiffness, measured by aortic pulse wave velocity (PWV), was significantly elevated in HF-PSF compared to both HF-RSF and control groups, implying that HF-PSF is indeed associated with greater arterial stiffness. In contrast, arterial diastolic waveform analysis failed to show any significant differences in derived parameters of arterial compliance between the three study groups, which may be due to the fact that all three groups were matched for underlying coronary heart disease, reducing the ability of the technique to differentiate between groups.

Using Laser Doppler iontophoresis, I demonstrated that HF-PSF and HF-RSF subjects have impaired microvascular responses to both acetylcholine and sodium nitroprusside. This suggests that, rather than being solely a primary disorder of endothelial function, impaired control of vascular tone in HF-PSF reflects significant vascular smooth muscle dysfunction. It is not certain if arterial smooth muscle and/or endothelial dysfunction is secondary to the inflammatory and neurohumoral activation associated with the heart failure syndrome, or a primary pathophysiological factor in the development of either form of heart failure.

As regards venous function, patients with HF-PSF had a lower venous capacitance than patients with HF-RSF (but similar venous capacitance to the controls). Increased venous capacitance may represent a compensatory response in heart failure that is less marked or absent in HF-PSF, compared to HF-RSF. Venous endothelial function was measured with the Aellig dorsal hand vein technique. It was not technically possible to complete an Aellig study in the whole patient cohort, resulting in fewer data being available for analysis. Despite this, both heart failure groups appeared to have impaired venodilatation in response to acetylcholine, compared to controls, although this apparent difference was not statistically significant.

While the studies of venous capacitance and endothelial function were not conclusive, they suggest that venous function may be abnormal in HF-PSF. The finding that endothelial and smooth muscle control of arterial tone was impaired in both HF-PSF and HF-RSF may indicate a similar primary pathophysiological process, or indeed a similar response to inflammatory and neurohumoral activation in heart failure. I conclude that the data presented in this thesis supports the hypothesis that HF-PSF is associated with increased arterial stiffness, which in combination with increased LV stiffness is likely to result in impaired ventriculo-vascular coupling. This process is likely to be an important pathophysiological factor in the development of HF-PSF.

# Chapter 1

## Introduction and Literature Review

## 1.1 The Heart Failure Syndrome

### 1.1.1 A definition of heart failure

There are several contemporary definitions of heart failure, most of which are unsatisfactory, as they do not encompass all aspects of the condition.

One of the most widely quoted definitions of heart failure was first proposed by Paul Wood in 1950, and adapted slightly by Braunwald et al in 1982. In Braunwald's definition, heart failure is described as "…a pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the requirements of the metabolising tissues or can do so only from an elevated filling pressure."[1]

Many modern texts define heart failure as a clinical syndrome characterised by typical symptoms, clinical signs and chest radiograph appearance, which may result from a number of different disease processes, rather than being a disease in its own right. Both European and American guidelines on the diagnosis and management of chronic heart failure advise evaluation of patient symptoms and clinical signs in conjunction with investigation results to make a diagnosis of heart failure.[2, 3] This is highlighted by the frequent use of clinical scoring systems, such as the Boston criteria,[4] to determine the likelihood of the presence of heart failure.

### 1.1.2 Epidemiology of heart failure

The importance of heart failure as a major public health problem has been described in multiple review articles, such as those written by McMurray et al [5] and Mehta et al.[6] The following section provides a summary of the most important published data on the epidemiology of heart failure.

### 1.1.3 Prevalence of heart failure

Heart failure is common, particularly in older individuals. The largest body of evidence available on the prevalence of heart failure is from large cohort studies carried out in the United States. In the Framingham heart study, prevalence of heart failure in 50-59 year-olds was 0.8% rising to 9.1% in those above 80 years of age.[7] Another extremely large cohort study, The United States National Health And Nutrition Examination Survey (NHANES), reported the prevalence of heart failure to be 8% in subjects over 65 years of age.[8]

### 1.1.4 Incidence of heart failure

Less information is available regarding the incidence of heart failure, with most data once again resulting from large population-based studies in the United States. In the Framingham heart study, the annual incidence of heart failure was 0.3% in men and 0.2% in women aged 50-59 years, rising to 2.7% in men and 2.2% in women aged 80-89 years.[7] Another population-based study conducted in the United States was the

Rochester epidemiology project.[9] In this study, investigators examined the incidence of heart failure during 1981 in Olmsted County, Minnesota. During the study period, 46 new diagnoses of heart failure were made in patients aged 0-74 years. Overall, this equates to an annual heart failure incidence of 110 cases per 100,000 population. As noted in the Framingham study, the incidence of heart failure in the Olmsted study was higher in males than in females (157 *versus* 71 per 100,000 population).

### 1.1.5 Prognosis of heart failure

Heart failure is lethal, with a particularly high early mortality, and carries a prognosis similar to many common forms of cancer.

The Framingham study showed that men with a diagnosis of heart failure have one-year, five-year and ten-year mortality rates of 43%, 75% and 89%, respectively. The corresponding mortality rates in women at one, five and ten years were slightly better at 46%, 62% and 79%, respectively.[10] The NHANES study also provided long-term follow-up mortality data in patients with heart failure. In this study population, ten-year mortality rate in those aged 25-74 years was 49.8% in men and 36% in women.[8] These mortality rates compare favourably with those reported from the Framingham study. This discrepancy has been attributed to the fact that symptoms of heart failure in this study were self-reported, rather than based purely on a score derived from clinical criteria, such as was employed in the Framingham study.[10] In addition, the patients were not hospitalised, implying that their symptoms were less severe.[5, 6]

The Rochester epidemiology project also found the survival after a diagnosis of heart failure to be poor. In the cohort presenting in 1981 mortality was 20% at six months and 34% at one year.[9] The same group has reported longer-term survival data on another cohort of patients who presented with heart failure ten years later, in 1991. The overall mortality in the 1991 cohort was 24% at one year and 65% at five years. [11]

In summary, several large epidemiological studies have repeatedly shown that heart failure is common and carries a poor prognosis, underlining the importance of the condition as an area where further research and development of new therapies are vital.

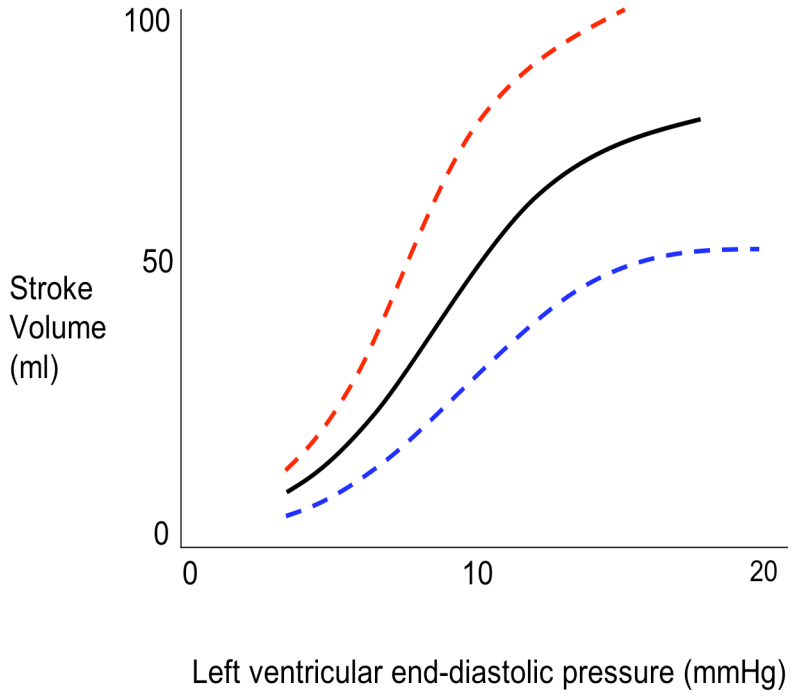
## 1.2 Pathophysiology of Heart Failure

### 1.2.1 Elevated intra-cardiac pressure

Central to the pathophysiology of heart failure is the development of elevated intra-cardiac pressure. The commonest precipitant is myocardial damage and the development of impaired LV systolic contraction following myocardial infarction or ischaemia. Impaired LV systolic contraction leads to a chain of compensatory mechanisms in order to maintain stroke volume and cardiac output. The most important compensatory mechanism is dilation of the left ventricle, increasing left ventricular end-diastolic pressure (LVEDP), or pre-load. According to Frank-Starling's law, increasing LV pre-load stretches myocardial fibres (increasing sarcomere length) and will increase force of contraction, stroke volume and cardiac output.[12]

However, with progressive deterioration of LV systolic contraction, the left ventricle will continue to dilate and the LVEDP and subsequent myocardial stretch ultimately exceed the level that will result in improved stroke volume (figure 1.1). During the diastolic phase of the cardiac cycle, high LV pressure results in elevation of the left atrial pressure. As left atrial pressure rises, pulmonary capillary hypertension develops, resulting in transudation of fluid from intra-vascular to extra-vascular compartments, causing pulmonary oedema. Over time, pulmonary vascular remodelling occurs and pulmonary vascular resistance rises. This increases right ventricular afterload and end-diastolic pressure, starting a cascade of compensatory mechanisms on the right side of the heart. When the right ventricle decompensates, the right atrial and systemic venous pressure rise and the characteristic features of peripheral oedema, hepatic congestion and ascites develop.

**Figure 1.1:**
Frank-Starling curve demonstrating that increased left ventricular end-diastolic pressure results in increased stroke volume up to a point when the curve plateaus and beyond which the left ventricle will decompensate and stroke volume may fall. The curve representing the normal resting heart is represented by the solid, black line. With increased venous return (preload) or increased left ventricular inotropy, the curve moves up and to the left (dashed red line). With increased left ventricular afterload, or decreased inotropy, the curve moves down and to the right (dashed blue line).



### 1.2.2 Neurohumoral activation

Heart failure is associated with marked vasoconstriction and retention of salt and water. Initially an adaptive response in an effort to improve arterial pressure and tissue perfusion, ultimately these processes become maladaptive. Vasoconstriction and volume overload in heart failure are secondary to neurohumoral activity. Specifically, increased plasma concentrations of angiotensin II, norepinephrine and endothelins all contribute to the increased vascular tone observed in heart failure. The resulting vasoconstriction exacerbates poor tissue perfusion and stimulates further neurohumoral activity. Volume overload results from the actions of aldosterone. Reduced cardiac output results in renal arterial under-filling. This stimulates the renin-angiotensin-aldosterone system (RAAS) to constrict peripheral vessels via action of angiotensin II to improve central arterial perfusion pressure, and to retain sodium and water via action of aldosterone. Arterial under-filling also stimulates baroreceptors, leading to activation of the sympathetic nervous system, increased norepinephrine activity and further activation of the RAAS. This over-activation of the RAAS ultimately becomes maladaptive, and fluid overload results.[13] In addition to angiotensin II and norepinephrine, endothelins circulate in high concentrations and exacerbate vasoconstriction in heart failure.[14]

As a compensatory mechanism, endogenous vasodilators are released from cardiac tissue, predominantly in response to circulating volume overload and myocardial stretch. These endogenous vasodilators are known as the natriuretic peptides and in addition to vasodilatation, stimulate salt and water excretion from the kidney.[15] One of the

natriuretic peptides, brain-type natriuretic peptide (BNP), is predictably found in higher plasma concentrations in patients with the clinical syndrome of heart failure. More recently, evidence has shown BNP concentration to be associated with disease severity and a powerful predictor of clinical outcome in both HF-RSF [16] and HF-PSF.[17] Heart failure is also an inflammatory process, associated with profound activation of cytokine pathways leading to production of reactive oxygen species. This inflammatory over-activity and free radical production has been implicated in the pathophysiology of vascular dysfunction in HF-RSF.[18]

There is profound neurohumoral activity in heart failure involving several different pathways. Excess neurohumoral activity in heart failure has been implicated as a potential cause or exacerbating factor in the peripheral vasoconstriction and vascular dysfunction seen in the heart failure syndrome.

## 1.3 Heart Failure with Preserved Left Ventricular Systolic Function

### 1.3.1 Definition of HF-PSF

Heart failure with preserved systolic function can be defined as: the syndrome of heart failure in the presence of normal LV systolic function and the absence of significant valvular heart disease, cardiac dysrhythmia, right ventricular dysfunction or high output states such as anaemia. This definition is straightforward but relies heavily on the statement that LV systolic function is 'normal'. The definition of normal LV systolic

function is not straightforward and has been the subject of debate. Most centres use LV ejection fraction (LVEF) to quantify LV systolic function. Due to the widespread availability of non-invasive imaging techniques, such as echocardiography and radionuclide imaging, LVEF can be obtained easily. As LVEF is expressed as a percentage of LV diastolic volume, it gives one a figure that is easy to understand and lends itself to comparison between groups with statistical analysis. These factors have greatly contributed to the widespread use of LVEF as a means of describing LV systolic performance. In the literature, the LVEF considered to represent 'normal' LV systolic function varies widely between 40 and 70%,[19-21] but most groups define preserved LV systolic function as a LVEF >50%. The LVEF value deemed to represent 'normal' LV systolic function obviously impacts on incidence and prevalence figures as detailed below in sections 1.3.3 and 1.3.4.

### 1.3.2 Epidemiology of HF-PSF

Much has been learned about the epidemiology of HF-PSF in the last ten years. It is now apparent from multiple epidemiological studies that patients with HF-PSF constitute a significant proportion of the total burden of heart failure. Comprehensive review articles on the epidemiology of HF-PSF have been published by Vasan et al [22] and more recently by Hogg et al.[23] The following section comprises an overview of the epidemiology of HF-PSF.

### 1.3.3 Prevalence of HF-PSF

Although the populations studied are heterogeneous, with variable definitions of preserved systolic function, the evidence from multiple cross-sectional, population-based studies tends to agree with incidence studies, and suggests that HF-PSF makes up a significant proportion of the overall burden of heart failure worldwide. Prevalence of HF-PSF (LVEF ≥50%) in the Framingham study was 51%.[24] These results are mirrored in the Cardiovascular Health Study, in which the prevalence of PSF (LVEF ≥50%) among patients with heart failure was 55%.[20] Another population-based study, looking at registry data of heart failure admissions to ten hospitals, was the Management to Improve Survival in Congestive Heart Failure (MISCHF) Study. In the MISCHF study, 42% of patients had a LVEF ≥ 40% and 24% of patients had a LVEF ≥ 50%.[25] The Helsinki Aging Study looked at older subjects (75-86 years) with heart failure. The threshold LVEF for 'preserved' systolic function was high at >70%. In spite of this stringent definition of preserved LV systolic function, a significant proportion of this cohort of patients (51%) had HF-PSF.[21] Follow-up data from the Rochester Epidemiology Project heart failure population indicate that the prevalence of HF-PSF is high, and has risen in the last 15 years. The prevalence of preserved systolic function (LVEF >50%) among patients with heart failure increased from 38% to 54% between 1987 and 2001,[26] although the authors note that this phenomenon is probably partly due to increased clinician awareness of HF-PSF in recent years, leading to more frequent recognition and diagnosis of HF-PSF.

### 1.3.4 Incidence of HF-PSF

As with epidemiological data on HF-RSF, there is less published information available about the incidence of HF-PSF than prevalence. The Rochester group found that of all patients admitted with a new diagnosis of heart failure over a one-year period (1991), 43% had preserved LV systolic function, defined as an ejection fraction of ≥50%.[11] Similarly, the Cardiovascular Health Study population had a high incidence of HF-PSF. Over the study period there were 597 incident cases of heart failure, diagnosed in the community, rather than in hospital, of which 60% had a LVEF of ≥55% at time of diagnosis.[20, 27] More recently, Bhatia et al have reported a study of 2802 patients admitted to 103 Canadian hospitals with a new diagnosis of heart failure. In this study population, the incidence of HF-PSF (LVEF ≥50%) was 31%.[28]

### 1.3.5 Prognosis of HF-PSF

Previously reported morbidity and mortality data from the Cardiovascular Health Study [29] and the CHARM study [19] suggest that HF-PSF is a more benign condition that HF-RSF. However, recently published data from Canada would indicate that outcome is similar in both HF-PSF (LVEF >50%) and HF-RSF (LVEF <40%) with one year mortality rates of 22% and 26%, respectively (p=0.08).[28]

## 1.4 Pathophysiology of HF-PSF

The pathophysiology of HF-PSF has not yet been fully established. A commonly held view is that HF-PSF is due to diastolic dysfunction of the left ventricle. More recently, it has been proposed that abnormalities of interaction between the left ventricle and the vasculature may lead to HF-PSF. In this section I will provide an overview of what is currently known about LV diastolic dysfunction and ventriculo-vascular interaction, and the relevance of both in the context of HF-PSF pathophysiology.

### 1.4.1 Diastolic dysfunction of the left ventricle

The term 'diastolic dysfunction' refers to abnormalities of relaxation and filling of the left ventricle, rather than contraction and emptying. It is a commonly held view that diastolic dysfunction of the left ventricle is the primary pathophysiological process in HF-PSF.[30, 31] Left ventricular diastolic function is a complex process that has been extensively studied. A comprehensive account of current information regarding the physiology of normal LV diastolic function and the pathophysiology of LV diastolic dysfunction is beyond the scope of this introduction. Kass et al have recently reviewed this subject.[32]

For the purposes of this introduction I will focus on a summarised description of LV diastolic function and dysfunction, and how LV diastolic dysfunction may be implicated in the pathophysiology of HF-PSF.

### 1.4.2 Active relaxation of the left ventricle

Left ventricular diastole consists of two phases: active relaxation and passive filling. Relaxation of the left ventricle is an active process, beginning at the end of ventricular systolic contraction. LV relaxation is a complex process, which relies on multiple events at molecular level, including myocyte calcium handling, active phosphorylation and actin-myosin cross-bridge detachment to name but a few. All of these events consume energy, and can therefore be attenuated during myocardial ischaemia, resulting in abnormal LV relaxation. This ischaemic failure of relaxation can be due to coronary heart disease, where normal myocardial oxygen requirements are not adequately met due to poor arterial supply. Left ventricular hypertrophy (LVH) also causes ischaemic failure of LV relaxation. The increased myocardial mass in LVH has abnormally high oxygen requirements and demand can outstrip supply even in the absence of coronary heart disease. Co-existing LVH and coronary heart disease can result in a powerful substrate for impaired LV relaxation.[33, 34] Left ventricular relaxation is also dependent on venous return and LV pre-load, which I discuss in section 1.5.7, and duration of systole and LV afterload, which I discuss in section 1.4.5.

Delayed onset or slow active relaxation results in attenuation of the normal fall in LV pressure during diastole, and a net rise in LV diastolic pressure.

