The Pathophysiology of Heart Failure with Preserved Ejection Fraction

By

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Dedication

This work is dedicated to my parents and to my loving wife

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Abstract

About 50% of patients with the clinical features of chronic heart failure suffer from heart failure with preserved ejection fraction (HfpEF); (1, 2) Previous data have demonstrated that patients with HfpEF had abnormal left ventricle (LV) relaxation and increased LV stiffness. Consequently the left ventricular diastolic pressure-volume relationship is shifted upwards and to the left. (1) In addition, enhanced left ventricular stiffness can result in increased end-diastolic pressure (EDP) during handgrip exercise. (2) Our studies demonstrate that patients with HfpEF have impaired myocardial energetics as indicated by the diminished *in vivo* myocardial PCr/ATP ratio. (3) Which might provide a scientific rationale for the potential use metabolic modulators such as perhexiline in HfpEF, which may improve cardiac energetics and therefore improve LV relaxation and exercise capacity. Data acquired during semi-supine cycling exercise indicates that patients with HfpEF had a dynamic impairment of LV active relaxation. (3) In addition, ventricular-vascular coupling ratio was unchanged during exercise in HfpEF patients in contrast to healthy controls where the ratio fell substantially during exercise, suggesting a failure of the normal increase in LV contractile function during exercise. In addition, we found in a group of HfpEF patients with normal LA dimensions that there was increased LA contribution during exercise in patients with HfpEF compared to matched controls. (4) This may be an attempt to compensate for the observed reduction in LV relaxation. Furthermore, we showed patients with HfpEF exhibits contractile inefficiency as well as systolic and diastolic dyssynchrony as measured by speckle tracking imaging (STI). And that the LV anterior wall appears to be the most delayed segment providing a probable target for a pacing site.

Another potential cause of exercise limitation is autonomic dysfunction. We studied a cohort of HfpEF patients who were not on heart rate (HR) limiting medication and found that patients with HfpEF exhibited chronotropic incompetence during peak exercise testing and abnormal HR recovery following exercise compared to age-gender-matched healthy controls and hypertensive patients. (5) In a separate study, we showed that changes in LV torsion, untwist and LV strain and strain rate in patients with HfpEF at rest were similar to changes found in normal aging. (6) Our studies suggest that the pathophysiology of the HfpEF is one of a dynamic process with complex interaction between various processes such as increased LV stiffness, abnormal myocardial energetic, increased central arterial tree stiffness, abnormal autonomic functions and other factors such as cardiac dyssynchrony and contractility inefficiency.

Contents

Introduction

Introduction

The prevalence of chronic heart failure (CHF) is increasing and it is a leading cause of morbidity and mortality in developed countries (7) and an emerging one in the developing world. (8) The mortality of CHF at five years remains about 50%, which is similar to the prognosis for many cancers.(9) Heart failure is, therefore, a major clinical problem. Attention has focused mainly on those patients with left ventricular (LV) systolic dysfunction, as evidenced by a reduced LV ejection fraction (LVEF). The epidemiology and pathophysiology of such patients is well described, and treatment has improved substantially over the past 10-15 years. There is increasing consensus that about 50% of patients with the clinical features of chronic heart failure suffer from heart failure with preserved ejection fraction (HFpEF); (10, 11) epidemiological observations from different populations confirm that the prevalence of HFpEF is increasing, especially in obese hypertensive females. HFpEF causes as many hospitalizations and incurs as severe morbidity as heart failure with reduced LVEF. Finally, HFpEF portends a significant and un-improving mortality. (12) Recent epidemiological studies suggests that the leading cause of death in patients with HfpEF is non-cardiovascular whereas it is coronary artery disease in patients with systolic heart failure (SHF), and that the proportion of cardiovascular death reduced over time in patients with HfpEF. (13)

The available epidemiological data suggests that these patients are, as a group, older, more commonly female, and more frequently have systolic hypertension (associated with stiff large arteries) than those patients with a reduced LVEF (14). Patients may present with

chronic symptoms of breathlessness and/or fatigue, and/or acutely in ‘flash’ pulmonary oedema (15).

Diagnostic criteria

Differentiation between systolic heart failure and HfpEF in patients presenting with chronic symptoms cannot be made at the bedside on the basis of the history, examination, ECG, or chest X-Ray alone (16). At present the epidemiology, pathophysiology and therapy of HFpEF is poorly understood, in part because of the absence of robust widely accepted diagnostic criteria.

The European Society of Cardiology proposed the following criteria for the diagnosis of HFpEF (17): (a) signs or symptoms of heart failure, (b) presence of normal or mildly abnormal LV systolic function, and (c) evidence of abnormal LV relaxation, filling, diastolic distensibility, and diastolic stiffness. (Fig. 1) This was a consensus document and it has not been validated or used in clinical studies.