### 1.4.3 Passive filling of the left ventricle

Passive filling of the left ventricle is the other important phase of effective diastolic function. It is dependent on the intrinsic characteristics of the LV wall, usually termed 'LV diastolic stiffness'.[32]

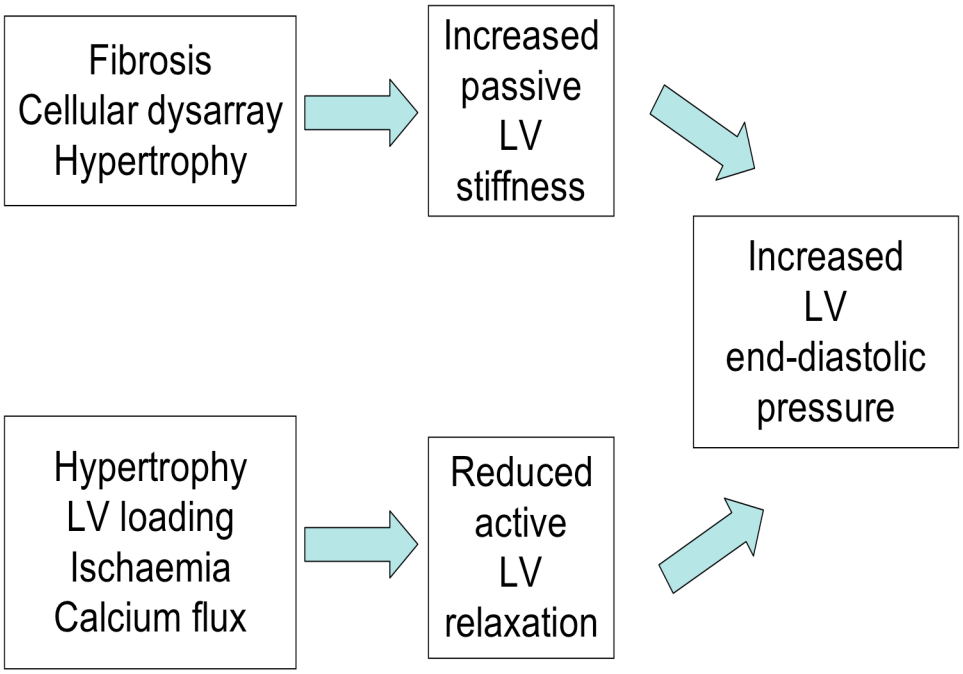
The diastolic stiffness of the left ventricle is determined by the pressure-volume relationship during diastole. Increased LV stiffness results in resistance to changes in volume in response to rising filling pressure.

The most common cause of increased LV diastolic stiffness is thought to be LVH secondary to essential hypertension. Left ventricular hypertrophy is particularly common in patients with HF-PSF, strengthening the argument that LV diastolic stiffness is an important factor in the development of HF-PSF.[35] The development of LVH is complex, being influenced by various mechanical and, in particular, neurohumoral factors.[36, 37] Left ventricular remodelling and hypertrophy occur in response to sustained elevation of pre-load or afterload in an attempt to maintain cardiac output. Ultimately these changes become maladaptive and deleterious to cardiac performance. There are two main pathophysiological processes in the development of LVH: myocyte hypertrophy and interstitial fibrosis. Both of these phenomena can result in LV diastolic dysfunction. Myocyte hypertrophy results in impaired active LV relaxation as described above. Interstitial fibrosis is the increased deposition of myocardial extracellular matrix, in the form of collagen, through the proliferation and action of fibroblasts. Interstitial fibrosis results in a poorly compliant LV chamber i.e., increased LV stiffness and abnormal passive filling of the left ventricle during diastole.[38]

### 1.4.4 Combined effects of abnormal relaxation and filling of the left ventricle

The mechanisms by which impaired LV relaxation and increased LV stiffness result in diastolic dysfunction are illustrated in figure 1.2.

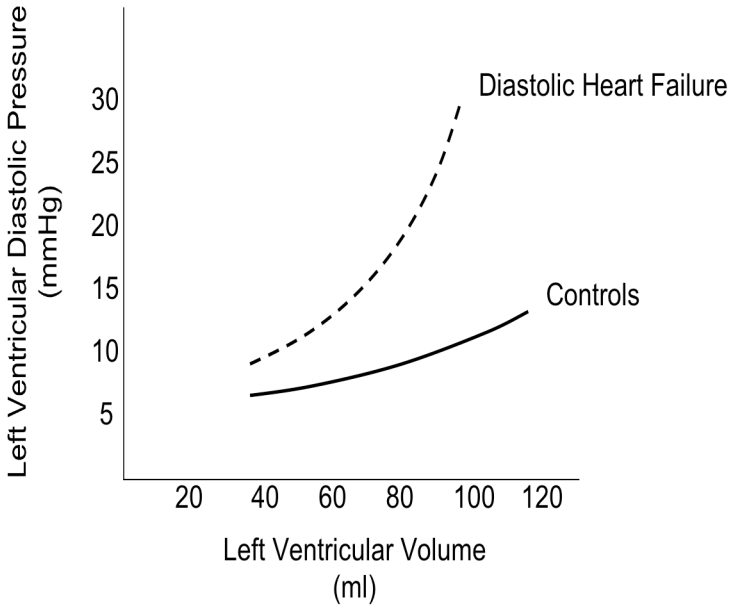
**Figure 1.2:**
Mechanism by which increased passive stiffness of the left ventricle and impaired active relaxation of the left ventricle combine to result in elevation of the left ventricular (LV) end-diastolic pressure. Adapted from Gaasch WH, in Braunwald's Heart Disease: a textbook of cardiovascular medicine.[1]



The end result of diastolic dysfunction is elevated LV diastolic pressure despite normal filling volume. Zile et al employed cardiac catheterisation to obtain invasive LV pressure-volume loops from patients with HF-PSF, demonstrating that the diastolic pressure rise is steeper per unit rise in volume that in subjects without HF-PSF (figure 1.3).[39, 40]

It is via this mechanism that diastolic dysfunction of the LV can lead to elevation of the LVEDP, left atrial hypertension, neurohumoral activation and the clinical features of heart failure. Therefore, LV diastolic dysfunction is implicated in the pathophysiology of HF-PSF.

**Figure 1.3:**
Left ventricular pressure - volume relationship in patients with HF-PSF (diastolic heart failure) and normal subjects (controls) demonstrating the steep rise in intra-ventricular pressure with rising diastolic volume. Figure adapted from Zile et al 2002 and 2004.[39, 40]



### 1.4.5 Is HF-PSF purely due to LV diastolic dysfunction?

The invasive cardiac catheter studies conducted by Zile et al [31] demonstrated that patients with HF-PSF have LV diastolic dysfunction, prompting the authors to suggest that formal assessment of diastolic dysfunction was unnecessary to make a diagnosis of 'diastolic heart failure'. Although HF-PSF patients, when assessed with invasive studies, have LV diastolic dysfunction, it does not necessarily follow that diastolic dysfunction will lead to the development of HF-PSF. Other groups have examined diastolic dysfunction in larger populations of patients, both with and without heart failure. Chen et al examined patients presenting with a first diagnosis of heart failure and found that only half of patients with HF-PSF had echocardiographic evidence of diastolic dysfunction. [41]

Petrie et al analysed echocardiograms of patients with a presumptive diagnosis of heart failure and found that there was poor correlation between echocardiographic markers of diastolic dysfunction and either the presence of heart failure or accepted risk factors for diastolic dysfunction, such as hypertension, LVH and myocardial ischaemia.[42] Large cross-sectional population echocardiographic studies [43, 44] have shown that diastolic dysfunction is at least as common in men as in women but, as discussed in section 1.3, HF-PSF is much more common in elderly females.

There are fundamental problems when comparing studies of LV diastolic dysfunction. Invasive assessment of LV function in all patients with suspected heart failure is not practical and echocardiographic measures of diastolic function can be unreliable. Diastolic properties of the left ventricle are load-dependent and therefore prone to

variation even within the individual.[45] It is therefore difficult to be certain how much 'diastolic dysfunction' is truly due to impaired active relaxation and/or increased passive stiffness of the left ventricle, and how much is influenced by pre-load conditions of the left ventricle.

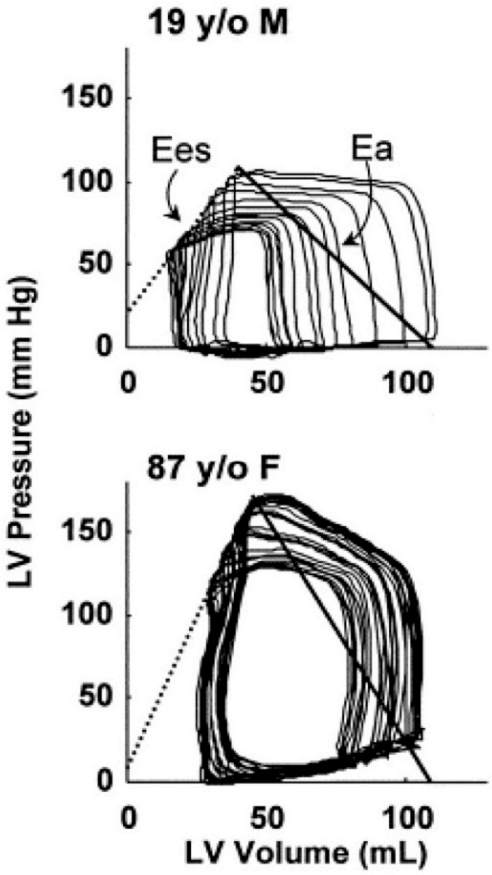
Although LV diastolic dysfunction certainly plays a role in the pathophysiology of HF-PSF, the poor correlation between diastolic dysfunction and HF-PSF seen in population-based studies has prompted consideration of other factors which may be implicated in the development of heart failure in this patient group.

### 1.4.6 Ventriculo-vascular interaction

Abnormal vascular function, and in particular the interaction between the systemic vasculature and the left ventricle, is a potential pathophysiological process in HF-PSF. Reduced aortic distensibility, or increased aortic impedance, has been known to increase LV afterload and adversely influence LV performance for more than 40 years.[46, 47] Since then, detailed studies of ventriculo-vascular interaction have been performed in both animal models and humans. Twenty years ago, Hori et al used a canine model to demonstrate that elevation of LV afterload during late systole directly impairs active LV relaxation during diastole.[48] These findings have been confirmed by more recent animal studies.[49] The possibility that late-systolic ventriculo-arterial interaction can adversely affect cardiac performance has been explored by other groups using human subjects.

Ventriculo-vascular interaction and, in particular, ventricular and vascular stiffness are commonly described in terms of LV end-systolic elastance (Ees) and effective arterial elastance (Ea), as discussed by David Kass in his 2005 review paper.[50] Ea is a measure of arterial load derived from the ratio of LV end-systolic pressure to stroke volume.[51] Ees represents the LV end-systolic pressure-volume relationship. The Ea/Ees ratio is representative of end-systolic ventriculo-arterial interaction. Ea and Ees are determined by invasively or non-invasively measuring the LV pressure-volume relationship throughout the cardiac cycle. An example of LV pressure-volume loops in young and elderly subjects, demonstrating Ea and Ees, is shown in figure 1.4.[52]

**Figure 1.4:**
Pressure volume loops and relations derived by preload reduction maneuver in a young and in an elderly patient. End systolic elastance (Ees) measures chamber systolic stiffness and is the slope of a line connecting the upper left-hand corners (end systole) from each pressure volume loop (dotted line). Effective arterial elastance (Ea) measures arterial load and stiffness and is depicted by the negative slope of the diagonal solid line shown. Figure adapted from Chen et al 1998.[52]



Kass, Redfield and colleagues have conducted several studies investigating ventriculo-vascular coupling in various patient groups, as detailed below.

Redfield et al used non-invasive techniques to show that the combination of ventricular and arterial stiffening is more common in elderly females,[53] the section of the population most likely to develop HF-PSF according to population-based studies.[22, 35, 41, 43] Although subjects with diabetes or manifest cardiovascular disease were excluded from analysis, almost 40% of the subjects were taking cardio-active medications, which could have influenced the results. Chen et al used cardiac catheterisation to demonstrate that elderly subjects without heart failure have increased end-systolic LV and arterial stiffening.[52] Once again, some aspects of the study group baseline characteristics are likely to have influenced the results. The groups studied were heterogeneous, with some patients having severe coronary heart disease or significant hypertension, and a similar proportion having normal coronary arteries.

These investigators have also conducted studies of ventriculo-vascular interaction in patients with HF-PSF. Kawaguchi et al,[54] used a combination of invasive and non-invasive methods to assess ventriculo-arterial interaction in HF-PSF. Both ventricular and arterial stiffening were markedly elevated in subjects with HF-PSF, compared with both young and age-matched controls without cardiovascular disease, and with age-matched subjects with hypertension. Unfortunately the most interesting comparison, between HF-PSF and hypertensive subjects, was made using different methodologies. Subjects with HF-PSF underwent cardiac catheterisation and subjects with hypertension

were assessed non-invasively with echocardiography. Again, patients with coronary heart disease were excluded making the results less applicable to all patients with HF-PSF. Melenovsky et al recruited subjects with HF-PSF, subjects with hypertensive LVH, and normal controls into their study.[55] They examined multiple parameters of cardiac and vascular function using non-invasive techniques. The study population was predominantly African-American females and there was a higher prevalence of coronary heart disease, renal dysfunction and diabetes mellitus in the HF-PSF group. They found that HF-PSF and hypertension/LVH groups had similar abnormalities of ventriculo-arterial stiffening and LV diastolic dysfunction but that LVH and left atrial dilatation were more marked in HF-PSF. They speculate that left atrial dysfunction may be involved in that pathophysiology of HF-PSF. Another important study from this group of investigators was reported by Lam et al.[56] A large cohort of patients with HF-PSF was recruited. Once again parameters of cardiac and vascular function were measured non-invasively in patients with HF-PSF and compared to hypertensives and normal controls. As in other studies, heterogeneity was present between groups: the HF-PSF patients had a higher prevalence of coronary heart disease and renal dysfunction compared to both other groups and had significantly lower blood pressures than the hypertensive group. Despite this, Ea and Ees were similarly increased in HF-PSF and hypertensive patients, compared to controls. In contrast with the findings of Melenovsky et al, diastolic dysfunction was more severe in HF-PSF subjects.

Another group who have investigated cardiac and vascular function in HF-PSF used cardiovascular magnetic resonance imaging to determine aortic stiffness and examined

the correlation between aortic stiffness and exercise tolerance in HF-PSF. Hundley et al [57] investigated patients with HF-PSF and found that reduced aortic distensibility correlated with exercise intolerance in their cohort, suggesting that reduced arterial compliance, and therefore elevated LV afterload, is implicated in the development of symptoms in HF-PSF. The patient selection process was robust, with clearly defined criteria to diagnose HF-PSF. However, subjects in the control group were normotensive and free of cardiovascular disease, which undoubtedly enhanced the differences in vascular and ventricular abnormalities observed between HF-PSF and control groups. In addition, patients with coronary heart disease were excluded and this may have led to an atypical cohort of patients with HF-PSF being studied. The same investigators have since published a similar study conducted in patients with HF-RSF.[58] They found that HF-RSF is also associated with reduced aortic distensibility and exercise intolerance. One could conclude from these two studies that reduced large artery compliance adversely influences LV performance and therefore symptoms, regardless of LV systolic function. Whether or not reduced arterial compliance causes HF-PSF is as yet unproven. The role of abnormal ventricular-arterial interaction in the pathophysiology of HF-PSF has yet to be fully established. Even so, the evidence presented by the above studies has cast doubt on the theory that HF-PSF is purely a disorder of LV diastolic function, with more than one group suggesting that the pathophysiology is in fact far more complex.[59, 60] The combination of ventricular stiffening and increased LV afterload has many implications when considering the pathophysiology of HF-PSF. Increased afterload increases cardiac work and oxygen requirements. Combined ventricular and

arterial stiffening results in labile haemodynamic responses to both exercise and changes in circulating volume. Elevation of LV afterload is a powerful stimulus for the development of LVH, which, as described in section 1.4.3, causes increased passive stiffness of the left ventricle during diastole.

Left ventricular chamber stiffness, diastolic dysfunction and afterload are closely linked via dynamic interaction and may all be implicated in the pathophysiology of HF-PSF.

## 1.5 Vascular Function

There are reasons to believe that vascular dysfunction is implicated in the pathophysiology of HF-PSF. Arterial stiffness is the main determinant of LV afterload and influences ventriculo-vascular coupling. Some groups have investigated arterial compliance in HF-PSF but overall very little is known about vascular function in HF-PSF. Control of arterial tone via arterial endothelial and smooth muscle function is likely to influence arterial stiffness and compliance, but has never been investigated in HF-PSF. Venous capacitance and control of venous tone are important determinants of LV preloading conditions that, if abnormal, could theoretically contribute to increased LVEDP and the clinical syndrome of heart failure. As with arterial endothelial and smooth muscle function, venous function has never been studied in HF-PSF.

Vascular function (and dysfunction) is dynamic and complex. There are multiple methods available to assess vascular function. In the following section, I will provide an

overview of the aspects of vascular dysfunction that I feel are most relevant to the pathophysiology of HF-PSF. I will discuss the concept, clinical relevance and assessment methods of: arterial stiffness and compliance; arterial endothelial and smooth muscle function; venous capacitance; and venous endothelial function.

### 1.5.1 Arterial stiffness and arterial compliance

The terms arterial stiffness and arterial compliance are often used to describe the properties of the arterial tree. It is a common belief that these terms are interchangeable, but they are actually slightly different. As outlined in the preceding section, arterial stiffening may have an important role to play in the pathophysiology of HF-PSF. Arterial stiffening is an all-encompassing term used to describe rigidity of the arterial tree. To understand pathological arterial stiffening, one must first appreciate the main mechanisms affecting blood flow through the arterial tree, one of which is arterial compliance.

The term 'arterial compliance' refers to the ability of large arteries, namely the aorta and its major branches, to distend in response to a given pressure. The aorta and major branches act as an elastic reservoir, buffering pulsatile flow from LV ejection, and transmitting less-pulsatile flow to the peripheral vasculature. This is known as windkessel theory.

The windkessel model of the circulation was originally proposed by Hales in 1733 [61] and further developed by Frank in 1899.[62] An analogy was made between the hand-operated fire-fighter water pump (windkessel in German) and the relationship between

the left ventricle, aorta and conduit vessels. The pump was primed with intermittent air injections. When the air pressure within the pump reached a certain level, a steady stream of water was forced out. Windkessel theory compares this sequence of events with the buffering action of the aorta, receiving pulsatile flow from the left ventricle and delivering relatively steady flow to the conduit vessels. This analogy enhanced early understanding of blood flow through the arterial tree.[63] Although windkessel theory helps to understand the importance of elastic and conduit characteristics of arteries, this model of the circulation assumes that the arterial tree has separate elastic and conduit compartments. In fact most of the arterial tree has both elastic and conduit properties, although one or other tends to predominate in any given arterial segment. For example, the ascending aorta is predominantly elastic and the peripheral, more muscular, arteries are predominantly conduit vessels.

### 1.5.2 Propagation of the arterial pressure wave

The second mechanism is propagation of the arterial pressure wave through the arterial tree. The speed at which the arterial pressure wave is transmitted is termed the pulse wave velocity. Young originally described the relationship between PWV and stiffness of the vessel wall in 1804. The concept was developed in the first half of the 20$^{th}$ Century when work by Frank in 1920 [64] and then Bramwell and Hill in 1922 [65] led to development of the Moens-Korteweg equation:

$$PWV=\sqrt{E_{inc}} \bullet h/2R\rho$$

(Where $E_{inc}$ is Young's Modulus of the arterial wall, h is wall thickness, R is end-diastolic radius and ρ is blood density).