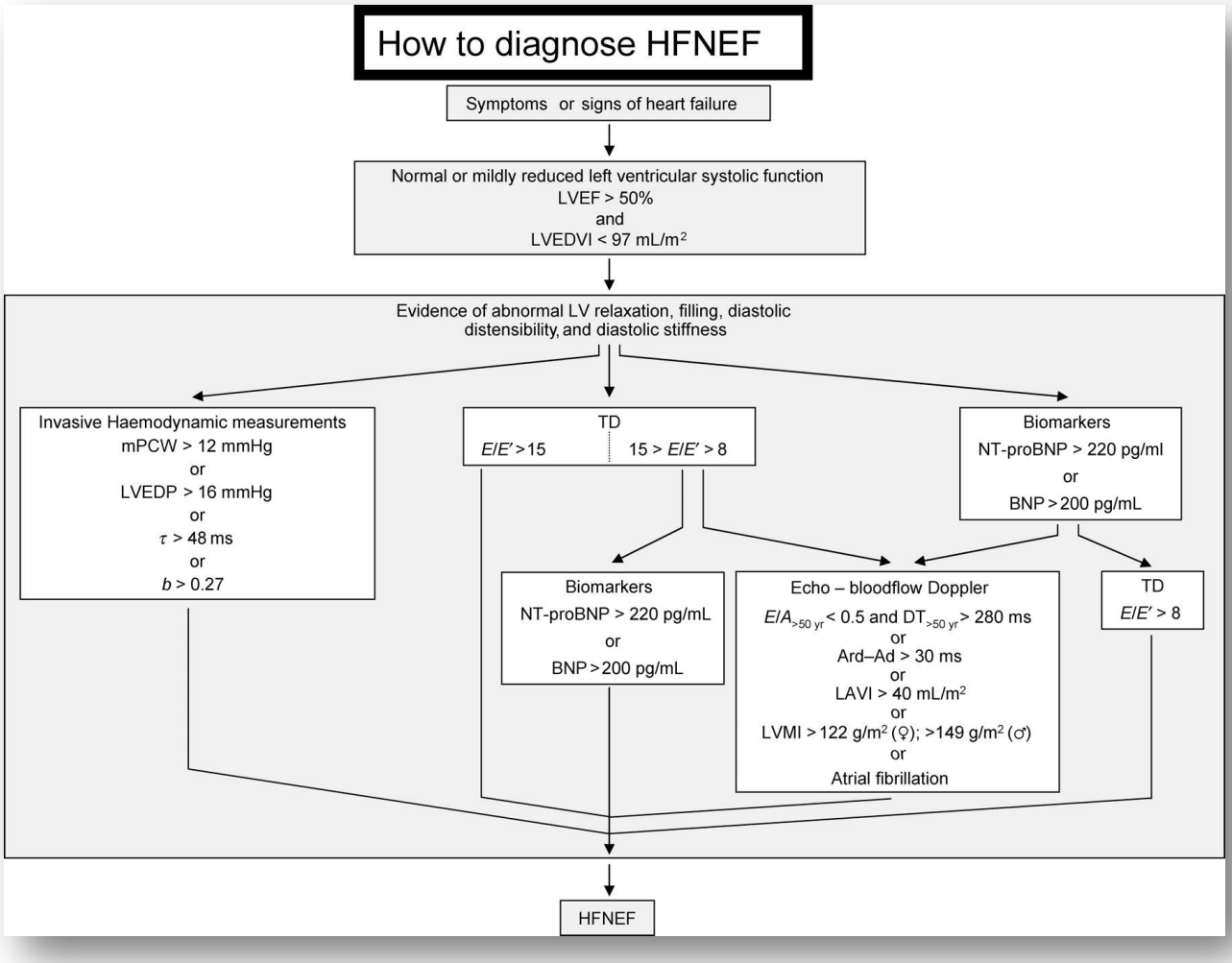

Figure 1: Flow chart from 2007 ESC guidelines 'How to diagnose diastolic heart failure'. (17)

The Vasan and Levy criteria for 'diastolic heart failure' (DHF) proposed a few years earlier were also slightly similar but placed less emphasis on the presence of normal ejection fraction (EF) as this potentially could lead to underestimation of heart failure. (18) They proposed criteria of definite, probable and possible DHF. (Table 1)

Table 1: Vasan and Levy Criteria for Diastolic Heart Failure
1. Reliable evidence of CHF (Framingham or Boston criteria)
2. Objective evidence of normal LV EF (EF >50% within 72 h of CHF event)
3. Evidence of LV diastolic dysfunction (cardiac catheterization is required)

If all 3 criteria are met, the diagnosis of "definite" diastolic heart failure is made. In the absence of cardiac catheterization data, a diagnosis of "probable" diastolic heart failure is made. If the EF was not measured near the time of the heart failure and the patient presented with CHF and catheterization was not performed, then a diagnosis of "possible" diastolic heart failure is made. (Table from Yturralde and Gaasch (19))

One of the main differences between the Vasan and Levy criteria and the ESC guidelines was that in the ESC guidelines they accepted diastolic dysfunction as determined by either catheterization or echocardiography, whereas in the Vasan and Levy criteria, cardiac catheterization was required for a definite diagnosis of DHF.

What both of these proposals have in common is that they require the evidence of abnormal LV relaxation, filling, diastolic dispensability, and diastolic stiffness. However, whether it is necessary to have evidence of diastolic dysfunction to have HFpEF is

controversial especially when measuring LV diastolic function is fraught with difficulties and most indices of diastolic function are load dependent. (20) To fully assess ventricular relaxation, high-fidelity measures of LV pressure must be obtained at cardiac catheterization. Because diastolic compliance falls in a curvilinear fashion with increasing pressure, assessment of passive diastolic stiffness requires measurement of end-diastolic pressure-volume relations over a variety of preloads (e.g. using IVC balloon occlusion) and is clearly impractical as a routine. Some believe that the measurements of relaxation rates have no diagnostic value. (21) Indeed, in a study of Zile et al (22), they showed that the diagnosis of HFpEF can be made without measurement of LV relaxation and passive stiffness and concluded that the measurement of LV diastolic dysfunction serves to confirm rather than establish the diagnosis of HFpEF.

Several features of diastolic dysfunction can be assessed on echocardiography. The most commonly used is transmitral flow on pulse-wave Doppler analysis. A ‘delayed relaxation’ pattern (reversal of the E/A ratio) is often referred to as indicating ‘diastolic dysfunction’. However, resting diastolic abnormalities are frequently present in healthy elderly subjects (23) and it does not necessarily predicts clinical heart failure (24) nor exertional dyspnoea (25). When LV end-diastolic pressure rises, a ‘pseudo normal’ or ‘restrictive’ pattern may supervene (26). (Fig 2)

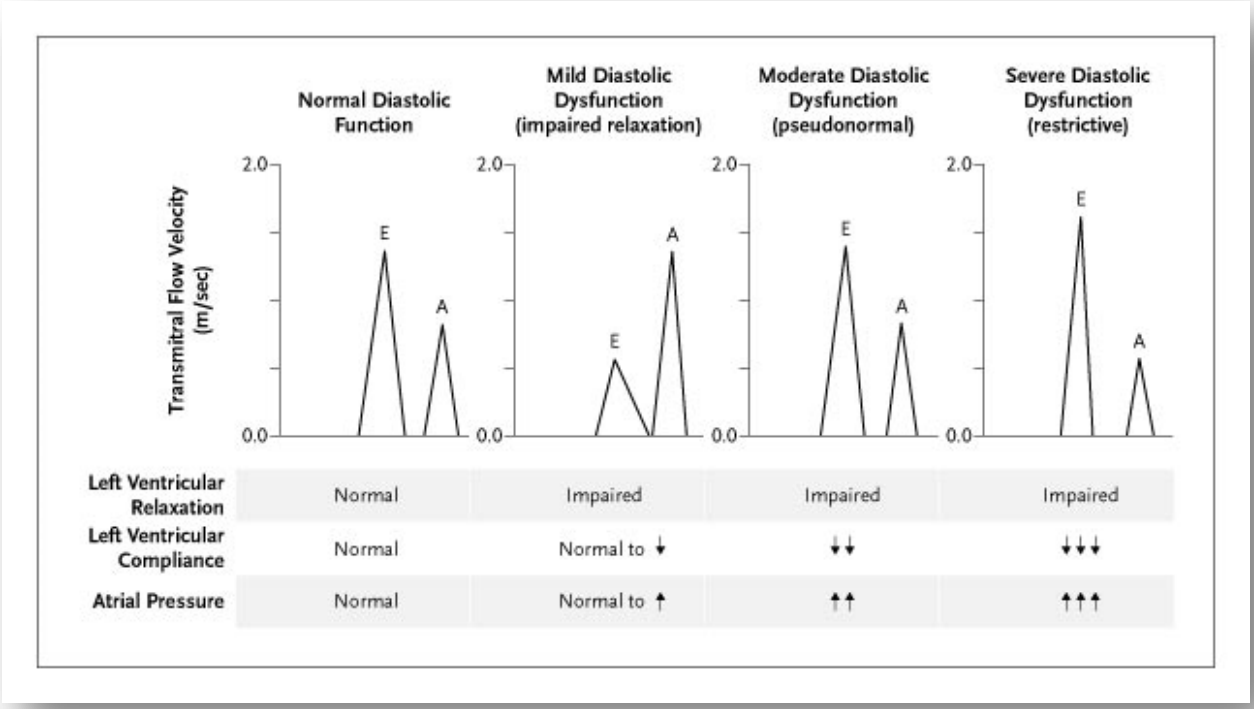

Figure 2: The abnormal relaxation pattern (mild diastolic dysfunction) is brought on by abnormally slow left ventricular relaxation, a reduced velocity of early filling (E wave), an increase in the velocity associated with atrial contraction (A wave), and a ratio of E to A that is lower than normal. In more advanced heart disease, when left atrial pressure has risen, the E-wave velocity and E:A ratio is similar to that in normal subjects (the pseudonormal pattern). In advanced disease, abnormalities in left ventricular compliance may supervene. In these latter two instances, the E wave of normal to high velocity is a result of high left atrial pressure and a high transmitral pressure gradient in early diastole. Therefore, the use of transmitral velocity patterns alone to estimate left ventricular filling pressures in patients with diastolic heart failure is problematic. Figure from Aurigemma and Gaasch. (27)

In fact, despite this, the presence of a 'delayed relaxation' pattern at rest appears not to identify elderly subjects with 'diastolic heart failure', but rather is associated with a significantly greater exercise capacity than the presence of a 'normal' filling pattern. (28) In

the Strong Heart Study of elderly subjects, the presence of a 'normal' E/A profile was associated with an increased risk of developing heart failure and of subsequent mortality compared with the 'delayed relaxation' profile, presumably because a high proportion of patients with a 'normal' profile actually had a 'pseudo normal' pattern (29).

Other echocardiographic measures include the deceleration time of the E wave, which is characteristically prolonged in patients with impaired relaxation, and IVRT (Isovolaemic relaxation time), which tends to parallel deceleration time. Assessment of pulmonary vein flow helps to differentiate between normal and 'pseudo normal' patterns (30). Tissue Doppler assessment of the mitral annulus during diastole provides a velocity profile of the movement of the mitral annulus, and is less preload-dependent than conventional indices of transmitral flow. The mitral annular E wave velocity (Em) is related to the rate of active relaxation, and is less influenced by filling pressures than the E wave velocity on transmitral flow. The ratio of transmitral E/Em has been shown to correlate well with LVEDP (31, 32). Whilst a high E/Em ratio in a patient with symptoms of chronic heart failure but normal LVEF is strongly supportive of heart failure, many of these patients have normal or near normal estimated LVEDP at rest, and the negative predictive value of a normal E/Em ratio at rest for HFpEF may therefore be relatively low. Importantly, as discussed below, diastole is highly dynamic and load-dependent, therefore standard resting measures may provide little information about diastolic function during exercise. Consequently, even sophisticated resting measures of diastolic function may not accurately identify patients with HFpEF.

Metabolic exercise testing can be useful to objectively measure exercise intolerance especially in patients with little evidence of congestion or physically deconditioned. As

argued by Coats in a recent review, metabolic exercise testing is the nearest one can achieve to a ''gold'' standard for evaluating the mechanism of exercise limitation in patients with breathlessness on exertion, especially in patients in whom cardiac and respiratory disease co-exist. (33, 34) Patients undergo full resting assessment of respiratory function, including measurement of MVV (maximum voluntary ventilation) and Transfer Factor (DL_{CO} – a measure of lung diffusion). Breath by breath assessment of gas exchange and finger oximetry is performed at rest and during incremental exercise. (33, 34)

In the Yturralde and Gaasch diagnostic criteria for diastolic heart failure (19) they incorporate the use of metabolic exercise testing and does not stipulate the presence of diastolic dysfunction as a major criteria. In the Yturralde and Gaasch criteria, emphasis is placed on clinical evidence of DHF and the presence of normal LVEF and LV chamber size. Confirmatory evidence such as the presence of LVH or LA enlargement or diastolic dysfunction (echocardiographic Doppler or catheterization) is then required to make a definite diagnosis of DHF. (Table 2)