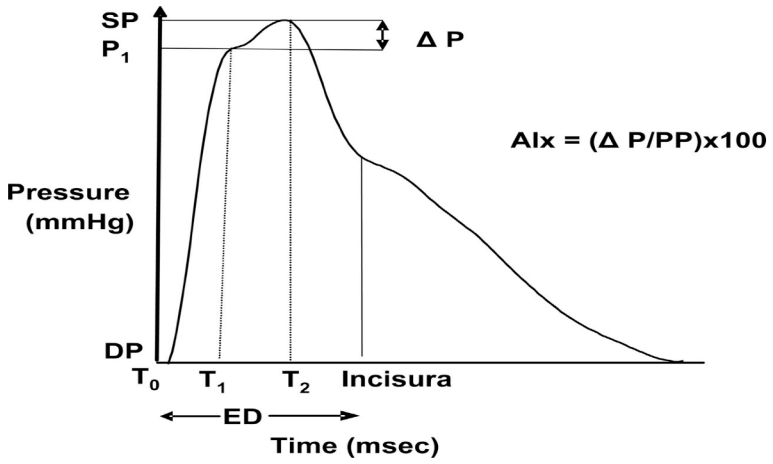
The velocity of the pulse wave is inversely proportional to the elasticity of the vessel wall. Pulse wave velocity may be measured by recording the pulse wave at a proximal and distal artery (e.g. carotid and femoral), either simultaneously or gated to the electrocardiogram (ECG), using pressure transducers, Doppler ultrasound probes or applanantion tonometry.[66] The distance travelled is determined by measuring the body surface distance between the proximal and distal arterial points. The resulting calculation gives a direct measure of arterial stiffness that is easily reproducible.[67, 68]

### 1.5.3 Arterial pressure wave reflection and diastolic waveform analysis

The arterial pressure waveform is a composite of incident and reflected waves. Wave reflection occurs at points of impedance mismatch, such as vessel bifurcation and is also influenced by the diameter and contractile properties of the resistance arterial bed.[69] The point at which the reflected wave returns to the proximal aorta is dependent upon the speed with which the incident wave travels along the aorta, i.e. the PWV. In health the reflected wave arrives in the proximal aorta in diastole, augmenting diastolic pressure and facilitating coronary artery flow. When, as a result of arterial stiffening, PWV increases, the reflected wave may arrive earlier, in systole. The reflected wave superimposes on the incident systolic wave, augmenting systolic blood pressure. This early return of the reflected wave during systole results in a steepening of the diastolic

decay contour of the pressure waveform, reduced diastolic blood pressure and widening of the pulse pressure.[70] The proportion of this augmented pulse pressure that results from wave reflection is termed the augmentation index (AIx). Therefore, aortic PWV, peripheral wave reflection from resistance arteries and pulse pressure are directly related. An example of the central aortic waveform is shown in figure 1.5.

**Figure 1.5:**
The central (aortic) pressure waveform consists of an incident wave, generated by systolic left ventricular ejection, and a reflected wave, from impedance points of the peripheral arterial tree. Haemodynamic parameters are derived by analysis of the central aortic pressure wave. T0 indicates the time at the start of the waveform; T1, duration from start of waveform to the first peak/shoulder (incident pressure wave); T2, duration from start of waveform to the second peak/shoulder (reflected pressure wave); ED, ejection duration, or duration from start of waveform to closure of the aortic valve (incisura); SP, central aortic systolic pressure; DP, central aortic diastolic pressure; P1, P1 height difference between the minimum pressure and the pressure at the first peak/shoulder (T1); augmentation (P), difference between maximal pressure (central aortic systolic pressure) and pressure at the fist peak/shoulder (P1 height); PP, pulse pressure; and AIx, augmentation index. Figure from Williams et al, 2006.[71]

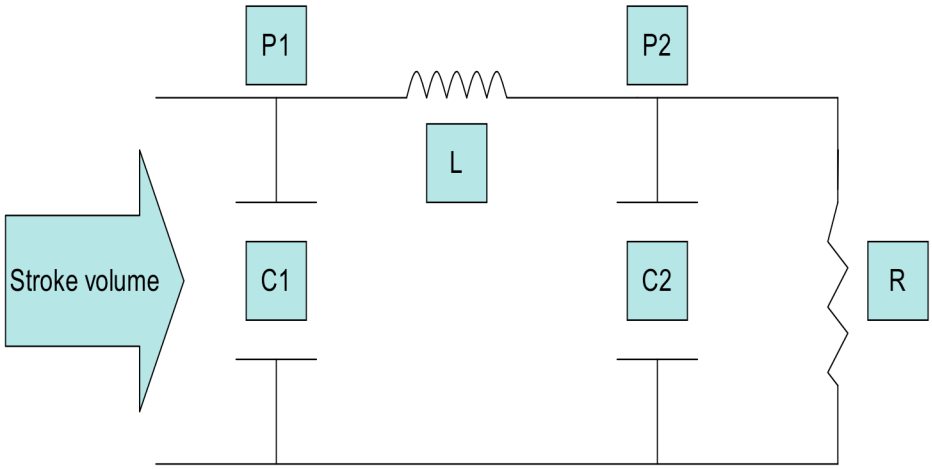


In an attempt to characterise the properties of resistance vessels, i.e. points of impedance mismatch and wave reflection, more advanced models of the circulation have been developed. Initial work by Hales and Frank [61, 62] on windkessel models of the circulation provided the building blocks for development of these more advanced models.

Flow within a blood vessel is determined by two factors: 1) the difference in pressure between both ends of the vessel, i.e. the pressure gradient forcing blood through the vessel; and 2) the resistance of the vessel to flow. In a two-element windkessel model, these two factors are described in terms of an electrical circuit, as a resistor and an inductor. The human circulatory system is clearly far more complex than a simple two-element model, and the concept was developed accordingly. A third-order windkessel model was proposed by Watt et al in 1976 [72] and includes two capacitors in the circuit, representing the total compliance of proximal large arteries, and distal small arteries, termed C1 and C2, respectively.

Cohn and colleagues combined this electrical analogy of the circulation with analysis of the arterial pressure waveform, in particular the diastolic portion of the waveform. They analysed the diastolic waveform in terms of two components. 1) Diastolic decay of the incident wave representing elastic recoil of the proximal, large arteries. This is termed capacitive compliance, or C1. 2) Fluctuations of the diastolic waveform due to reflected waves from small, peripheral resistance vessels. This is termed oscillatory compliance, or C2 (figure 1.6).[73]

**Figure 1.6:**
Modified windkessel model used for analysis of vascular properties. C1 indicates capacitive compliance; C2, oscillatory compliance; R, systemic vascular resistance; L, inertia of the blood; P1, proximal pressure; and P2, distal pressure. Figure adapted from Cohn J et al, 1995.[73]



### 1.5.4 Clinical significance of arterial stiffness

There are many factors that affect arterial stiffness *in vivo*. The proportion of elastin to collagen fibres in the vessel wall is extremely important, with the number and integrity of elastin fibres being closely related to the elastic properties of the aorta and major branches.[74] Another factor which must be considered when evaluating arterial stiffness is the distending pressure of blood within the vessel lumen. With increasing pressure, there is increased recruitment of less elastic collagen fibres in the vessel wall.[75] Therefore, increased distending pressure, for example in systolic hypertension, results in increased arterial stiffness through two mechanisms: 1) increased distending intra-luminal pressure; and 2) arterial remodelling, with thinning and fragmentation of elastin fibres, which are eventually replaced by collagen, fibrosis and in advanced stages, calcification.[76, 77] This process results in a positive feedback loop of increased pressure resulting in increased stiffness from vessel distension and arterial remodeling, elevation of PWV, early return of reflected pressure waves, further augmentation of systolic pressure and vascular damage (figure 1.7).[70]

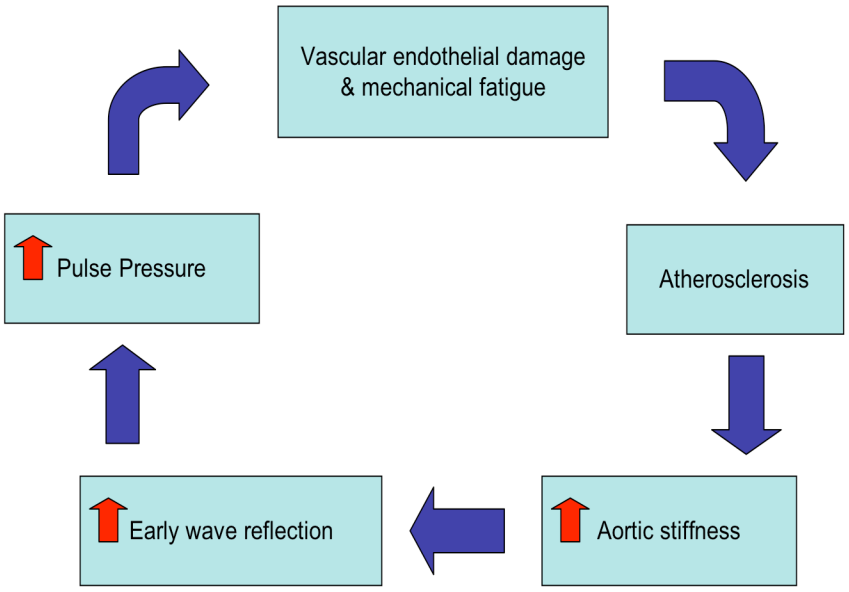
Other important influences on arterial stiffness include the bulk and tone of smooth muscle in the vessel wall, the latter being partly controlled by endothelium [78] and neurohumoral factors.[79] As the body ages, the aorta undergoes significant structural change. With increasing age, there is a linear relationship with elevation of the systolic blood pressure in association with reduction of the diastolic blood pressure and widening of the pulse pressure.[80] Pulse pressure is related to the development of surrogate markers of cardiovascular risk, such as LVH.[81] Pulse pressure itself is an independent

predictor of cardiac events, as evidenced by data from the Framingham study [82, 83] and from large longitudinal studies in France [84] and Germany.[85] As with PWV, pulse pressure rises with age [80] and is an important factor in the development and clinical course of cardiovascular diseases, including heart failure.[86-88] A landmark study comparing pulsatile haemodynamics non-invasively between patients with HF-RSF and controls without heart failure, was performed by Mitchell et al.[89] This study demonstrated that patients with HF-RSF have both wider pulse pressure and increased characteristic aortic impedance (termed Zc – a measure of proximal aortic stiffness) compared to controls, indicating that properties of the arterial tree are likely to be implicated in the pathophysiology of heart failure.

Perhaps unsurprisingly, PWV has also been shown to be an independent predictor of cardiovascular risk. Blacher et al demonstrated that elevated PWV predicts cardiovascular mortality in patients with end-stage renal disease.[90] Pulse wave velocity also predicts cardiovascular events in those with traditional cardiovascular risk factors, such as patients with hypertension,[91] diabetes,[92] and in subjects over 70 years of age.[93]

Analysis of diastolic pressure waveforms has shown that diabetes is associated with abnormal peripheral arterial function,[94] and that elderly subjects display abnormalities of both central and peripheral arterial function.[95] There are, as yet, no data on the ability of diastolic pressure waveform analysis to predict cardiovascular outcomes.

**Figure 1.7:**
Positive feedback loop indicating the pathogenesis of arterial stiffness. Figure adapted from Dart AM, Kingwell BA, 2001.[70]



### 1.5.5 Assessment of arterial stiffness

There are many ways of assessing arterial stiffness *in vivo*, both invasively and non-invasively. In recent years there has been much interest in the non-invasive assessment of arterial function, particularly in view of the risks to patients associated with invasive instrumentation. Non-invasive assessment of arterial stiffness lends itself to a wide range of uses in the research arena, and there has been a large amount of literature published in this area, as reviewed by Oliver and Webb.[96] In a joint venture of the European Society of Cardiology and the European Society of Hypertension, the European Network for Non-invasive Assessment of Large Arteries was formed and has reviewed methods currently used to assess arterial stiffness non-invasively, deeming carotid-femoral PWV to be the 'gold standard' method.[67] In view of the correlation between increased arterial stiffness and increased risk of cardiovascular events, non-invasive assessment of arterial stiffness is gathering momentum as a means of estimating cardiovascular risk in the clinical setting.

### 1.5.6 Arterial endothelial function – relevance to heart failure

As demonstrated nearly 40 years ago by Zelis et al [97] patients with heart failure have impaired peripheral vasodilatation in response to various stimuli. Over the years, it has become apparent that arterial endothelial dysfunction plays an important role in the attenuation of peripheral vasodilatation observed in heart failure patients.[78] Arterial endothelial dysfunction has been well documented in HF-RSF [98-102] and is due to multiple factors, including maladaptive overactivity of the RAAS system,[79]

circulating endothelin [103] and oxidant stress.[18, 104, 105] The vascular endothelium is integral to maintaining normal vessel tone and function and, therefore, influences arterial stiffness.[76] It could be argued that abnormal arterial endothelial function increases arterial stiffness and may adversely affect ventriculo-vascular interaction: a probable key factor in the pathophysiology of HF-PSF. Impaired arterial endothelial function is associated with reduced arterial compliance in older individuals,[106] which may be relevant in patients with HF-PSF who tend to be elderly. However, no such studies have been performed in subjects with HF-PSF and whether or not HF-PSF is associated with arterial endothelial dysfunction is not known.

### 1.5.7 Methods of assessing arterial endothelial function

There are several methods available for the assessment of arterial endothelial function. Much of our original information about vascular function, including the role of arterial endothelial function, was obtained with *in vitro* (or *ex vivo*) studies of blood vessels mounted on strain gauges and exposed to vasoactive agents. This method was used to demonstrate that vascular endothelium plays a role in vasodilatation [107] and that endothelium-derived relaxing factor, later proven to be nitric oxide, is the key endogenous vasodilating agent released by vascular endothelium.[108] A related method, termed wire myography, has been used more recently using human vessels taken from patients with cardiovascular diseases, such as heart failure and coronary artery disease.[109]

*In vivo* studies of vascular function obviously have the advantage of providing information on 'real life' arterial endothelial function in the context of the disease process under investigation.

The most widely used technique involves cannulation of the brachial artery and intra-arterial infusion of vasoactive agents, most commonly the endothelial-dependent vasodilator acetylcholine and the endothelial-independent vasodilator sodium nitroprusside, combined with forearm venous occlusion plethysmography to assess changes in forearm blood flow.[100-102, 110]

An alternative method of measuring changes in forearm blood flow is referred to as flow-mediated dilatation. Flow-mediated dilatation can be measured with a variety of techniques, one of the commonest being high fidelity ultrasound of the brachial or radial artery. Changes in diameter of the forearm resistance arteries correspond closely to forearm blood flow. This technique can be used with post-ischaemic reactive hyperaemia, which is predominantly influenced by arterial endothelial function,[99] or with intra-brachial infusions of endothelium-dependent and –independent vasodilators.[111]

Another non-invasive method of assessing endothelial function involves administering vasoactive agents, typically acetylcholine and sodium nitroprusside, to the microvasculature of the skin using low voltage electrical current. This method of drug delivery is known as iontophoresis. The corresponding vasodilatory effect is then measured with Laser Doppler imaging, with increased flow of red blood cells during vasodilation giving higher intensity Laser Doppler readings, or 'flux'.[112] In the

literature this technique of measuring microvascular responses to vasoactive agents is termed 'Laser Doppler iontophoresis', 'Laser Doppler fluximetry' or 'Laser Doppler flowmetry' and has been used to assess microvascular endothelial function in patients with HF-RSF [113] and in patients with various cardiovascular risk factors.[114-116] Laser Doppler iontophoresis has been shown to be reproducible [117] and results correlate well with those obtained by alternative methods of measuring arterial endothelial function, such as wire myography [118] and flow-mediated dilatation.[116, 119]

### 1.5.8 Venous capacitance

The venous system has been studied far less than the arterial system in cardiovascular research. As a result, there is limited information on the role of the venous system in cardiovascular disease. The venous system is best described in terms of pressure-volume relationships.

Venous capacitance refers to the ability of the venous bed to increase in volume in response to pressure. Venous capacitance is predominantly influenced by two factors:

1) Intra-luminal venous pressure and the corresponding effect on vessel wall tension. Veins are thin-walled and extremely distensible at low pressures. However, at the peak of the pressure-volume curve veins become less compliant and the curve plateaus out (figure 1.8).[120]

2) Changes in venous tone. Venous tone is actively controlled by mural smooth muscle, which in turn is influenced by venous endothelium. These mechanisms are discussed in more detail in section 1.5.10.

Most of the circulating volume lies in the venous bed, which acts as a capacitance reservoir. Changes in venous capacitance can have dramatic effects on LV filling pressure, or pre-load, and stroke volume.[121]

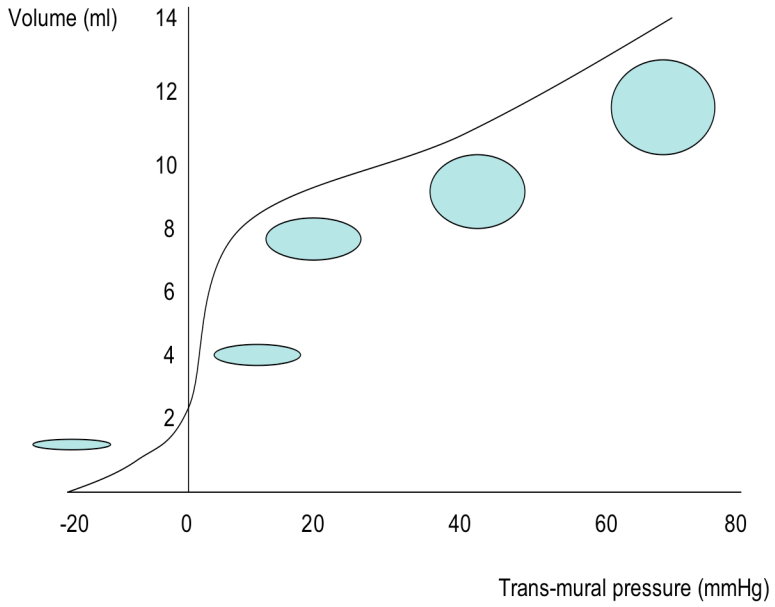
Assessment of venous capacitance has predominantly been performed by measuring changes in either upper or lower limb volume in response to occluding venous return from the limb. The assumption being that changes in volume will be attributable to venous distension and pooling, as bone and muscle have a relatively constant volume at rest.