Table 2: Yturralde and Gaasch diagnostic Criteria for diastolic heart failure
Major criteria
1. Clinical evidence of heart failure
Framingham or Boston criteria
Plasma BNP or chest x-ray
Cardiopulmonary exercise testing
2. Normal LV EF and chamber size
Data are contemporary with item 1
Confirmatory evidence
1. LV hypertrophy or concentric remodeling
2. Left atrial enlargement (in absence of atrial fibrillation)
3. Echocardiographic Doppler or catheterization evidence of diastolic dysfunction

If the 2 major criteria are met and there is evidence of LV hypertrophy or left atrial enlargement, the diagnosis of definite diastolic heart failure can be made. In the absence of hypertrophy or left atrial enlargement, it can be appropriate to make a tentative diagnosis of probable diastolic heart failure and require confirmatory evidence before making a diagnosis of definite diastolic heart failure. Valvular heart disease should be excluded. Table adapted from Yturralde and Gaasch for diastolic heart failure (19)

Acute pulmonary oedema in HFpEF

Patients with HFpEF often have markedly labile symptoms, and can present not only with chronic symptoms of breathlessness and/or fatigue, but also in pulmonary oedema, which may occur with very rapid onset (‘flash’ pulmonary oedema). Recent studies have shown that up to 30-40% of patients presenting to the emergency department with clear cut acute pulmonary oedema have normal LVEF measured within 2 hours of arrival, and Gandhi et al reported no significant difference between LVEF measured before and after stabilization

(35). This does not entirely exclude the possibility of transient systolic dysfunction that had fully recovered by the time of initial echocardiography. Some episodes of 'flash' pulmonary oedema in patients with HFpEF may be precipitated by acute myocardial ischaemia by one of three mechanisms: (a) transient systolic dysfunction, (b) ischaemia-mediated diastolic dysfunction, or (c) ischaemia-mediated mitral regurgitation (36, 37). Other precipitating factors may include arrhythmias (especially paroxysmal atrial fibrillation), and bilateral renal artery stenosis (38). However, many cases remain unexplained.

A majority of patients with HFpEF often have normal LV end-diastolic volume (39) and LVEF with a low stroke volume and reduced cardiac output. (22) Studies have also found that LA volume indexed to body surface area was a stronger predictor of cardiovascular event in the elderly than LV mass index or LV diastolic dysfunction, (40) and that LA volume is a marker of LV diastolic dysfunction in patients with heart failure and normal LVEF. (41, 42) LA volume has also been shown to predict cardiovascular event in patients with diastolic dysfunction. (43, 44) In a study of a predominantly African American cohort of patients with hypertensive left ventricular hypertrophy (LVH), it was shown that those with and without features of HFpEF had similar systolic, diastolic and vascular function, however the cohort with features of HFpEF differed predominantly because they had evidence of left atrial dilatation and left atrial 'failure'. (45) In a separate community study, LA fractional area change (a measure of LA emptying volume) was found to be reduced at rest in community older patients (≥65) with diastolic heart failure. (46)

The problem with atrial dilatation is that it can contribute to alterations in LA pressure and therefore reduced early diastolic filling. (47) Furthermore LA dilatation in HFpEF is

associated with reduced LA function (45), reduced LA strain (during systole) and increased LA stiffness (48), the combination of which might compromise atrial contraction and therefore might compromise or worsens diastolic filling. Indeed, when these HFpEF patients develops atrial fibrillation which is not uncommon (11, 12), the consequence is more severe diastolic dysfunction as well as increase hospitalization or death. (49)

Systolic abnormalities in HFpEF

Recently, the concept that diastolic dysfunction is responsible for HFpEF has been challenged. The term 'diastolic' heart failure is often used on the assumption that diastolic dysfunction underlies the pathophysiology, but more recently the term 'heart failure with preserved ejection fraction' (HFpEF) has become popular because it makes no presumptions about the pathophysiology. Many consider HFpEF to be a disorder of diastolic function (50), whilst others believe that it may be due to a combination of diastolic abnormalities with subtle disturbances of systolic function that are insufficient to reduce LVEF (51). Whilst echocardiographic LVEF is preserved in these patients, regional wall motion abnormalities may be missed and abnormal 'long axis' systolic function has also been reported (52, 53) Impairment of long axis systolic function is initially compensated for by enhanced radial function, therefore global LVEF is preserved (52-55). In addition, studies using tissue Doppler imaging (TDI) have demonstrated the presence of diastolic and/or systolic dyssynchrony in patients with HFpEF (56, 57). According to this paradigm, diastolic heart failure is a misnomer and represents a phase in the evolution of heart failure where the LVEF is still within the 'normal' range. Advocates of this view argue that the increased diastolic LV stiffness reported in patients with HFpEF might be the result rather than the cause of the raised LVEDP, because compliance decreases as diastolic pressure increases. As

noted above, assessment of 'passive' LV stiffness requires the evaluation of LV pressure and volume across a wide range of filling conditions. 'Passive' LV stiffness is not consistently increased in patients with HfpEF (58). Table 3 summarises the similarities and differences between diastolic heart failure and systolic heart failure.

Table 3: Characteristics of Diastolic Heart Failure as Compared with Those of Systolic Heart Failure.*

Characteristic	Diastolic heart failure	Systolic heart failure
Clinical features		
Symptoms (e.g., dyspnea)	Yes	Yes
Congestive state (e.g., edema)	Yes	Yes
Neurohormonal activation (e.g., brain natriuretic peptide)	Yes	Yes
Left ventricular structure and function		
Ejection fraction	Normal	Decreased
Left ventricular mass	Increased	Increased
Relative wall thickness†	Increased	Decreased
End diastolic volume	Normal	Increased
End diastolic pressure	Increased	Increased
Left atrial size	Increased	Increased
Exercise		
Exercise capacity	Decreased	Decreased
Cardiac output augmentation	Decreased	Decreased
End diastolic pressure	Increased	Increased

* The clinical features of diastolic heart failure are similar to those of systolic heart failure, but left ventricular structure and function are distinctly different.

† The descriptor of left ventricular geometry is the relative wall thickness, defined as the ratio of left ventricular wall thickness to the radius of the left ventricular cavity. (Table adapted from Aurigemma and Gaasch(27)).

Left ventricular stiffness in HFpEF

Diastolic function is influenced by passive elastic properties of the LV and by energy dependent process of active relaxation. Increased myocardial mass or changes in extra-

myocardial collagen (50) network can cause increased LV passive diastolic stiffness at rest (1). Recently there has been increased interest in the molecule titin which is a large cytoskeletal protein which contributes to resting stiffness of the myocardium. (59)Titin is a giant molecule which spans the entire half-sarcomere from the M-line to the Z-line. It is responsible for passive and restoring forces within the myofilament during sarcomere elongation and compression respectively (60). Titin appears to be the major determinant of passive force development at shorter slack length (61, 62). Titin also acts as a spring during shortening below slack length, generating a restoring force which opposes further shortening (61). It is expressed in two major isoforms via differential splicing. This results in two isoform transcripts; N2BA (long isoform) and N2B (short isoform), which may coexist within the same sarcomere (63). The expression ratio of these isoforms demonstrates interspecies variation, as well as variability in different locations within the heart (63). Changes in isoform expression have been demonstrated in certain pathological states. A recent study showed that in patients with dilated cardiomyopathy there was a shift towards increased expression of the longer N2BA isoform, which correlated with both lower LV stiffness and an improvement in diastolic function (64).

In the canine rapid pacing heart failure model a change in isoform expression towards the shorter N2B isoform has been reported, which may account in part for the higher passive ventricular stiffness which develops in this model (65). Spontaneously hypertensive rats were shown to have a higher N2B/N2BA expression ratio than the normotensive WKY rat, potentially explaining the higher passive ventricular stiffness of the former. (66) A recent study by van Heerebeek et al examined shifts in titin isoform expression between patients with systolic and diastolic heart failure. (67) In this small, highly selected group (n=4 with

DHF, n=5 with SHF) they demonstrated a shift towards the stiffer N2B isoform in the DHF group and a shift towards the longer N2BA isoform in the SHF group when compared with previously publishes results from healthy controls (68). These healthy control samples were obtained from normal donor hearts. However, the authors do not state the mean age of this group, and it is unlikely that they were age-matched. Although invasive haemodynamic studies were performed in the group with DHF, there was no mentioned of measures of LV end-systolic elastance or arterial elastance, and their correlation to the titin isoform expression ratio.

Cardiac myocytes that express predominantly N2BA have a longer extensible region and are predicted to develop less force than those that express predominantly N2B. Passive stiffness of cardiac myocytes is much higher in N2B-expressing myocytes than in N2BA myocytes, thus a change in titin isoform expression may (together with increased collagen content) contribute to the increase in passive LV diastolic and systolic stiffness in patients with HFpEF. A shift to expression of the shorter N2B isoform in response to increased arterial stiffness would increase 'contractility' (to compensate for increased aortic impedance) at the price of increased LV systolic stiffness (and leading in turn to dynamic impairment of LV active relaxation during acute increases in blood volume and/or exercise).

When titin is phosphorylated by protein A, the LV stiffness is reduced which improves LV diastolic compliance and diastolic filling during sympathetic stimulation (i.e. during exercise). (59, 69) In patients with HFpEF, van Heerebeek *et al* found a reduced ratio of N2BA to N2B titin isoforms because of the greater increased in the stiffer N2B which may contribute to the observed high diastolic stiffness in HFpEF. (67) Indeed, in patients with

dilated cardiomyopathy the converse has been observed that is increased levels of the less stiff N2BA isoform. (64)

Zile *et al* studied 47 patients and 10 controls to assess LV diastolic function by means of cardiac catheterization to assess diastolic pressure-volume relationship. (1) They demonstrated that patients with HFpEF had abnormal LV relaxation and increased LV stiffness. The diastolic pressure-volume relationship was also shifted up and to the left. (Figure 3)

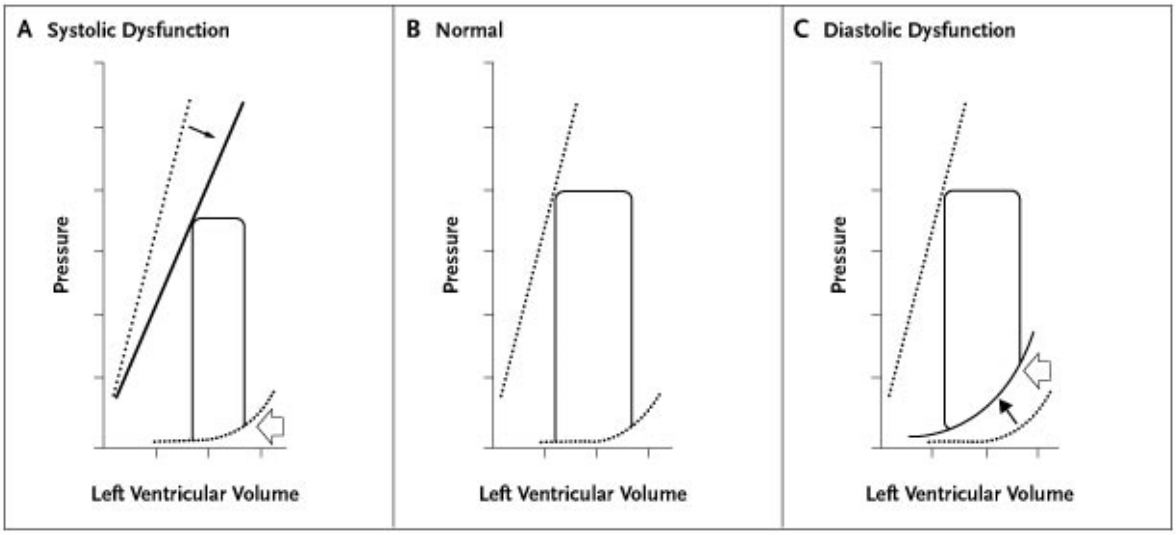

Figure 3: Left Ventricular Pressure–Volume Loops in Systolic and Diastolic Dysfunction.