This investigative technique is termed venous occlusion plethysmography. Essentially, circumferential changes in limb circumference during venous congestion are measured and pressure/volume data is extrapolated to give a measurement of venous capacitance. The basic principles of performing venous occlusion plethysmography in the limbs were first described by Hewlett and van Zwaluwenburg in 1909 [122] and have become more sophisticated over time. Changes in limb circumference were originally recorded using a fluid-filled cuff to enclose the whole forearm. The rise in forearm volume caused deflection of a column of water that was then measured. In the late 1940's, a mercury-filled rubber gauge was developed to measure changes in limb circumference. The gauge forms part of a constant current electrical circuit and will provide a recordable

linear output that responds to tension on the gauge. This technique was introduced by Whitney [123] and further developed by Hokanson and colleagues.[124]

More recently, radionuclide plethysmography has been developed. Although technically more challenging, radionuclide plethysmography facilitates the assessment of multiple vascular beds, such as pulmonary and splanchnic, in addition to the vasculature of the upper and lower limbs.[120]

**Figure 1.8:**
Relationship between trans-mural pressure and volume in veins, demonstrating marked compliance at low pressure but relatively low compliance at higher pressures. Figure adapted from Schmitt M et al, 2002.[120]



### 1.5.9 Venous capacitance – relevance to heart failure

Venous capacitance has been studied in the context of HF-RSF. Reduced venous capacitance is associated with reduced exercise capacity in patients with HF-RSF.[125] Reduced venous capacitance has also been demonstrated to correlate with more severe symptoms of heart failure and elevated pulmonary artery pressure in patients with HF-RSF.[126] There are also data, from canine models of heart failure, to suggest that reduced LVEF can induce marked baroceptor-mediated venoconstriction resulting in a dramatic increase in LVEDP.[127] Frenneaux's group have completed multiple studies using radionuclide plethysmography to assess venous function in patients with HF-RSF and in normal subjects. They have demonstrated that venous capacitance responds appropriately to endothelial-dependent vasodilatation, despite evidence of impaired arterial endothelial function.[98] Their data also show that venous capacitance is under the control of natriuretic peptides both in patients with HF-RSF [128] and in healthy subjects,[129] but that exogenous aldosterone has little effect on venous capacitance in HF-RSF.[130]

Reduced venous capacitance may also play an important role in the pathophysiology of HF-PSF. Patients with HF-PSF are more sensitive to the effects of vasodilators and diuretics on LV filling pressure, suggesting that venous capacitance is reduced.[131, 132] Although there is evidence to support the theory that changes in venous capacitance can influence both symptoms and LVEDP in HF-RSF, there have been no studies of venous capacitance in patients with HF-PSF.

### 1.5.10 Venous endothelial function

The most accurate method of assessing venous endothelial function is with the dorsal hand vein technique, introduced by Nachev, Collier and Robinson in 1971 [133] and modified by Aellig ten years later.[134]

This technique involves placing a sensor, attached to a linear variable transformer, directly over a dorsal hand vein. The vein is then cannulated to allow vasoactive substances to be administered locally. The venous return from the limb is occluded intermittently and compliance of the vein during venous congestion is measured. In this manner, the effects of vasoactive drugs on venous compliance can be assessed in real time.[135, 136] By using endothelium-dependent and –independent vasodilators, a measure of venous endothelial and vascular smooth muscle function can be derived.[137] Most studies using the dorsal hand vein technique have been carried out in healthy adults. Some of these studies have focused on the effects of vasoactive agents which are potentially important in the pathophysiology of HF-RSF, such as angiotensin I and II,[138] endothelin,[139] atrial natriuretic peptide [140] and norepinephrine.[141]

A few groups have performed dorsal hand vein studies in patients with HF-RSF. Love et al characterised the venoconstrictive effects of endothelin receptor agonists in patients with HF-RSF.[142] Dzeka et al demonstrated that patients with HF-RSF have venous endothelial dysfunction and an exaggerated venoconstriction response to prostaglandin inhibition.[143] It has also been shown that HF-RSF patients display marked venoconstriction in response to neuropeptide Y, a sympathetic neurotransmitter, in comparison with healthy controls.[144]

The venous endothelium plays an important role in maintaining venous tone and, therefore, modulates venous capacitance. Previous studies suggest that HF-RSF is associated with venous endothelial dysfunction. The venous endothelium has never been studied in patients HF-PSF and therefore it is not known whether or not venous endothelial dysfunction plays a role in the pathophysiology of HF-PSF.

The interaction of the vascular system with the heart is dynamic and complex. Abnormal vascular function, and increased arterial stiffness in particular, has been shown to adversely affect left ventricular morphology and function, for example the development of LVH in systolic hypertension. As LVH and LV diastolic dysfunction have been associated with HF-PSF, it is possible that abnormal vascular function leads to the development of HF-PSF. However, the role of vascular dysfunction in the pathophysiology of HF-PSF has not yet been established.

## 1.6 Summary

Heart failure is a clinical syndrome that can occur in the presence of reduced or preserved LV systolic function. The investigation of the pathophysiology of HF-PSF to date has answered some questions about potential mechanisms involved but the subject remains an area of controversy and debate. From reviewing the evidence available, diastolic dysfunction of the left ventricle appears to play a key role in the development of heart failure in these patients, as HF-PSF subjects usually have abnormalities of LV

relaxation and filling on invasive assessment.[39] However, the idea that HF-PSF is purely due to diastolic dysfunction has been challenged. The main basis for questioning the 'diastolic heart failure' theory is that parameters of diastolic function vary widely depending on loading circumstances of the left ventricle and do not always correlate with presence of heart failure in population-based studies.[42]

An alternative theory is that impaired vascular function adversely affects LV performance and ultimately leads to development of the heart failure syndrome.

There are some data to support the hypothesis that HF-PSF is causally associated with impaired ventriculo-arterial interaction: both increased stiffness of the left ventricle during systole and reduced arterial compliance have been demonstrated in previous studies.[54, 57] These results are perhaps not surprising, given that LV afterload, i.e. large artery compliance, has long been accepted as a determinant of LV mass and systolic and diastolic performance (section 1.4).

I propose that the role of vascular function in the pathophysiology of HF-PSF has not yet been fully established. Previous studies have suggested that reduced arterial compliance is frequently present in patients with HF-PSF, but the groups included for study have been heterogeneous, with a high percentage of patients having significant co-morbidities. Co-morbidities varied between groups compared and conditions which are known to be associated with reduced arterial compliance, such as diabetes mellitus and coronary heart disease, were not matched for during patient selection. Concomitant medications were also extremely variable between groups, which may have confounded results from previous studies. Furthermore, vascular function in HF-PSF and HF-RSF

has not been directly compared. It is therefore uncertain whether or not vascular dysfunction is secondary to the 'heart failure syndrome', with compensatory activation of neurohumoral and inflammatory systems resulting in abnormalities of vascular function and tone, or if vascular dysfunction is a primary pathophysiological mechanism in the development of HF-PSF. There are other unanswered questions regarding the role of the vasculature in the pathophysiology of HF-PSF. Mechanisms controlling arterial tone and function, such as endothelium and smooth muscle, have been extensively investigated in HF-RSF, but have never been evaluated in HF-PSF. Most investigators of heart failure pathophysiology have neglected to study the venous bed. Although there are some data on venous function in HF-RSF, venous capacitance has not been examined in HF-PSF and may be important in determining LV preload conditions. To date, nothing is known about endothelial control of venous tone in HF-PSF.

My hypothesis is that abnormalities of vascular function are more extensive and severe in HF-PSF than in HF-RSF, supporting the theory that vascular dysfunction plays a key role in the complex pathophysiology of HF-PSF. The studies described in this thesis were designed to test this hypothesis.

# Chapter 2

## Materials and Methods

## 2.1 Patient Selection

This project conforms to the principles outlined in the Declaration of Helsinki and was approved by the West Medical Ethics Committee, Western Infirmary, Glasgow, UK. All patients gave written, informed consent.

### 2.1.1 Study groups

Three groups of patients were recruited:

**HF-PSF** group

Patients with coronary heart disease and HF-PSF.

**HF-RSF** group

Patients with coronary heart disease and HF-RSF.

**Control** group

Patients with coronary heart disease, preserved LV systolic function, and no evidence of heart failure.

### 2.1.2 Definitions and inclusion criteria

***Heart failure was defined as*:**

a) Relevant symptoms/ signs/ radiographic findings as indicated by Boston criteria (table 2.1).[4]

b) Clinical requirement for diuretic therapy

c) Increased plasma N-terminal pro b-type natriuretic peptide (NT-proBNP) concentration (section 2.2.8).

Subjects had to fulfill all three criteria to be included in either heart failure group.

**Table 2.1:**

Boston criteria for diagnosis of heart failure. Each parameter has a corresponding score. Scores are added to give a total. If the total Boston score > 8, the diagnosis of heart failure is deemed probable. JVP: jugular venous pressure; PA: postero-anterior.

| CRITERION | SCORE (if present) |
|---|---|
| ***History*** | |
| Rest dyspnoea | **4** |
| Orthopnoea | **4** |
| Paroxysmal nocturnal dyspnoea | **3** |
| Dyspnoea walking on flat | **2** |
| Dyspnoea walking on incline | **1** |
| ***Examination*** | |
| Pulse 91-110bpm | **1** |
| Pulse > 110bpm | **2** |
| JVP > 6cm | **2** |
| JVP > 6cm + hepatomegaly or peripheral edema | **3** |
| Rales – basilar | **1** |
| Rales – basilar and elsewhere | **2** |
| Wheeze | **3** |
| Third heart sound | **3** |
| ***Chest Radiograph*** | |
| Alveolar oedema | **4** |
| Interstitial oedema | **3** |
| Bilateral effusions | **3** |
| Cardiothoracic ratio > 50% (PA film) | **3** |
| Upper lobe venous diversion | **2** |

***Echocardiographic parameters:***

a) Preserved LV systolic function was defined as:

LVEF of ≥0.50, measured by echocardiography.

b) Reduced LV systolic function was defined as:

LVEF of < 0.40, measured by echocardiography.

Echocardiograms were performed by a single operator, who was blinded to subject group allocation, on a routine basis for clinical reasons in all subjects. Left ventricular ejection fraction was calculated using semi-quantitative assessment with 16 segment wall motion scoring.[145]

***Coronary heart disease was defined as:***

a) Previous myocardial infarction.

AND/OR

b) Symptoms of angina pectoris with exercise electrocardiogram or myocardial perfusion scan evidence of reversible myocardial ischaemia.

AND/OR

c) Evidence of significant coronary artery disease at coronary angiography.

### 2.1.3 Exclusion criteria

a) Significant valvular heart disease (defined as at least moderate dysfunction of one or more heart valves at echocardiography).

b) Atrial fibrillation.

c) Diabetes mellitus.

d) Renal failure (defined as serum creatinine >250μmol/L).

e) Uncontrolled hypertension despite antihypertensive therapy (systolic BP >140 or diastolic BP >90mmHg).

### 2.1.4 Screening process

All emergency admissions to the Western Infirmary Glasgow with a primary diagnosis of heart failure, admitted between August 2003 and June 2005, were screened for potential enrolment in the study.

The central database comprising all attendees of the Heart Function Clinic at the Western Infirmary was screened for patients who could potentially be recruited into the study.

In order to be eligible for the study patients had to be able and willing to undertake two whole mornings of vascular function studies. Recruitment of subjects into the HF-PSF group was therefore slow as a high proportion of the patients were elderly, with multiple co-morbidities, and thus unsuitable. A significant number of patients with HF-PSF also had specific exclusions such as diabetes mellitus or atrial fibrillation.

To speed up recruitment into the HF-PSF group I incorporated twice-weekly review of the echocardiography laboratory log at the Western Infirmary into my screening methods. All patients whose indication for echocardiography was heart failure and who had preserved LV systolic function were screened for potential recruitment into the study.

In total 935 patients were considered for recruitment into the HF-PSF group. Of these 935 patients, 803 were excluded. The commonest reason for exclusion was inadequate objective evidence of heart failure to achieve a Boston score of >8. The second commonest reason for exclusion was the presence of multiple co-morbidities and/or general frailty, making it impractical for patients to undergo two full mornings of vascular function studies. The remaining exclusions were on the basis of pre-defined exclusion criteria, most frequently diabetes mellitus or atrial fibrillation. Of the 132 patients who were suitable for recruitment into the HF-PSF group, 112 declined to participate. 8 patients agreed to participate after initial invitation but later cancelled or did not attend for vascular function studies. Therefore, 12 patients were successfully recruited into the HF-PSF group.

Control subjects and HF-RSF subjects were identified and recruited by screening patients attending general cardiology and Heart Function clinics at the Western Infirmary.

## 2.2 Study Design

This study was conducted in the Clinical Investigation and Research Unit (CIRU), Gardiner Institute, Western Infirmary, Glasgow, UK. Subjects attended the CIRU on two occasions in the morning following a light breakfast. Subjects were asked to abstain from caffeine and tobacco for 12 hours and to omit their morning medication prior to their visit.

### 2.2.1 Schedule of experiments

***First visit***

*The experimental protocol was as follows:*

1) An equilibration period of 30 minutes supine rest in a temperature-controlled (21-23$^{o}$C) vascular study room.

2) Applanation tonometry: carotid-femoral (aortic) PWV.

3) Applanation tonometry: radial arterial diastolic waveform analysis. This experiment includes heart rate and brachial blood pressure measurement.

4) Laser Doppler iontophoresis.

5) Venous occlusion plethysmography.

***Second visit***

1) Equilibration period of 30 minutes supine rest in a temperature-controlled (21-23$^{o}$C) vascular study room.

2) Aellig dorsal hand vein study.

3) Venepuncture for NT-proBNP, renal biochemistry and total cholesterol.

Both visits were conducted under identical conditions, as described above, one week apart.

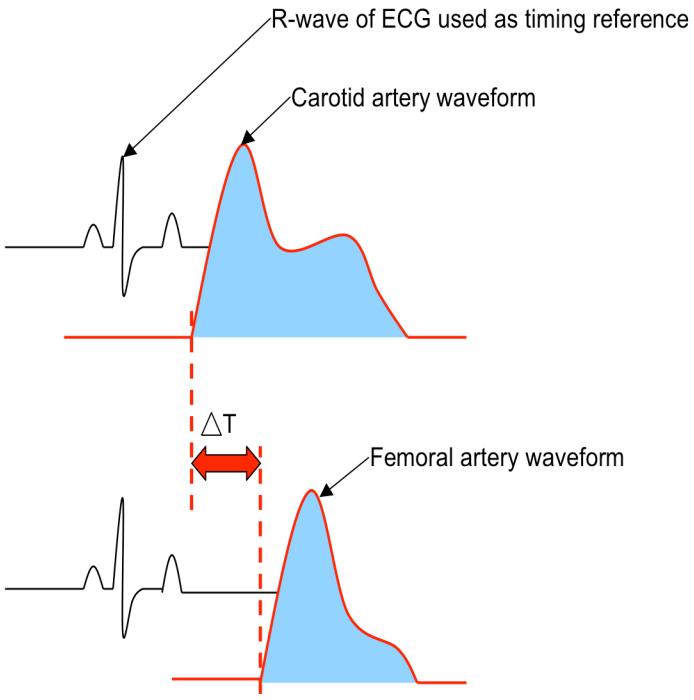
The duration of the first visit was approximately 4 hours and the duration of the second visit was between 3 and 4 hours. Offline processing of raw data combined with subsequent statistical analysis took between 12 and 13 hours per patient studied. Therefore the total time to perform vascular function studies including processing and analysing the data was between 19 and 21 hours per patient.

### 2.2.2 Aortic pulse wave velocity

Aortic pulse wave velocity (PWV) was measured using a high fidelity micromanometer (SPC-301; Millar Instruments, Texas, USA) coupled with the SphygmoCor™ system (SphygmoCor™ BPAS; PWV Medical, Sydney, Australia). The body surface distance between points of maximal palpable pulsation at carotid and femoral arteries was measured in millimetres. A hand-held micromanometer-tipped probe was applied to the skin overlying the carotid and femoral arteries in turn, at the point of maximal palpable pulsation. Arterial waveforms were recorded for a minimum of 12 seconds at both carotid and femoral sites. The pulse wave recorded at each point was gated to a simultaneous electrocardiogram. The time delay between initial wave reflection at the proximal (carotid) artery and the distal (femoral) artery was measured with the "foot-to-foot" method to give a value in milliseconds (figure 2.1). The time difference between

successive arterial wave reflections and the body surface distance between the measurement points of carotid and femoral arteries were then assimilated by the software program to calculate PWV in metres per second (m/s). The quality control parameters integral to the SphygmoCor™ software were used to determine the quality of arterial waveform and ECG recordings. The foot of the waveform and the R-wave of the ECG must be easily identified from at least three successive beats at each measurement point to calculate PWV. The variation in heart rate between carotid and femoral recordings must be less than 6%. PWV measurements must have a standard deviation of 10% or less. Three PWV measurements meeting these quality control criteria were recorded for each subject and averaged for analysis. Pulse wave velocity data were transferred to a personal computer and collated using Microsoft Excel software for Windows (Microsoft Corp., Seattle, Washington) before undergoing statistical analysis with Prism Graphpad software for Windows (Graphpad Software Inc., San Diego, California, USA).

**Figure 2.1:**
Aortic pulse wave velocity (PWV) calculation using the "foot-to-foot" method; the arterial pressure waveform at the carotid and femoral arteries is gated to the R-wave of the electrocardiogram (ECG).
PWV = L / Δ T (L = Length between surface measurement sites in mm, Δ T = transit time in m/s.)



### 2.2.3 Arterial diastolic waveform analysis

Arterial pressure waveform analysis was performed and central and peripheral arterial compliance, derived from a modified windkessel model, measured using the Hypertension Diagnostic incorporated (HDI) system (HDI pulse wave CR-2000; Hypertension Diagnostics Inc., Egan, Minnesota, USA). As mentioned below, this device was also used to measure heart rate and brachial blood pressure in all subjects. An acoustic transducer was applanated over the radial artery of the dominant arm. A wrist stabiliser was positioned on the wrist to gently immobilise the wrist and stabilise the radial artery making it accessible for optimum placement of the arterial pulse pressure sensor. The pressure sensor was positioned over the radial artery and held in place using a holding and positioning device on a manually adjustable shaft. Pulse pressure waveforms were recorded by placing the device over the point of maximal arterial pulsation. The applanating pressure was adjusted by rotating the knob on the top of the device until optimal waveforms were obtained. A sphygmomanometer cuff was placed around the upper non-dominant arm. Heart rate, blood pressure and arterial pressure waveforms were recorded for 30 seconds and analysed with integrated HDI software. Three measurements were taken and averaged for off-line analysis. Using curve-fit analysis of the diastolic pulse waveform and a modified windkessel model of the circulation, the HDI software calculates derived measurements of large (central or 'capacitive') artery compliance, small (peripheral or 'oscillatory') artery compliance and systemic vascular resistance, along with averaged heart rate and brachial blood pressure

values. Averaged values for large and small artery compliance, systemic vascular resistance, heart rate and blood pressure data was transferred to a personal computer and collated using Microsoft Excel software for Windows (Microsoft Corp., Seattle, Washington) before undergoing statistical analysis with Prism Graphpad software for Windows (Graphpad Software Inc., San Diego, California, USA).

### 2.2.4 Heart rate and brachial blood pressure measurement

The Hypertension Diagnostic Inc. system (HDI pulse wave CR-2000; Hypertension Diagnostics Inc., Egan, Minnesota, USA) was used to measure heart rate and blood pressure in all subjects. As described above, heart rate was recorded for 30 seconds during each measurement of arterial pressure waveforms. Also during this time, a brachial blood pressure reading was taken using a semi-automated sphygmomanometer. Heart rate and brachial blood pressure were recorded three times and averaged.

### 2.2.5 Microvascular function

Microvascular function was measured with Laser Doppler imaging and iontophoresis of endothelial-dependent and -independent vasodilators.[112, 146]

***Iontophoresis***

Two iontophoresis chambers were attached to the skin of the volar aspect of the right forearm by means of double-sided adhesive discs, avoiding hair, broken skin and superficial veins.