In systolic dysfunction, left ventricular contractility is depressed, and the end-systolic pressure–volume line is displaced downward and to the right (Panel A, black arrow); as a result, there is a diminished capacity to eject blood into the high-pressure aorta. In diastolic dysfunction, the diastolic pressure–volume line is displaced upward and to the left (Panel C, black arrow); there is diminished capacity to fill at low left-atrial pressures. In systolic dysfunction, the ejection fraction is depressed, and the end-diastolic pressure is normal

(Panel A, open arrow); in diastolic dysfunction, the ejection fraction is normal and the end-diastolic pressure is elevated (Panel C, open arrow). (Figure from Aurigemma and Gaasch (27)).

A subsequent study by Westermann et al (2), involving pressure-volume loop analyisis with and without atrial pacing in 70 HFpEF patients and 20 matched-controls, found that enhanced left ventricular stiffness can result in increased end-diastolic pressure (EDP) during handgrip exercise. In addition during atrial pacing, pacing with HFpEF displayed decreased stroke volume, bearing in mind that these patients were resting in supine position and thus to artificially increase HR by atrial pacing might not reflect true physiological exercise conditions. At the very least, these data suggest that left ventricular stiffness can modulate cardiac function in HFpEF patients.

Systolic and diastolic function during exercise

Diastolic abnormalities may involve active ventricular relaxation and/or passive ventricular filling. The physiological increase in the rate of LV active relaxation during exercise is a consequence of sympathetic activation, via cAMP-dependent protein kinase (PKA) mediated phosphorylation of key proteins including Troponin I, Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) and Titin. (70-72) Consequently, in health even at high heart rates associated with maximal exercise, it has been shown that the rate of LV relaxation should not limit LV filling (73).

Exercise data in HFpEF is scarce. In a small study by Kitzman *et al* 7 patients with HFpEF and 10 matched controls underwent invasive cardiopulmonary exercise testing. HFpEF patients

had a shift of the LV end-diastolic pressure volume relation upward and to the left at rest, however during exercise increases in LV filling pressure during exercise were not accompanied by increases in end-diastolic volume index (EDVi), indicating limitation to LV filling during exercise and a failure of the Frank-Starling mechanism. (74) More recently a study conducted by Kawaguchi et al, reported in a relatively small number of HFpEF patients (n=10) a dynamic impairment of left ventricular active relaxation during isometric (handgrip) exercise. (58) This abnormal relaxation during dynamic exercise has previously been described in other related conditions. In a study of patients with asymptomatic essential hypertension, it was found that hypertensive patients (with LVEF either increased by <5% or decreased with exercise) had impaired diastolic filling during exercise. (75) In another study conducted by our group in patients with non-obstructive hypertrophic cardiomyopathy, a classic paradigm of diastolic heart failure, we found that exercise left ventricular diastolic filling characteristics were major determinant of peak exercise capacity. (76)

In a study by Borlaug *et al* (77) , 17 HFpEF patients and 19 matched controls were studied at rest and during exercise using radionuclide ventriculography to assess chronotropic, systemic vasodilatation, and cardiac output responses to exercise. They found that HFpEF patients had impaired chronotropic and systemic vasodilatation responses to exercise compared to matched-controls. Ennezat *et al* (78), studied 25 HFpEF patient and 25 hypertensive matched-controls using exercise echocardiography. They found that patients with HFpEF had reduced arterial elastance response to exercise which was accompanied by reduced systolic function as measured by LVEF, stroke volume and cardiac output. In another study by Ha *et al* (79), 141 patients with abnormal LV relaxation as defined by echocardiographic mitral inflow Doppler measurements (mitral E/A <0.75 and/or

deceleration time >240 ms) underwent exercise echocardiography with respiratory gas analysis. They found that patients with low LV diastolic function reserve index had higher ventricular elastance during exercise and reduced exercise capacity.

Ventricular-vascular coupling

The interaction between the heart and the systemic vasculature, termed ventricular-vascular coupling (VVC) is essential for the heart to achieve maximal cardiac work, power and chamber efficiency while maintaining physiological blood pressures and cardiac outputs. (80, 81) Figure 4 demonstrates the relationship between stroke work and VVC coupling in isolated canine hearts.

VVC is indexed by the ratio arterial elastance/end-systolic elastance. End-systolic elastance (Ees) is calculated from the slope of the end-systolic pressure-volume relation. Arterial elastance (Ea) is a measure of impedance and is determined by the ratio of end-systolic pressure/stroke volume. The VVC ratio in normal humans ranges between 0.7 and 1.0, the range of optimal function.(82, 83) In patients with chronic heart failure (CHF) this VVC ratio is typically high as Ees decreases (poor ventricular contractile function) and a rise in Ea (arterial resistance). Such high ratio leads to poor ventricular performance and metabolic efficiency. (82, 83) (Figure 4)

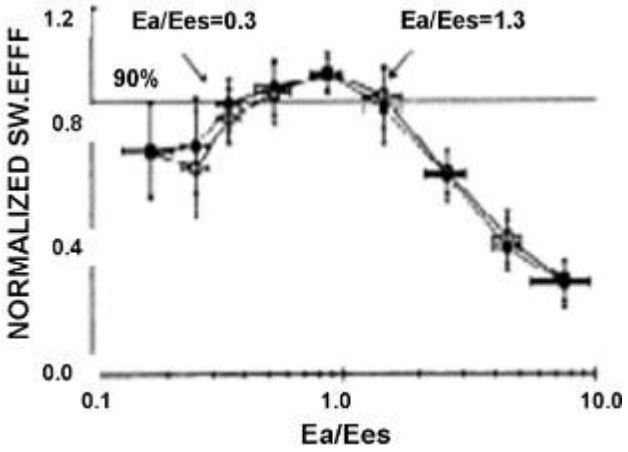

Figure 4: Mean data obtained in isolated canine hearts, demonstrating the dependence of left ventricular stroke work on ventricular-vascular coupling (as indexed by the ratio of Ea/Ees). Over an Ea/Ees ratio spanning 0.3 to 1.3, left ventricular stroke work and cardiac metabolic efficiency is near optimal, whereas both decline at much higher or lower ratios. From De Tombe et al (84)

Aging is associated with increases in Ea (85) which in turn increases the load on the heart by increasing systolic wall stress. (86) Aging is also associated with increases in Ees which therefore preserve the VVC ratio and therefore preserving cardiac chamber power, stroke work and efficiency. (85) However, in women the VVC ratio declines modestly because of a disproportionate increase in Ees compared to Ea. (87) VVC ratio typically falls with exercise, as Ees (contractility) increases more than the increase in Ea (afterload) to augment cardiac output and blood pressure. (88) With aging, the exercise drop in VVC ratio becomes compromised, which in part contributes to the age-dependent reduction in aerobic capacity. (88)

However, there are consequences to an increased ventricular-vascular stiffness which includes reduced reserve mechanisms and hemodynamic instability. The hemodynamic consequences of an increased ventricular-vascular stiffness are reduced left-sided circulatory compliance which can lead to large changes in LV end-systolic pressure for a given change in ejected volume. This means for the heart, any modest rise in central blood volume (e.g. venoconstriction or increased blood volume) can result in exaggerated changes in systolic pressure. Furthermore, increased vascular stiffness can result in dramatic alterations in arterial pressures for a given change in cardiac output from the heart. (85) Ea usually rises because of increased heart rate and increased arterial pulsatility despite a fall in peripheral vascular resistance. (86) Elevated Ea can exacerbate systolic hypertension during exercise which increases energetic demand on the heart and raises myocardial oxygen consumption. (86) Enhanced LV contractility is reflected by a rise in Ees, however when there is elevated basal Ees and Ea there is consequently less reserve.

Many patients with HFpEF are hypertensive, typically with isolated ‘systolic’ hypertension. Systolic hypertension is predominantly due to increased large artery stiffness and results in an increased pulsatile left ventricular afterload (impedance) often leading to left ventricular hypertrophy. A recent study showed that in older patients with ‘diastolic’ heart failure, impaired exercise tolerance correlated with aortic stiffness, which was significantly increased compared with age-matched controls (89). In a separate study, patients receiving verapamil was found to have increased exercise capacity, in association with a reduced resting pulse wave velocity and carotid pressure augmentation, implying improved large artery distensibility (90).

In patients with HFpEF, the VVC ratio falls compared to younger individuals (85) but similar to asymptomatic hypertensive elderly patients (45, 91) and falls within a range where cardiac work and efficiency are not compromised. (84) However, although the VVC ratio is preserved in patients with HFpEF, the absolute values of Ees and Ea are considerably elevated. HFpEF have increased vascular and ventricular stiffness in both systole and diastole. (1, 91) (Figure 5). Table 4 summarises the harmful hemodynamic effects of increased ventricular and vascular stiffness.

Studies in healthy subjects have found that during exercise, VVC coupling decreases in both genders but to a lesser extent in older healthy subjects. (88) (Figure 6) This lead to the postulation that sub-optimal VVC coupling may be associated with blunting of maximal exercise ejection fraction. To date, no dynamic exercise data exists for VVC coupling in patients with HFpEF. However we can get some idea from a study that used hand grip exercise during invasive pressure-volume loop studies to derive the VVC coupling.