The iontophoresis chambers (ION 6; Moor Instruments Ltd., Axminster, UK) are constructed from Perspex with an internal platinum wire electrode. Following application to the skin, the chambers were connected to the anode and cathode connections of the iontophoresis controller. A digital multimeter was connected in parallel to monitor voltage across the chambers (figure 2.2). As a constant current source was used, resistance values were calculated from the recorded voltages using Ohm's law.

Control of current delivery has been pre-programmed into the software for the Laser Doppler imager such that the current is switched on at the beginning of a scan and remains on until the start of the following scan. The current then either remains on for the next scan or is switched off once the total charge has been delivered. Current duration was determined by the time taken to complete each scan (50s) multiplied by the total number of scans. To limit the iontophoresis dose, low currents were used. The protocol involves incremental current delivery of four scans at 5 microamps, four at 10 microamps, four at 15 microamps and two at 20 microamps, giving a total charge of 8 mC.

Drugs used: 2.5 ml of 1% acetylcholine chloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) was introduced into the anodal chamber; 2.5ml of 1% sodium nitroprusside (Sigma) was introduced into the cathodal chamber. The vehicle for both drugs was 0.5% sodium chloride solution.

Fluid was prevented from escaping by placing circular 32 mm coverslips over the chambers. Thus both agents were delivered simultaneously during each period of current administration.

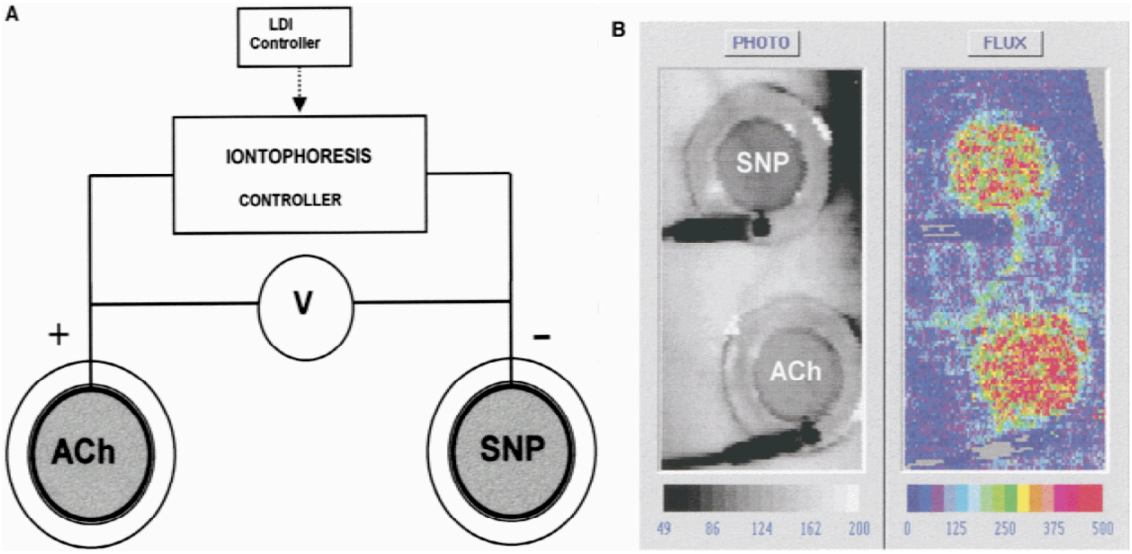
***Perfusion measurements***

Non-invasive measurement of skin perfusion was performed with a Laser Doppler Imager (Moor Instruments Ltd.) equipped with a red laser (wavelength 633 nm, power 1 mW, beam diameter 1 mm). The laser was scanned in a raster fashion over both chambers and through the coverslips. The backscattered light was collected by photodetectors and converted into a signal proportional to perfusion in arbitrary perfusion (flux) units that are displayed as a colour-coded image on a monitor (figure 2.2). Perfusion measurements were obtained using the imager manufacturer's image analysis software. Analysis of perfusion measurements were performed off-line to yield the median flux value across approximately 700 measurement points. Twenty sequential scans were taken. The first was taken as a control prior to current administration, followed by the protocol of fourteen scans described above, followed by five scans with no current administration. The biological zero was measured with a single scan taken during occlusion of arterial blood flow by inflation of a sphygmomanometer cuff to supra-systolic levels (200 mmHg). During off-line analysis, the colour-coded images were used to trace the two-dimensional area of each chamber in turn to generate a region of interest (ROI) for each scan. The integrated software then calculated median flux values from acetylcholine and sodium nitroprusside chambers for each scan. These raw

data were collated with Microsoft Excel software. Professor William Ferrell, University of Glasgow, performed statistical analysis of the data using Microsoft Excel software, as discussed below.

Using this method, I compared skin microvascular responses to endothelial-dependent (acetylcholine) and endothelial-independent (sodium nitroprusside) vasodilators.

**Figure 2.2:**
A) Schematic of experimental setup. Acetylcholine (ACh) was placed in the anodal chamber and sodium nitroprusside (SNP) in the cathodal chamber. A digital voltmeter (V) was connected between the two chambers. B) Backscattered light intensity provides the photo image and the Doppler-shifted component provides the flux image. Flux is colour-coded with lowest perfusion in dark blue and highest in dark red. Figure from Ramsey et al, 2002.[112]



### 2.2.6 Venous capacitance

Forearm venous capacitance was measured with venous occlusion plethysmography using mercury-in-silastic strain gauges (D.E Hokanson inc., Bellevue, USA) placed around the non-dominant forearm approximately 10 cm from the olecranon process at the point of greatest circumference. Just prior to measuring venous capacitance, the hand was excluded from the circulation with a wrist cuff inflated to 220 mmHg. Venous capacitance was measured using the equilibration method – inflating the upper-arm cuff to 45 mmHg for 2 minutes and 30 seconds. Rapid cuff inflators were used to inflate the cuffs. Venous capacitance was recorded 3 times in each patient and averaged. The voltage readings from the plethysmograph were transferred through a MacLab/8E (ADInstruments, Hastings, United Kingdom) to a Macintosh computer (Apple Computers Inc., Cupertino, California, US) for analysis using Chart software (version 3.2.8, ADInstruments) and then transferred to a personal computer for statistical analysis as detailed below.[147]

### 2.2.7 Venous endothelial function

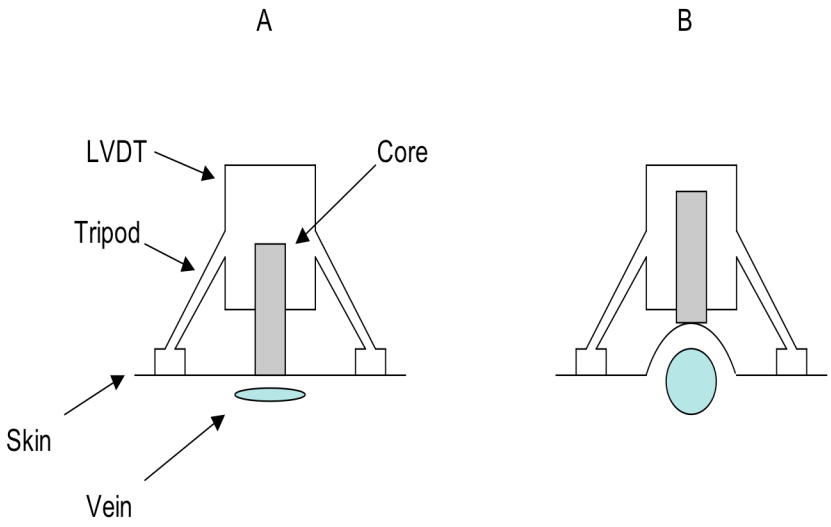
Venous endothelial function was assessed using the modified Aellig dorsal hand vein technique.[134] Figure 2.3 details a schematic representation of the experimental setup. A suitable dorsal hand vein was selected and cannulated with a 34-gauge butterfly cannula. Physiological (0.9% NaCl) saline was infused into the dorsal hand vein at a rate of 0.3 ml/minute for 30 minutes.

After this period, the transducer was mounted over the selected vein via a tripod and venous dilatation in response to congesting pressure of 45 mmHg, applied with a brachial sphygmomanometer cuff, was measured. This measurement was repeated three times and the average value used as the baseline range of venodilatation. The saline infusion rate was then reduced to 0.2 ml/minute. A second line was added to the butterfly cannula and a concomitant infusion of phenylephrine was commenced to constrict the vein. The dosage of phenylephrine was increased as follows in a stepwise fashion between 1 and 2500 ng/minute. Each dose was infused at 0.1 ml/minute for 6 minutes and the venous response measured between the $4^{th}$ and $6^{th}$ minute. The phenylephrine dose that produced 70% venoconstriction from baseline (ED70) was determined and infused for the remainder of the study. To ensure stable venoconstriction, the ED70 was infused for 12 minutes with venous responses being measured between 4 to 6 and 10 to 12 minutes. When stable venoconstriction had been established, the saline infusion was reduced further to 1 ml/minute and a third line was added to the cannula.

To assess endothelial-dependent venodilatation, a concomitant infusion of acetylcholine was commenced and increased sequentially at the following doses: 0.1, 1, 5, 10, 50 nmol/minute up to a maximum dose of 100 nmol/minute. Each dose was infused at 0.1 ml/minute for 6 minutes, with the venous response being measured between the $4^{th}$ and $6^{th}$ minute. Acetylcholine causes endothelial-dependant venodilatation at low doses and smooth muscle-dependent venoconstriction at higher doses. The dose of acetylcholine was increased until venodilatation had peaked and then diminished to 20% below the

peak, at which point all infusions were discontinued and the butterfly cannula removed. The maximum venodilatation by acetylcholine was then calculated to give a measure of venous endothelial function. The raw data were transferred to a personal computer and collated using Microsoft Excel software for Windows (Microsoft Corp., Seattle, Washington) before undergoing statistical analysis with Prism Graphpad software for Windows (Graphpad Software Inc., San Diego, California, USA).

**Figure 2.3:**
Schematic of experimental setup for the Aellig modified dorsal hand vein technique. A) Shows the mobile core over the collapsed vein. B) Shows the core position after vein dilatation in response to inflation of a sphygmomanometer cuff around the upper arm to a congesting pressure of 45 mmHg. Movement of the core within the linear variable differential transformer (LVDT) is converted into an output in volts that is graphed to give venous dilatation measurements.
Adapted from Aellig WH, 1981.[134]



### 2.2.8 Blood tests

All serum samples were taken from the antecubital fossa with the patient in the supine position for a minimum of 30 minutes prior to venesection.

Serum samples for urea and electrolytes and total cholesterol were analysed in the Biochemistry Laboratory at the Gartnavel General Hospital Glasgow, using standard methods.

Blood samples for plasma NT-proBNP were taken directly to the research laboratory in the Western Infirmary and spun directly at 4°C in a centrifuge for 15 minutes at 3,000 rpm. The supernatant was removed and put into separate freezer containers labelled with the patient subject number and stored in the freezer at -20°C until the assays were analysed.

The Roche Elecsys proBNP (Roche Diagnositics, East Sussex, England) immunoassay was used to analyse NT-BNP (proBNP). The Elecsys method used was an electrochemiluminescent immunoassay on an Elecsys 2010 autoanalyser. This has a within-assay and between-assay variability of 2.7 and 3.2% respectively. The measuring range of the assay is 5–35,000 pg/ml. The analytical sensitivity of the assay is 5 pg/ml. From work conducted on a healthy population, the following 95$^{th}$ percentile figures were established as normal ranges dichotomised for age and sex (table 2.2).[148] An elevated NT-proBNP was taken to be a value greater than the 95$^{th}$ percentile for each age and sex category.

The NT-proBNP immunoassays were completed by Dr Ian Morton who was blinded to clinical details, including LV systolic function. The results were recorded on a Microsoft Excel spreadsheet separate from any other clinical details or patient identifiers.

**Table 2.2:**

95$^{th}$ percentile according to age and sex of NT-proBNP for a healthy population.

| | **NT-proBNP concentration pg/ml** | | | |
|---|---|---|---|---|
| **Age (years)** | ≤ 64 | 65-69 | 70-74 | ≥ 75 |
| **Female** | 213.4 | 314.2 | 338.5 | 355.3 |
| **Male** | 122.6 | 112.6 | 236 | 295.7 |

## 2.3 Power Calculation, Data Collection and Statistical Analysis

### 2.3.1 Power calculation

Dr Chris Weir from the Department of Statistics at the University of Glasgow provided statistical advice on power calculation and data analysis. I performed the power calculation for this study using data from a prior validation study in our department using the SphygmoCor™ device to measure PWV (Dr Arthur Doyle – personal communication). Ten subjects over 60 years of age with hypertension, normal renal function and no other manifest cardiovascular disease underwent carotid-femoral PWV assessment. Data from this study are appropriate for use in my power calculation as patients with hypertension are at risk of developing heart failure. The standard deviation of PWV in Dr Doyle's sample was 0.45 m/s. Therefore to detect a difference in PWV between groups of at least 0.66 m/s, with 90% power, using analysis of variance (ANOVA), eleven subjects per group were required. As multiple comparisons were to be made, Bonferroni post-test correction was to be employed in statistical analysis. Significance level was set at 5% (between group differences were considered significant if p value $<0.05$).

### 2.3.2 Data collection and statistical analysis

Data were collated with Microsoft Excel Software for Windows (Microsoft Corp., Seattle, Washington). Statistical analysis and figure preparation was performed using

Prism Graphpad software (Graphpad software Inc., San Diego, California, USA) apart from microvascular endothelial function data (Laser Doppler iontophoresis), which were analysed with Microsoft Excel. Baseline characteristics of patients were summarised by mean (standard deviation) for continuous variables and by frequency for categorical variables. Comparisons of continuous variables between groups were made using t-tests and ANOVA with Bonferroni correction for multiple comparisons. Data are presented as mean (standard deviation) in the text and as box (median and inter-quartile ranges) and whisker (range) plots in figures apart from microvascular and venous endothelial function data, which are presented as dose-response graphs.

During analysis of Aellig dorsal hand vein data, it was apparent that there was marked variability in baseline vein diameter between subjects. There was also significant variability in responses to pre-constriction with phenylephrine and subsequent dilatation with acetylcholine, both within and between study groups. Although stable venoconstriction was achieved in all studies included in analysis, exact ED70 (70% constriction from baseline) was not achieved in all studies. Similarly, there was variability of venodilatation in response to acetylcholine within and between groups, with some subjects achieving peak venodilatation at low doses of acetylcholine, and some requiring much higher doses to achieve significant venodilatation. As a result, there was substantial variation of both baseline venoconstriction values, and of acetylcholine concentrations used to achieve venodilatation. Consideration of the raw data led to the decision to use percentage change venodilatation from baseline in response to acetylcholine for analysis. The data are presented for each study group as

percentage venous dilatation from baseline at each dose of acetylcholine, and were compared using analysis of variance with Bonferroni post-test correction for multiple comparisons.

When considering the patient baseline characteristics, it is clear that there are differences in potentially confounding variables between study groups. Specifically, there are differences in age and gender between groups, and the use of some medications such as nitrates, potassium channel activators and calcium antagonists varies between groups. Unfortunately the small sample size does not allow for multivariate analysis to correct for these variables. It must be acknowledged that less stringent inclusion/exclusion criteria would have resulted in recruitment of a larger sample size and the option to apply multivariate analysis if necessary.

# Chapter 3

## Aortic Pulse Wave Velocity

## 3.1 Introduction

As discussed in chapter 1, the cause of heart failure in patients with HF-PSF is an area of controversy. The most commonly held view is that most have diastolic dysfunction, i.e. a disorder of LV relaxation and filling, as opposed to contraction and emptying.[40, 149] More recently, it has been suggested that these patients have abnormal ventriculo-vascular coupling, due to a combination of ventricular and vascular stiffening.[50, 53, 54, 57, 59, 60, 150]

An important determinant of LV afterload, and integral to ventriculo-vascular coupling, is large artery (aortic) stiffness. As discussed in chapter 1, section 1.5.2, PWV has long been accepted as an excellent surrogate of aortic stiffness, and is generally considered to be the 'gold standard' method of assessing aortic stiffness non-invasively.

Previous studies have compared vascular function between patients with HF-PSF and patients with systemic hypertension and/or normal subjects. Co-morbidities such as diabetes mellitus and coronary heart disease were rarely matched between study groups during patient selection. Vascular function has never been compared between HF-PSF and HF-RSF.

In this chapter I present patient characteristics, blood pressure and heart rate data, and the results of PWV experiments.

## 3.2 Methods

See chapter 2 for comprehensive documentation of methods employed in this study.

### 3.2.1 Patient selection

The aim of patient selection in this project was to allow comparison of vascular function between both common types of HF-PSF and HF-RSF and an appropriate 'real-life' control group. Therefore all subjects selected had coronary heart disease and patients with co-morbidities known to adversely affect vascular function, such as diabetes mellitus, uncontrolled hypertension and renal failure, were excluded.

Three groups of patients (n=12 each) were selected, matched for the presence of coronary heart disease: 1) HF-PSF, 2) HF-RSF and 3) controls with preserved LV systolic function and no evidence of heart failure.

### 3.2.2 Heart rate and blood pressure measurement

The heart rate and one brachial blood pressure measurement was recorded over a 30 second time period using the HDI device, as described in chapter 2. This measurement was performed three times for each subject and averaged.

### 3.2.3 Aortic pulse wave velocity measurement

Pulse wave velocity was measured using the SphygmoCor$^{TM}$ applanation tonometry system as described in chapter 2.

Briefly, a sensor probe was used to measure the arterial waveform at carotid and femoral arteries. The upstroke of the arterial waveform was gated to the R wave of the ECG using the 'foot-to-foot' method to calculate transit time of the arterial pulse wave along the aorta, $\Delta T$. This value, together with the measured surface body distance in millimitres (mm) between both measurement points (carotid and femoral arteries), L, can be used to calculate PWV in m/s with the equation: $PWV = L/\Delta T$.

## 3.3 Results

### 3.3.1 Patient baseline characteristics

Patient baseline characteristics and concomitant medications are shown in table 3.1.

**Table 3.1:**

Baseline characteristics of subjects, summarised by mean (standard deviation) for continuous variables and by frequency for categorical variables. Diuretic use classified by type: Thiazide (T) or Loop (L). Five patients in the control group were taking thiazide diuretics as anti-hypertensives.
LVEF – left ventricular ejection fraction; NYHA – New York Heart Association; ACEI – angiotensin-converting enzyme inhibitor; ARB – angiotensin receptor blocker; HMG CoA – 3-hydroxy-3methylglutaryl coenzyme A

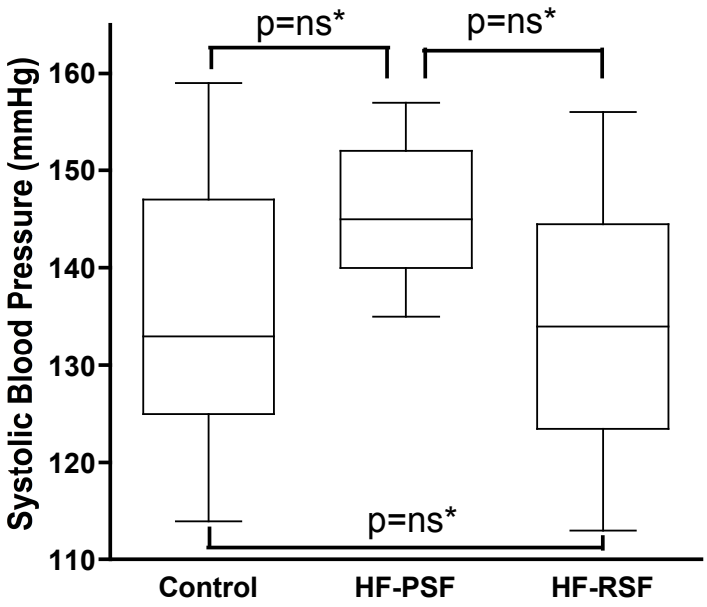
| **GROUP** | **Control** n=12 | **HF-PSF** n=12 | **HF-RSF** n=12 |
|---|---|---|---|
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** |
| **Age** | 69.7 (8.3) | 77.3 (5.9) | 73.9 (6.3) |
| **Boston Score** | N/A | 13.4 (2.1) | 12.8 (1.7) |
| **Cholesterol** | 4.62 (1.18) | 4.56 (1.12) | 4.7 (1.26) |
| **NT-proBNP** | 186.9 (113.4) | 894.2 (446.9) | 1416.1 (1033.7) |
| **Systolic BP (mmHg)** | 135.4 (14.1) | 146.2 (7.1) | 134.0 (13.5) |
| **Diastolic BP (mmHg)** | 70.1 (20.5) | 69.5 (7.3) | 72.8 (10.6) |
| **Mean BP (mmHg)** | 91.9 (26.5) | 95.0 (6.2) | 93.2 (8.5) |
| **LVEF (%)** | 63.2 (4.8) | 62.7 (8.1) | 28.0 (9.4) |
| **Sex M/F** | 5/7 | 4/8 | 8/4 |
| **History of hypertension** | 10 | 10 | 9 |
| **NYHA Class (n)** | N/A | II (11) III (1) | II (10) III (2) |
| **MEDICATION** | **n** | **n** | **n** |
| **ACEI/ARB** | 10 | 10 | 12 |
| **B-Blocker** | 9 | 9 | 10 |
| **HMG CoA** | 12 | 11 | 12 |
| **Diuretic (Thiazide/Loop)** | 5 (T) | 12 (L) | 12 (L) |
| **Aspirin/Antiplatelet** | 12 | 10 | 12 |
| **Nitrate** | 1 | 2 | 4 |
| **Ca Channel Blocker** | 6 | 2 | 2 |
| **K Channel Activator** | 6 | 1 | 2 |

### 3.3.2 Blood pressure

There were no significant differences in systolic, diastolic or mean blood pressure between patient groups (figures 3.1, 3.2, and 3.3, respectively).

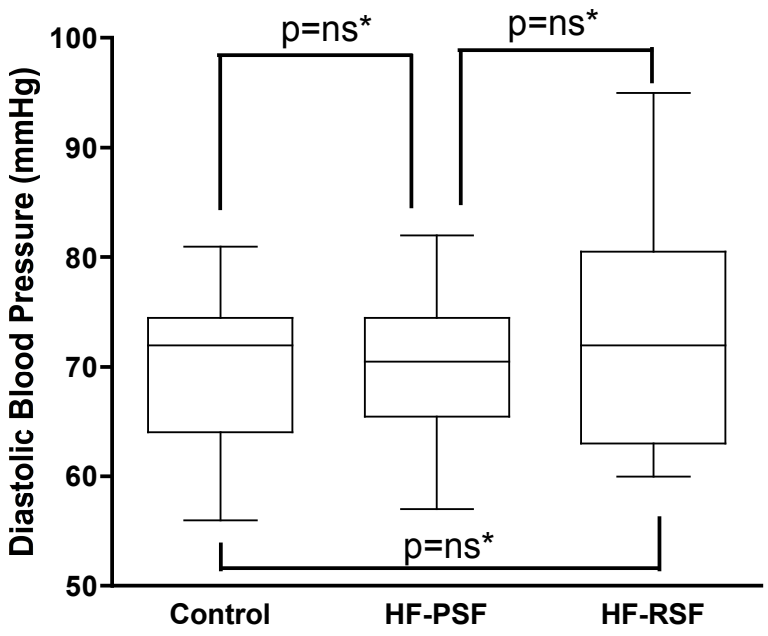
Pulse pressure was significantly higher in the HF-PSF group compared with the HF-RSF group but not compared with controls (figure 3.4).

**Figure 3.1:**
Systolic blood pressure compared by patient group with analysis of variance (ANOVA).



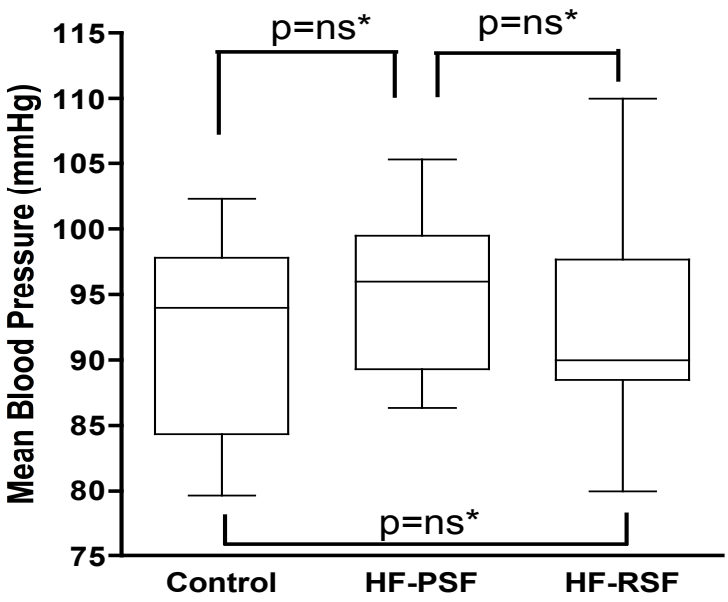

*ANOVA with Bonferroni correction

**Figure 3.2:**
Diastolic blood pressure compared by patient group with analysis of variance (ANOVA).



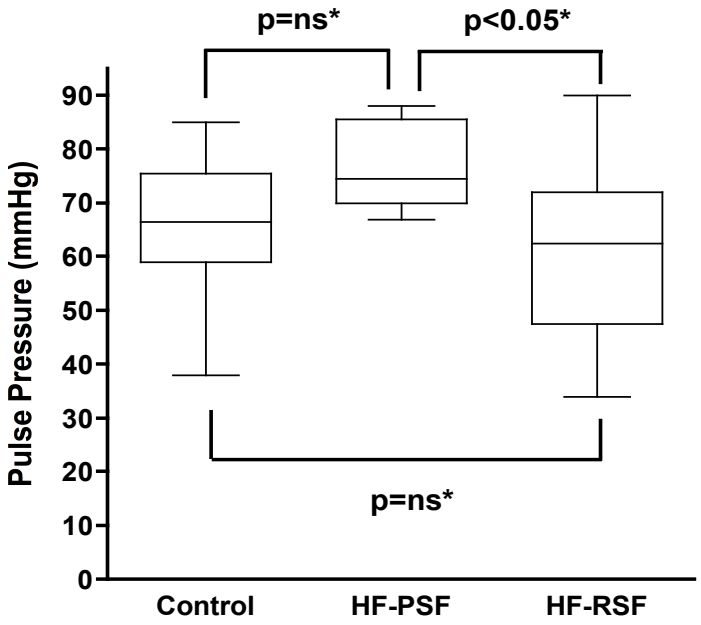

*ANOVA with Bonferroni correction

**Figure 3.3:**
Mean blood pressure compared by patient group with analysis of variance (ANOVA).




*ANOVA with Bonferroni correction

**Figure 3.4:**
Pulse pressure compared by patient group with analysis of variance (ANOVA).

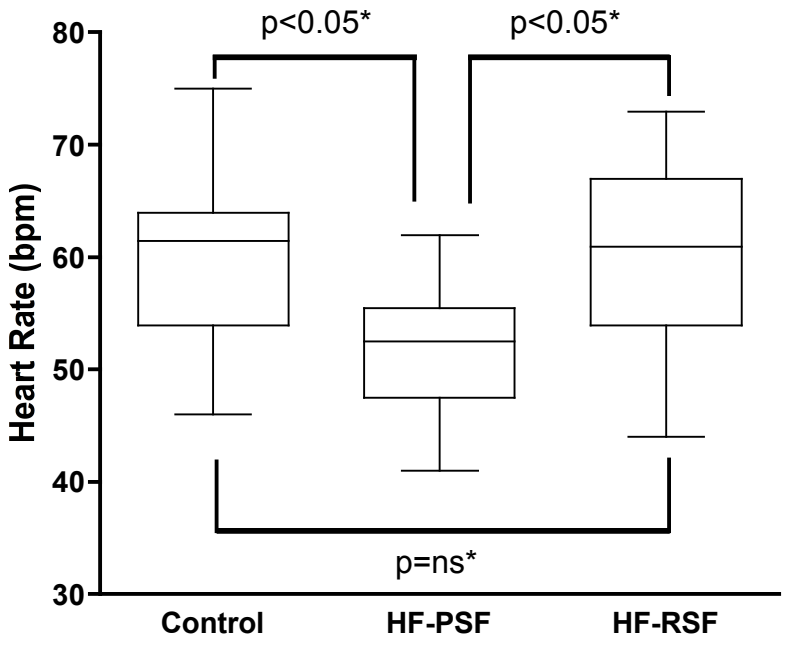



*ANOVA with Bonferroni correction

### 3.3.3 Heart rate

Subjects with HF-PSF had a significantly lower mean heart rate than both HF-RSF and control subjects: 51.6 (6.1); 60.3 (8.5) and 60 (8) beats per minute respectively, $p<0.05$, (figure 3.5).

**Figure 3.5:**
Heart rate compared by patient group with analysis of variance (ANOVA).



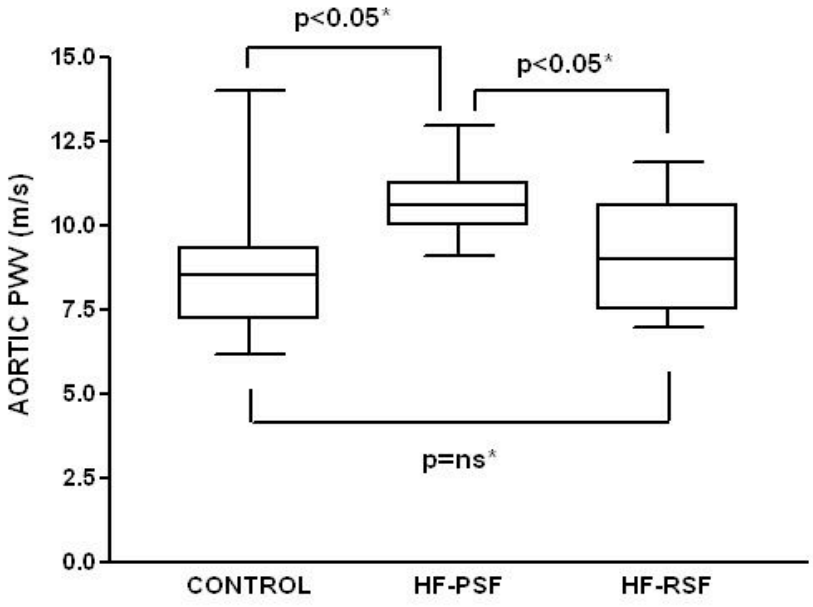

*ANOVA with Bonferroni correction

### 3.3.4 Aortic pulse wave velocity

Pulse wave velocity was not recordable in one male patient from the HF-RSF group.

Pulse wave velocity was significantly higher in HF-PSF subjects than in both HF-RSF and control groups: 10.7 (1.1); 8.9 (1.7) and 8.6 (2.1) m/s respectively, $p<0.05$ (figure 3.6).

**Figure 3.6:**
Aortic pulse wave velocity (PWV) compared by patient group with analysis of variance (ANOVA).



## 3.4 Discussion

The proportion of women was higher in the HF-PSF group than in both the HF-RSF and control groups. Patients with HF-PSF were older than controls, but not patients with HF-RSF. The use of medications known to modify vascular smooth muscle and endothelial function, particularly angiotensin-converting-enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), 3-hydroxy-3-methylglutaryl coenzyme A (HMG Co-A) reductase inhibitors, aspirin and beta-blockers, was similar between groups. The main difference in medications between groups was the higher use of diuretics in both heart failure groups, compared with controls. All three groups had similar total serum cholesterol levels. NT-proBNP was significantly elevated in both the HF-PSF and HF-RSF groups compared with controls ($p<0.05$), in keeping with the clinical diagnosis of heart failure (table 3.1). There was a trend to a higher mean plasma concentration of NT-proBNP in patients with HF-RSF than was observed in the HF-PSF group (1416.1 [1033.7] pmol/l *versus* 894.2 [446.9] pmol/l, respectively). However, this difference was not statistically significant ($p=0.12$, t-test) and was accounted for by one outlier in the HF-RSF group, who had a plasma NT-proBNP concentration of 4319 pmol/L. New York Heart Association functional class and Boston score were similar in both heart failure groups.

Heart failure with preserved LV systolic function was associated with elevation of PWV, which is related to the properties of the vessel wall by the Moens-Korteweg equation,

and has long been accepted as a surrogate marker of arterial stiffness and vascular remodelling.[65] Increased PWV in HF-PSF has not previously been reported. More importantly, PWV was significantly elevated in my cohort of patients with HF-PSF, compared to controls and patients with HF-RSF, despite all three groups of patients being matched for underlying arterial disease (i.e. having coronary heart disease), implying that HF-PSF is indeed associated with greater arterial stiffness.

Patients with HF-PSF had a significantly higher pulse pressure than patients with HF-RSF, independent of mean arterial pressure. Pulse pressure is closely related to PWV. As discussed in section 1.5: when PWV rises, pulse waves are reflected from the peripheral vasculature to the proximal aorta earlier in the cardiac cycle, augmenting central arterial pressure during systole rather than diastole, resulting in elevated central pulse pressure.[70] Pulse pressure is also influenced by height and age, with central pressure augmentation being more marked in shorter, older individuals.[151] This may be important in HF-PSF patients who are more likely to be older and female, as noted in epidemiological studies.[22, 23] On review of the blood pressure data, patients with HF-PSF displayed a trend towards higher systolic blood pressure, accounting for the observed difference in pulse pressure. Another factor influencing pulse pressure, albeit to a lesser degree than PWV, is heart rate, with slower heart rates resulting in higher pulse pressures.[70] My patients with HF-PSF had slower heart rates than the other two groups and this may have contributed to the elevated pulse pressure values observed in this particular cohort.

The PWV and pulse pressure data presented above support my hypothesis that patients with HF-PSF have increased aortic stiffness. Increased aortic stiffening is likely to be implicated in the pathophysiology of HF-PSF by causing widening of the pulse pressure, reduced coronary perfusion, increased LV wall stress, development of LVH and increased myocardial oxygen demand. All of these factors may result in impaired ventriculo-vascular coupling, elevation of LVEDP and the clinical syndrome of HF-PSF.

# Chapter 4

## Analysis of Arterial Diastolic Pressure Waveforms

## 4.1 Introduction

As I discussed in section 1.5.3, the arterial pressure waveform is a composite of incident and reflected waves. Wave reflection occurs at points of impedance mismatch, such as vessel bifurcation and is also influenced by the diameter and contractile properties of the resistance arterial bed. The point at which the reflected wave returns to the proximal aorta is dependent upon the speed with which the incident wave travels along the aorta, i.e. PWV. In health the reflected wave arrives in the proximal aorta in diastole, augmenting diastolic pressure and facilitating coronary artery flow. However, with progressive arterial stiffening PWV increases and the reflected wave arrives earlier in diastole and may arrive during systole. The reflected wave superimposes on the incident systolic wave, augmenting systolic blood pressure. This early return of the reflected wave during systole results in reduced diastolic blood pressure and widening of the pulse pressure. As I mentioned in chapter 1, section 1.4.6, this phenomenon has profound detrimental effects on ventriculo-vascular coupling, which is likely to be implicated in the pathophysiology of HF-PSF.

Using a modified windkessel model of the circulation, it is possible to analyse the contour of the arterial pressure waveform during diastole. With this method, one can determine the relative contributions of incident and reflected waveforms making up the contour of the composite arterial pressure waveform during diastole. At the beginning of diastole, following aortic valve closure, the incident arterial pressure wave decays as pressure in the proximal aorta falls. The elastic recoil of the aorta determines the speed

of decay. In healthy young individuals the aorta is rich in elastic fibres, buffering the incident wave and resulting in slow decay of the diastolic arterial pressure waveform. When the aorta loses elasticity, for example due to aging or systemic hypertension, its buffering effect is reduced. As a result the incident wave rises sharply during systole and decays rapidly in diastole. When described in the context of a windkessel model of the circulation, the elastic recoil of the large arteries during diastole is termed 'capacitive compliance'.

The second component of the arterial pressure waveform during diastole is the reflected pressure wave. As mentioned above, the point at which the reflected wave arrives in the proximal aorta is dependent on the speed at which the incident wave is conducted along the aorta, the PWV, and the contractile properties of the peripheral vessels. In the windkessel model of the circulation, the contractile properties of the peripheral vessels are termed 'oscillatory compliance'.

In this chapter, I will detail the results of diastolic pressure waveform analysis in patients with HF-PSF, HF-RSF and controls.

## 4.2 Methods

Full details of the methods employed in this study are documented in chapter 2.

Arterial diastolic pressure waveform analysis was performed using an applanation tonometry technique and a specifically designed analysis and software system (HDI

Pulse wave CR-2000; Hypertension Diagnostics Inc.), as described in more detail in chapter 2. Arterial waveforms were recorded from the radial artery over a 30 second time period. This protocol was performed three times and the data analysed automatically by the integrated software. Using curve-fit analysis of the diastolic pressure waveform and a modified windkessel model of the circulation, the HDI software calculates derived measurements of large (central or 'capacitive') artery compliance, small (peripheral or 'oscillatory') artery compliance and systemic vascular resistance.

## 4.3 Results

### 4.3.1 Patient baseline characteristics

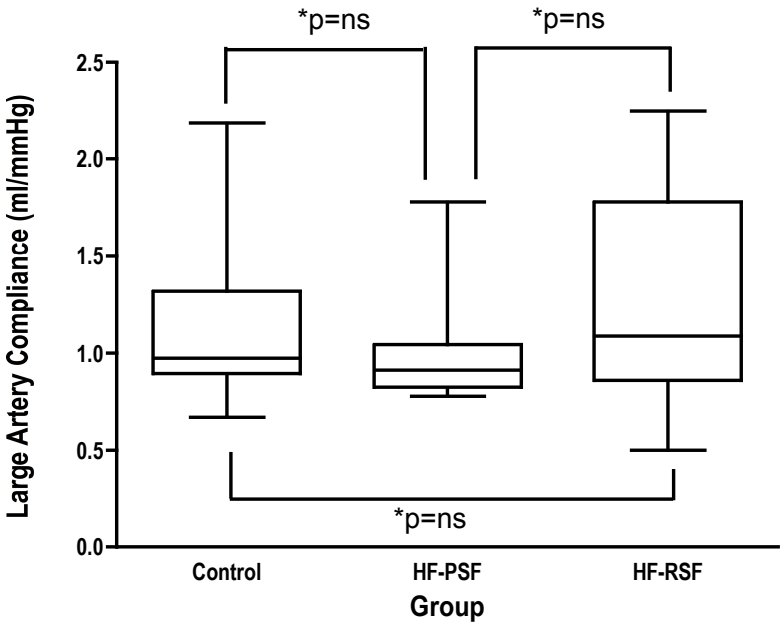
Patient baseline characteristics along with heart rate and blood pressure data are presented in chapter 3.

### 4.3.2 Arterial diastolic pressure waveform analysis

Acceptable waveforms were not obtainable in one subject from the HF-RSF group.

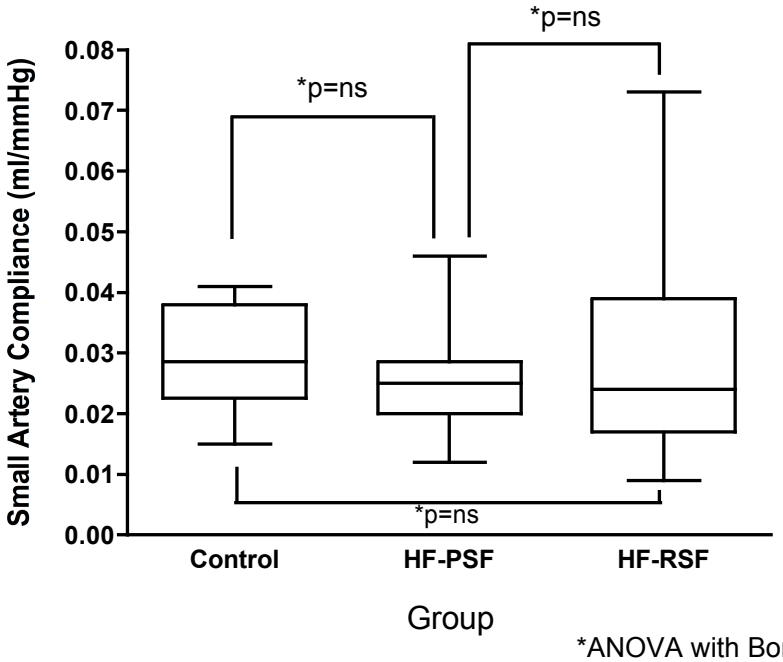
Derived values for large (central) arterial compliance (termed C1), small (peripheral) arterial compliance (termed C2) and systemic vascular resistance were compared between subject groups. No significant differences were detected between groups (figures 4.1, 4.2, 4.3. and table 4.1.).

**Figure 4.1:**
Large artery compliance compared by patient group with analysis of variance (ANOVA).



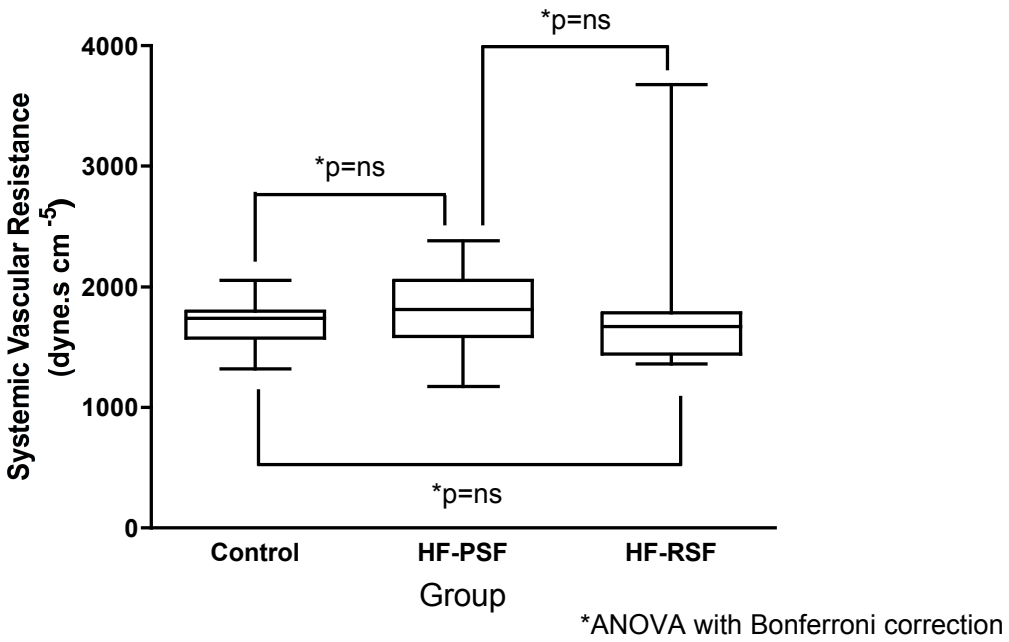

*ANOVA with Bonferroni correction

**Figure 4.2:**
Small artery compliance compared by patient group with analysis of variance (ANOVA).




*ANOVA with Bonferroni correction

**Figure 4.3:**
Systemic vascular resistance compared by patient group with analysis of variance (ANOVA).



## Table 4.1:

Large artery compliance (C1), small artery compliance (C2) and systemic vascular resistance compared by patient group with analysis of variance (ANOVA).

| PARAMETER | CONTROL n=12 | HF-PSF n=12 | HF-RSF n=11 | p-value |
|---|---|---|---|---|
| | **Mean (SD)** | **Mean (SD)** | **Mean (SD)** | |
| **Large Artery Compliance (C1) (ml/mmHg)** | 1.103 (0.42) | 1.004 (0.29) | 1.289 (0.55) | ns |
| **Small Artery Compliance (C2) (ml/mmHg)** | 0.029 (0.009) | 0.025 (0.009) | 0.033 (0.019) | ns |
| **Systemic Vascular Resistance (dyne.s cm-5)** | 1705.8 (197.4) | 1798.8 (336.3) | 1822.1 (659.1) | ns |

## 4.4 Discussion

Pulse wave analysis using the HDI device yielded values below the reported normal range for both large and small vessel compliance in all three patient groups, but no significant differences between groups. I found this surprising in light of the PWV results in the same subject groups, demonstrating that HF-PSF subjects have significantly higher PWV, and therefore, arterial stiffness.

The discrepancy between HDI and PWV results is likely to be due to the different applanation tonometry techniques. The HDI technique has predominantly been used in previous studies to identify abnormalities of arterial compliance in patients at cardiovascular risk, e.g. diabetics,[94] or patients with established heart failure [152] compared with normal subjects. It has also been employed effectively to demonstrate changes in arterial compliance in response to pharmacological therapies.[153, 154]

The fact that we used the HDI device to compare patients who all had established atherosclerotic disease may have reduced the ability of the technique to differentiate between groups and resulted in no significant inter-group differences being detected. Another factor that may account for the discrepancy between PWV and HDI results is the data analysis method. PWV is determined using a simple equation. On the other hand, the HDI system uses curve-fit analysis of the diastolic pressure waveform and estimates other variables such as stroke volume and cardiac output from the systolic upstroke of the arterial pressure wave, heart rate, brachial blood pressure, age and body

surface area. As I discuss in chapter 3, there are differences in age and gender between groups. These differences are likely to confound the data upon which the HDI software bases calculations of stroke volume and cardiac output, and subsequent calculations of arterial properties. Finally, a more simple explanation is that there may not have been enough patients studied to detect a difference between groups using this technique.

# Chapter 5

## Microvascular Endothelial and Smooth Muscle Function

## 5.1 Introduction

The vascular endothelium is integral to maintaining normal vessel tone and function and, therefore, normal ventriculo-vascular interaction.[76] As mentioned in section 1.5.6, arterial endothelial dysfunction has been well documented in HF-RSF [98-102] and is thought to be secondary to neurohumoral and inflammatory activation.[155-157] Whether HF-PSF is associated with endothelial dysfunction is, however, unknown. In this chapter I detail the results of microvascular function compared between patients with HF-PSF, HF-RSF and controls.

## 5.2 Methods

Iontophoresis combined with Laser Doppler imaging was performed as previously described in chapter 2.

Acetylcholine chloride was introduced into the anodal chamber and sodium nitroprusside was introduced into the cathodal chamber. Current applied to these chambers results in iontophoresis of both agents across the skin to exert their effects on the microvasculature. During iontophoresis, a laser scanned both chambers to determine skin perfusion – a measure of vasodilatation.

The data were analysed off-line and study groups were compared using repeated measures 2-way ANOVA (Microsoft Excel software for Windows).

## 5.3 Results

### 5.3.1 Patient characteristics

Baseline patient characteristics along with heart rate and blood pressure data are presented in chapter 3.

### 5.3.2 Laser Doppler iontophoresis

Vasodilatation in response to acetylcholine was similar in both heart failure groups. Both heart failure groups displayed an impaired vasodilator response to acetylcholine compared with controls (HF-RSF *versus* control p=0.0003, HF-PSF *versus* control p=0.00099, HF-PSF *versus* HF-RSF p=ns, ANOVA) as illustrated in figure 5.1. The heart failure groups also had similar peripheral vasodilatation responses to sodium nitroprusside. These endothelium-independent responses were also significantly reduced when compared with controls (HF-RSF *versus* control p=0.012, HF-PSF *versus* control p=0.006, HF-PSF *versus* HF-RSF p=ns, ANOVA), as demonstrated in figure 5.2.

**Figure 5.1:**
Peripheral microvascular responses to acetylcholine. Doppler measurement of vasodilatation is expressed as arbitrary perfusion units (PU). Cumulative charge in micro-Coulombs (mC) is the total charge delivered during iontophoresis.

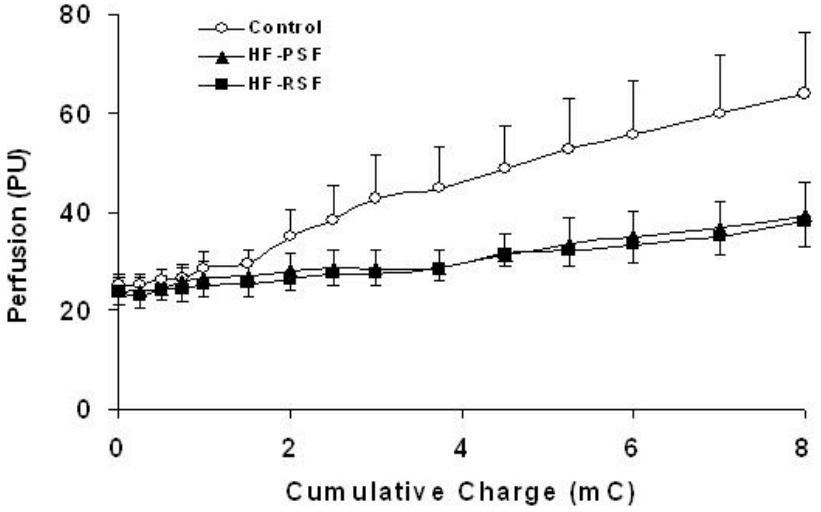
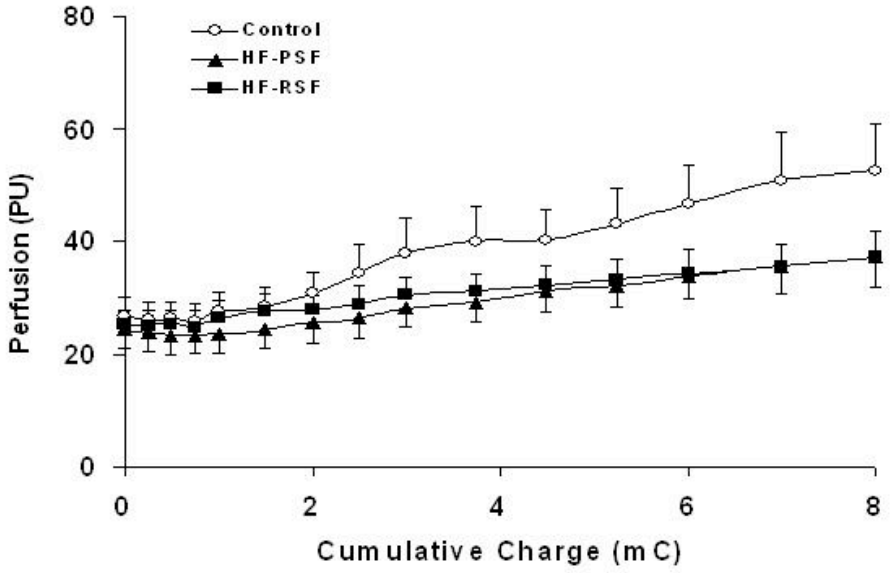


**Figure 5.2:**
Peripheral microvascular responses to sodium nitroprusside. Doppler measurement of vasodilatation is expressed as arbitrary perfusion units (PU). Cumulative charge in micro-Coulombs (mC) is the total charge delivered during iontophoresis.



## 5.4 Discussion

The data presented in this chapter are the first to demonstrate that HF-PSF subjects have impaired microvascular responses to both acetylcholine and sodium nitroprusside. This suggests that, rather than being solely a primary disorder of endothelial function, impaired control of vascular tone in HF-PSF also reflects significant vascular smooth muscle dysfunction. It should be emphasised that all patients in this study had coronary artery disease, which is known to be associated with impaired peripheral vascular responses to vasodilators.[158] These data show that patients with HF-PSF have impaired peripheral microvascular vasodilator function over and above what would be expected with atherosclerotic disease alone. These results also show that the degree of microvascular dysfunction is similar in both HF-PSF and HF-RSF patients, implying that the vascular abnormalities seen in heart failure are not exclusively due to reduced LV systolic function. Apart from the difference in LV systolic function, the heart failure groups were otherwise well matched. They had similar clinical symptoms and signs, as evidenced by NYHA class and Boston scores and both displayed significant neurohumoral activation, with equivalent elevation of plasma NT-pro-BNP concentration.

Impaired responses to both endothelial-dependent and -independent vasodilators, have been previously demonstrated in some,[100, 113, 159-161] but not all [78, 101, 102] studies in patients with HF-RSF. One of the most important potential mechanisms causing endothelial dysfunction is oxidative stress, leading to production of reactive

oxygen species and inactivation of nitric oxide, a key factor in the control of vasomotor tone.[162] There is a significant body of evidence suggesting that oxidative stress is an important pathophysiological process in the development of arterial endothelial dysfunction in HF-RSF.[18, 105] Other factors, such as maladaptive overactivity of the RAAS [79] and circulating endothelin [103] have been implicated as potential causes of arterial endothelial dysfunction in HF-RSF. The vascular endothelium is integral to maintaining normal vessel tone and function and, therefore, influences arterial stiffness.[76] Impaired arterial endothelial function is associated with reduced arterial compliance in older individuals,[106] which may be relevant in patients with HF-PSF who tend to be elderly. It is not yet known whether or not any of these pathophysiological processes are responsible for vascular endothelial and smooth muscle dysfunction in patients with HF-PSF

Notwithstanding the mechanisms causing it, it would appear that the 'heart failure syndrome', regardless of aetiology, is associated with impaired peripheral microvascular vasodilator function.

To date, peripheral vascular function in HF-PSF has not been extensively studied. The results in this chapter show that patients with HF-PSF have profoundly reduced peripheral microvascular responsiveness. In other words, HF-PSF is associated with a markedly impaired capacity to regulate vascular tone and function. If this phenomenon is present across multiple vascular beds it may be implicated in the pathophysiology of HF-PSF by potentially reducing coronary artery flow, increasing peripheral vasoconstriction and impairing ventriculo-vascular coupling.

This raises the possibility that modification of vascular endothelial and/or smooth muscle function will prove to be a useful therapeutic strategy in the future management of patients with HF-PSF.

Although it is possible that the mechanisms causing impaired vascular reactivity are similar in both types of heart failure, evidence is currently lacking. Further studies of the mechanisms causing vascular endothelial and smooth muscle dysfunction in HF-PSF are required to clarify this issue.

# Chapter 6

## Venous Function

## 6.1 Introduction

In this chapter I present the results of venous function studies. Results of both venous capacitance and venous endothelial function experiments are detailed and discussed.

As I discussed in section 1.5.9, venous capacitance and control of venous tone are important determinants of LV pre-loading conditions that, if abnormal, could theoretically contribute to increased LVEDP and the clinical syndrome of heart failure. Venous capacitance has been studied in the context of HF-RSF, where reduced venous capacitance is associated with reduced exercise capacity in that patient group.[125] Reduced venous capacitance has also been demonstrated to correlate with more severe symptoms of heart failure and elevated pulmonary artery pressure in patients with HF-RSF.[126] There is no information regarding venous capacitance in patients with HF-PSF. Patients with HF-PSF are more sensitive to the effects of vasodilators and diuretics on LV filling pressure, suggesting that venous capacitance is reduced.[131, 132] There have, until now, been no studies of venous capacitance in patients with HF-PSF.

As mentioned above, reduced venous capacitance may be an important pathophysiological factor in the development of HF-PSF as there is evidence to suggest that venous capacitance influences LVEDP and symptoms in patients with HF-RSF. The venous endothelium is integral to control of venous tone and, therefore, venous capacitance. The most widely accepted method of assessing venous endothelial function is the modified Aellig dorsal hand vein technique. In chapter 1, I provide an overview

of the development of the dorsal hand vein technique and a summary of the data from previous studies of venous endothelial function. Most of these studies were performed in healthy individuals, however there are some data indicating that patients with HF-RSF have impaired venous endothelial function. Venous endothelial function has never been assessed in patients with HF-PSF.

In the following text I will detail the methodology and results of venous capacitance and venous endothelial function measurement in subjects with HF-PSF, subjects with HF-RSF and appropriate controls.

## 6.2 Methods

Full details of the methodology used to measure venous capacitance and venous endothelial function are described in chapter 2. Briefly, forearm venous capacitance was measured with venous occlusion plethysmography using mercury-in-silastic strain gauges. Just prior to measuring venous capacitance, the hand was excluded from the circulation with a wrist cuff inflated to 220 mmHg. Venous capacitance was measured using the equilibration method – inflating the upper-arm cuff to 45 mmHg for 2 minutes and 30 seconds. Venous capacitance was recorded three times in each patient and averaged.[147]

Venous endothelial function was measured using the modified Aellig dorsal hand vein technique.[134] A suitable dorsal hand vein was cannulated. Baseline venous dilatation

in response to an upper arm sphygmomanometer cuff at 45 mmHg was measured. The vein was pre-constricted with phenylephrine, infused in incremental doses until 70% venoconstriction from baseline was achieved (ED70). While maintaining the phenylephrine infusion to achieve steady-state ED70, a concomitant infusion of acetylcholine was commenced and incremented to stimulate endothelial-dependent venodilatation. As acetylcholine causes smooth muscle-dependent venoconstriction at high doses I pre-determined that each study would be discontinued when maximal venodilatation had peaked and then fallen by 20% of the maximum vein diameter achieved.

## 6.3 Results

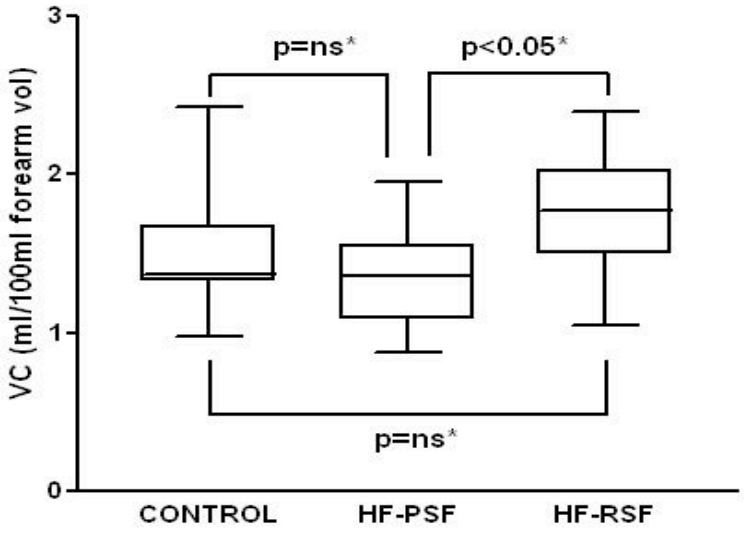
### 6.3.1 Patient baseline characteristics

Patient baseline characteristics along with heart rate and blood pressure data are presented in chapter 3.

### 6.3.2 Venous capacitance

Venous capacitance was higher in HF-RSF subjects compared to HF-PSF subjects: 1.75 (0.41), 1.34 (0.34) ml/100 ml forearm volume respectively, $p<0.05$, ANOVA (figure 6.1).

**Figure 6.1:**
Venous capacitance (VC) compared by patient group with analysis of variance (ANOVA).




*ANOVA with Bonferroni correction

### 6.3.3 Venous endothelial function

It was not technically possible to conduct a dorsal hand vein study on two patients from the HF-PSF group, and on 1 patient from each of the HF-RSF and control groups. There was marked variability in baseline vein diameter between subjects. There was also significant variability in responses to pre-constriction with phenylephrine and subsequent dilatation with acetylcholine, both within and between study groups. Although stable venoconstriction was achieved in all studies included in analysis, exact ED70 (70% constriction from baseline) was not achieved in all studies. Similarly, there was variability of venodilatation in response to acetylcholine within and between groups, with some subjects achieving peak venodilatation at low doses of acetylcholine, and some requiring much higher doses to achieve significant venodilatation. As a result, there was substantial variation of both baseline venoconstriction values, and of acetylcholine concentrations used to achieve venodilatation. Consideration of the raw data led to the decision to use percentage change venodilatation from baseline in response to acetylcholine for analysis. The data are presented for each study group as percentage venous dilatation from baseline at each dose of acetylcholine, and were compared using analysis of variance with Bonferroni post-test correction for multiple comparisons. The number of subjects receiving each dose of acetylcholine is shown in table 6.1.

Venodilatation in response to acetylcholine appeared to be similarly poor in both heart failure groups compared with controls, although there were no statistically significant differences in responses between groups (figure 6.2). Both heart failure groups

displayed a trend towards more pronounced venoconstriction at high dose acetylcholine compared with controls.

**Figure 6.2:**
Venous dilatation with acetylcholine (ACh) following pre-constriction with phenylephrine compared by patient group. Responses are presented as percentage change from baseline with each dose of ACh.

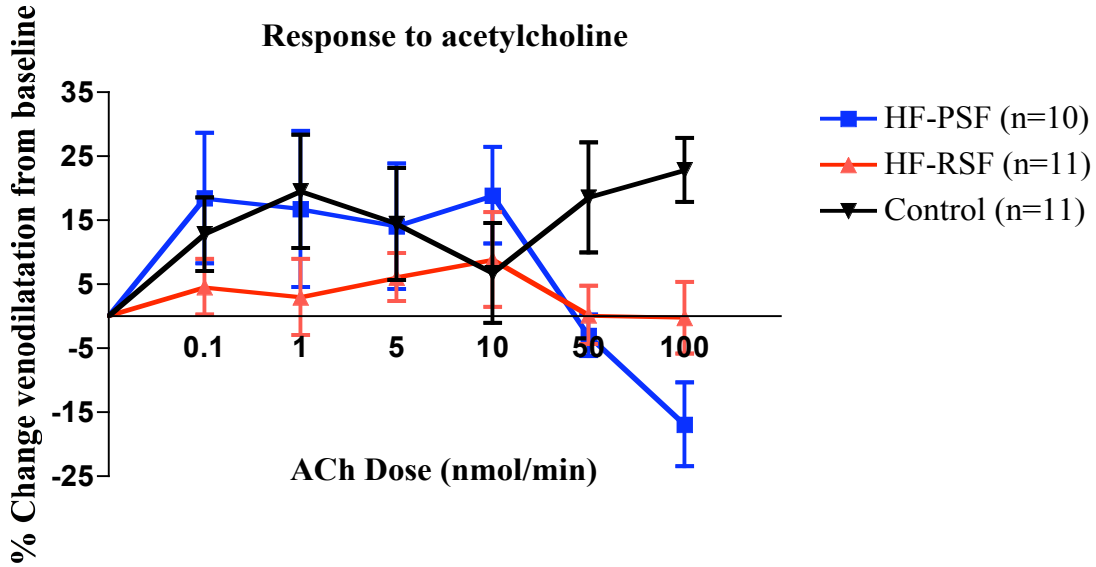



**Table 6.1:**
Number of patients per study group at each acetylcholine (ACh) dose.

| **ACh dose (nmol/min)** | 0.1 | 1 | 5 | 10 | 50 | 100 |
|---|---|---|---|---|---|---|
| **Number per study group** | | | | | | |
| HF-PSF | 10 | 10 | 9 | 9 | 7 | 4 |
| HF-RSF | 11 | 11 | 11 | 11 | 9 | 4 |
| Control | 11 | 11 | 11 | 9 | 6 | 3 |

## 6.4 Discussion

Venous capacitance appeared to be lower in the controls than in patients with HF-RSF. Perhaps the more interesting finding was that the patients with HF-PSF had a similar venous capacitance to the controls and lower venous capacitance than patients with HF-RSF. As I mentioned in the introduction to this chapter, patients with HF-PSF are more sensitive to the effects of vasodilators and diuretics on LV filling pressures, suggesting an abnormality of venous capacitance.[131, 132] Higher venous capacitance will result in a greater capacity of the circulation to accommodate a higher circulating volume without resulting in elevation of the LVEDP.[120] It has been shown that patients with HF-RSF display a preserved venodilator response to atrial natriuretic peptide [128] and an appropriate venous capacitance rise in response to nitric oxide [98] despite impairment of arterial endothelial function. Patients with HF-PSF may have a reduced venous response to endogenous nitric oxide leading to a failure to increase venous capacitance - which could then be considered to be a dysfunctional response. Another possibility is that this cohort of patients with HF-PSF had higher resting venous pressure than patients with HF-RSF. This would result in measurements starting at a steeper point on the venous compliance curve, and less change in venous volume in response to congesting pressure.

The data from the Aellig experiments suggest that patients with HF-PSF and HF-RSF may have impaired venous endothelial function compared to control subjects. However, these apparently different responses to acetylcholine did not reach statistical significance.

Both heart failure groups displayed a trend towards more pronounced venoconstriction at high dose acetylcholine compared with controls. Despite the graph appearance, it must be borne in mind that not all patients in each group received high dose acetylcholine, due to the pre-determined end-point of a 20% fall from peak venodilatation being reached at lower doses of acetylcholine for most patients. The point where the difference between heart failure groups and the control group is most apparent is during infusion of 100 nmol/minute acetylcholine. The number of patients who received this dose in each group was 6 HF-PSF patients, 4 HF-RSF patients and 3 control patients (table 6.1).

The fact that it was not technically possible to perform a dorsal hand vein study in all patients, coupled with the variability of responses to both constricting and dilating agents, makes it difficult to draw firm conclusions from these results.

The findings of these studies of venous function raise the possibility that patients with HF-PSF have abnormal venous capacitance and/or impaired venous endothelial function. However, I do not claim to have defined the exact role of the venous bed in the pathophysiology of HF-PSF. Although it remains possible that patients with HF-PSF have impaired venous function, the venous capacitance results presented above are not conclusive and the Aellig experiments do not have enough statistical power to prove

or disprove the hypothesis that HF-PSF is associated with venous endothelial dysfunction. Further, larger studies are required to investigate venous function in HF-PSF.

# Chapter 7

## Final Summary and Discussion

Heart failure has been recognised as an important public health burden for decades. Multiple epidemiology studies have indicated that a significant proportion of patients with heart failure have preserved LV systolic function.[22, 23] Recently published data indicate that HF-PSF has increased in prevalence in the last 15 years,[26] and carries a similar prognosis to HF-RSF.[28] Prior to these epidemiology studies, it was widely accepted that, in the absence of significant valvular heart disease, heart failure was caused by reduced LV systolic function. The pathophysiology of HF-RSF has been extensively studied and the basic principles are easy to understand, reinforcing the widespread assumption that HF-RSF is the predominant form of heart failure.

The realisation that approximately half of all patients with heart failure have preserved LV systolic function has caused controversy. The pathophysiology of HF-PSF is not easily explained and has been studied far less than HF-RSF. Acceptance within the medical community of HF-PSF as a condition in its own right has been slow, confounding the relative lack of research in the field. In fact, increased awareness of the condition among doctors has probably contributed to the apparent increase in prevalence of HF-PSF over the last 15 years.

Initial investigation into the pathophysiology of HF-PSF, most notably by Zile and colleagues, demonstrated that subjects with HF-PSF have marked abnormalities of LV diastolic function, i.e. LV relaxation and filling.[31] On the basis of this important finding, heart failure without significant impairment of LV systolic function was termed

'diastolic heart failure', a definition which was widely adopted. Despite the compelling evidence presented by Zile's group, some questions remained regarding whether or not LV diastolic dysfunction is the only pathophysiological process causing HF-PSF.

For example, diastolic dysfunction of the left ventricle is also very common in HF-RSF,[43] suggesting that the volume overload associated with the heart failure syndrome may impair LV diastolic function, rather than LV diastolic dysfunction causing heart failure *per se*. Supporting this view is the finding from several studies that non-invasive and invasive measures of LV diastolic function and pressure are load-dependent.[54, 163] This characteristic renders measures of diastolic function less reproducible, and there is poor correlation between LV diastolic dysfunction and the presence of heart failure in population-based echocardiography studies.[42]

Another issue is that population-based studies have revealed the paradox that LV diastolic dysfunction is as common in males as it is in females, whereas HF-PSF is more common in women.[43]

Although LV diastolic dysfunction is likely to play an important role in the development of HF-PSF, it is unlikely to be the only pathophysiological factor.

An alternative explanation is that patients with HF-PSF have impaired ventriculo-vascular interaction, i.e. stiffening of both the left ventricle and the vasculature, leading to elevation of LVEDP and the heart failure syndrome. Results of some studies would appear to support this hypothesis as combined ventricular and arterial stiffening has been demonstrated in patients with hypertension and elderly females who, according to epidemiology data, are more likely to develop HF-PSF.[53] Kawaguchi et al conducted

one of the few studies investigating this concept in patients with HF-PSF.[54] They demonstrated combined ventriculo-arterial stiffening in HF-PSF when compared with both hypertensive and normal individuals. Although not conclusive, this growing body of evidence indicates that HF-PSF is unlikely to be purely a disorder of LV diastolic dysfunction, and may be due to a combination of ventricular and vascular abnormalities, as has been proposed by both Burkhoff and Kass.[59, 60]

Increased arterial stiffness may result in abnormal ventriculo-arterial coupling, increased LV wall stress, reduced coronary flow and aggravate or even cause the clinical syndrome of heart failure. If this concept is expanded, one must consider that abnormalities of many facets of the vascular tree could potentially contribute to the development of heart failure.

The vascular endothelium is integral to maintaining normal vessel tone and function and, therefore, normal ventriculo-vascular interaction.[76] Vascular endothelial dysfunction has been well documented in HF-RSF.[98-102] Whether HF-PSF is associated with endothelial dysfunction is, however, unknown.

Additionally, reduced compliance of veins, leading to reduced venous capacitance, may also play an important role in the pathophysiology of HF-PSF. Venous capacitance has been shown to be a determinant of LV pre-load conditions [121] and patients with HF-PSF are particularly sensitive to the effects of vasodilators and diuretics on LV filling pressure, suggesting that venous capacitance is reduced.[131, 132] If venous capacitance can influence LV pre-load conditions then it follows that control of venous tone, via endothelial and smooth muscle activity, could also exert an effect on LV pre-

load and potential to develop heart failure. There have been no previous studies of venous function in patients with HF-PSF.

The studies presented in this thesis were designed to further define the extent of vascular dysfunction in HF-PSF and to test the hypothesis that vascular dysfunction is more severe in HF-PSF than in HF-RSF and appropriate controls. I aimed to investigate not only arterial stiffness and endothelial function, but also parameters of venous capacitance and endothelial function. The studies were designed specifically to compare vascular function between patients with HF-PSF and HF-RSF. I chose this method to determine whether or not vascular dysfunction is similar in both heart failure groups, suggesting that it is secondary to the heart failure syndrome regardless of aetiology, or more severe in HF-PSF, indicating that vascular dysfunction is potentially implicated in the aetiology of HF-PSF.

As a control group, I recruited patients with coronary heart disease and preserved LV systolic function, rather than normal subjects. It is extremely common for investigators to compare vascular function between patients with established cardiovascular disease, such as heart failure, and normal subjects. This method is well recognised, but undoubtedly over-emphasises abnormalities of vascular function in the group under study. I felt that for the purposes of this study it would be more appropriate to use a control group of patients at risk of developing heart failure, i.e. patients with coronary heart disease, who might also be expected to have endothelial dysfunction and/or abnormal arterial compliance. This method of patient selection provides a comparison of vascular function between patients who are representative of contemporary clinical

practice, and will help to determine if HF-PSF is truly associated with vascular dysfunction, over and above what would be expected from coronary heart disease alone. The vascular experiments were chosen to provide a comprehensive, non-invasive assessment of both arterial and venous function.

Previous studies have suggested that arterial stiffness is increased in HF-PSF, but arterial stiffness has never been compared between HF-PSF and HF-RSF, as investigated in this study. I also aimed to investigate aspects of vascular function that have never been assessed in HF-PSF, such as arterial endothelial function, venous capacitance and venous endothelial function.

The results proved interesting. Arterial stiffness, measured by aortic PWV, was significantly elevated in HF-PSF compared to both HF-RSF and control groups, despite all three groups of patients being matched for underlying arterial disease (i.e. having coronary heart disease), implying that HF-PSF is indeed associated with greater arterial stiffness. These data were consistent with the observation that patients with HF-PSF had a significantly higher pulse pressure than patients with HF-RSF, independent of mean arterial pressure. In contrast, the results of arterial diastolic waveform analysis were not especially illuminating, with no significant differences in derived parameters of arterial compliance being detected between the three study groups. This was surprising, as abnormal arterial compliance might be expected to be reflected in both increased PWV and abnormal arterial waveforms. However, as I discuss in chapter 4, the complexity of the diastolic waveform analysis equation to derive measures of arterial compliance, coupled with the fact that all three groups had established arterial disease (coronary heart

disease), is likely to have reduced the ability of the technique to differentiate between groups in this study. It may also be possible that a larger sample size was required to demonstrate true differences between groups.

The Laser Doppler iontophoresis experiments revealed that HF-PSF subjects have impaired microvascular responses to both acetylcholine and sodium nitroprusside. This suggests that, rather than being solely a primary disorder of endothelial function, impaired control of vascular tone in HF-PSF reflects significant vascular smooth muscle dysfunction. Although a novel finding, subjects in the HF-RSF group displayed a similar pattern of microvascular dysfunction. Therefore it is not certain if arterial smooth muscle and/or endothelial dysfunction is secondary to the inflammatory and neurohumoral activation associated with the heart failure syndrome, as has been suggested in studies of HF-RSF, [155-157] or a primary pathophysiological factor in the development of either form of heart failure. It is possible that endothelial and arterial smooth muscle dysfunction is both cause (in HF-PSF) and effect (in HF-RSF) – reflecting different pathophysiologies in the two conditions

Venous capacitance appeared to be lower in the controls than in patients with HF-RSF. An interesting finding was that patients with HF-PSF had a similar venous capacitance to the controls and lower venous capacitance than patients with HF-RSF. As I outline in chapter 6, the low venous capacitance in patients with HF-PSF may be representative of failure to increase venous capacitance - which could then be considered to be a dysfunctional response. Another possibility is that my cohort of patients with HF-PSF had higher resting venous pressures than patients with HF-RSF, resulting in

measurements starting at a steeper point on the venous compliance curve, and less change in venous volume in response to congesting pressure.

The Aellig dorsal hand vein experiments provided some interesting information on endothelial control of venous tone. Both heart failure groups appeared to have impaired venodilatation in response to acetylcholine compared with controls, however this trend did not reach statistical significance. Unfortunately it was not technically possible to complete an Aellig study in the whole cohort of patients, resulting in relatively small numbers used for data analysis. This makes it difficult to draw any firm conclusions about venous endothelial function in HF-PSF on the basis of the data presented in chapter 6.

In conclusion, the studies presented in this thesis have added to current knowledge regarding the pathophysiology of HF-PSF. After initially recognising HF-PSF as a separate form of heart failure and appreciating the epidemiology, we have begun to understand the complex pathophysiology of HF-PSF. Previous studies have shown that there is strong association between HF-PSF and LV diastolic dysfunction.

This study has reinforced the theory that HF-PSF is associated with increased arterial stiffness, which in combination with increased LV stiffness is likely to result in impaired ventriculo-vascular coupling. This process is likely to be an important pathophysiological factor in the development of HF-PSF. A novel finding presented in this thesis is that endothelial and smooth muscle control of arterial tone, measured with Laser Doppler iontophoresis, was impaired in both HF-PSF and HF-RSF. This may indicate a similar primary pathophysiological process, or indeed a similar response to

inflammatory and neurohumoral activation in heart failure. The studies of venous capacitance and endothelial function I have presented suggest that venous function may be abnormal in HF-PSF. The venous function data are not conclusive but may provide stimulus for further research in this rarely investigated field.

Further studies are required, not only to investigate the pathophysiology of HF-PSF, but also to seek effective therapies for this extremely common form of heart failure. Examination of arterial stiffness, endothelial function and venous function during different cardiovascular loading conditions could provide interesting information on the generation of symptoms in HF-PSF. This could be achieved by conducting vascular function studies during exercise or acute decompensated fluid overload. As regards treatment of HF-PSF, there is a paucity of data. The CHARM-Preserved trial showed that treatment with the angiotensin receptor antagonist Candesartan resulted in mild clinical benefit in HF-PSF.[164] Treatment of HF-PSF with angiotensin receptor antagonists is the subject of other large, randomised, controlled trials such as I-PRESERVE (comparing Irbesartan with placebo) and full results are awaited.[165] Novel therapies for HF-PSF are also being developed. For example, compounds which break glycation cross-links can attenuate the development of both left ventricular hypertrophy and arterial stiffness, an attractive combination when one considers potential pathophysiological factors in the development of HF-PSF. These compounds, such as Alagebrium [166] and ALT-711 [167], have been investigated in small studies but are not yet ready for use in larger clinical trials. It is my hope that the studies presented in this thesis will stimulate future studies into this important area of heart failure research

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