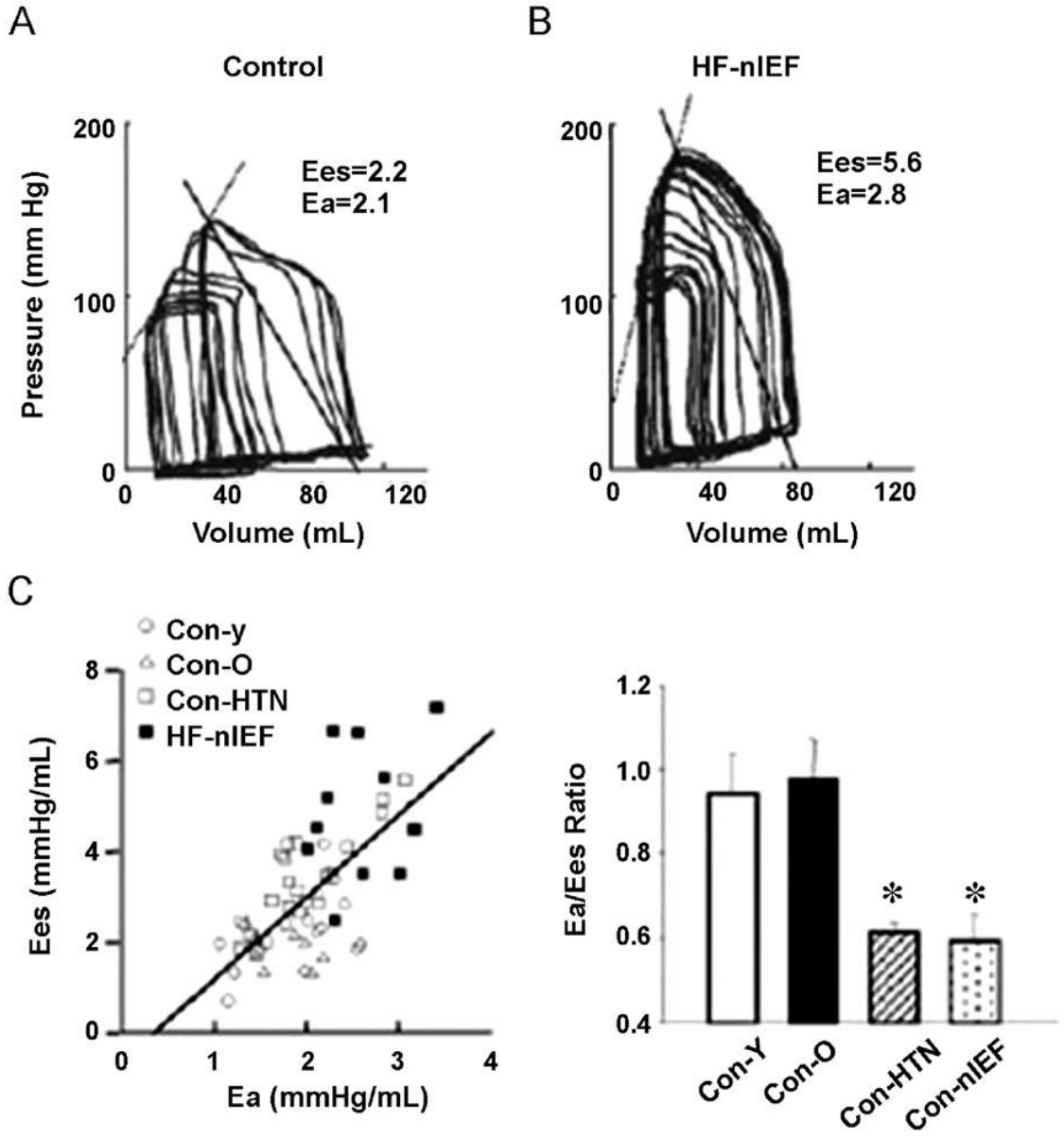

Figure 5: Increased Ees and Ea in a patient with Heart Failure normal Ejection Fraction (B) compared with an age matched healthy control (A). There is near matching of Ees and Ea in the control subject, compared with a disproportionate increase in Ees compared with Ea in the Heart Failure normal Ejection Fraction patient. (C) Group data showing the correlation between Ea and Ees (left) and the Ea/Ees coupling ratio for each subgroup (right). The HfpEF

patients show a lower coupling ratio compared with normotensive age-matched controls. Reproduced from Kawaguchi M et al (58))

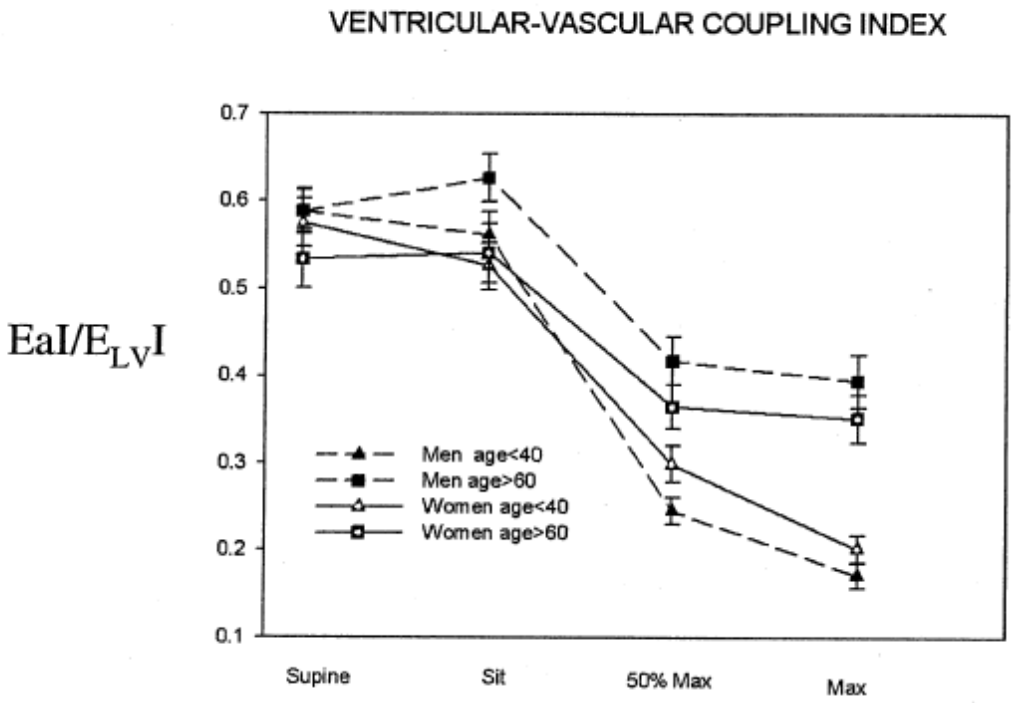

Figure 6: Ventricular-vascular coupling at rest and during exercise in healthy subjects defined by age and gender. (Figure from Najjar SS et al (88))

Table 4: The pathophysiology of ventricular vascular stiffening		
Abnormality	**Hemodynamic consequences**	**Clinical relevance**
Increased Ventricular Systolic Stiffness	Exaggerated change in blood pressure for a given change in preload or afterload	Hypotension and oliguria with slight over-diuresis or the addition of a new vasodilator agent
	Lower contractile reserve	Modest volume infusion leads to hypertension and/or acute pulmonary edema
	Lower stroke volume reserve	Impaired exercise tolerance and functional disability
	Greater energetic cost to eject a given stroke volume	Increased myocardial oxygen demand and ischemia
Increased Arterial Stiffness	Exaggerated change in blood pressure for a given change in preload or contractility	Hypotension and oliguria with slight over-diuresis or the addition of a new vasodilator agent
	Increased total afterload, wave reflections and late systolic load	Modest volume infusion leads to hypertension and/or acute pulmonary edema
	Greater dependence upon systolic pressure for coronary flow	Impaired relaxation and decreased LV diastolic compliance, prolonged systole, abbreviated diastole
	Abnormal endothelial mechanotransduction	Increased ischemia and infarct size for a given drop in systolic blood pressure
		Endothelial dysfunction, abnormal vasodilation response to stress

Table adapted from Borlaug and Kass (92)

In a recent invasive hemodynamic study (58), a small group of patients with HFpEF were compared with healthy age and gender matched controls. LV pressure volume loops were constructed before and during acute balloon occlusion of the inferior vena cava. Ees was

then calculated by measuring the slope of this pressure/volume relation. Ees is a measure of end-systolic LV elastance (ΔP/ΔV). Ea was calculated as the ratio of end-systolic pressure/stroke volume, and is a measure of impedance (being influenced by static and pulsatile afterload and by heart rate). In HFpEF patients both of these relations were considerably steeper than in controls (i.e. for a given increase in systolic volume these patients demonstrated much larger increases in LV end-systolic pressure (LVESP))(58). Active cardiac relaxation is slowed by large acute increases in LV 'afterload' (93), and, consistent with this, during handgrip exercise

Patients with HfpEF demonstrated an increase in time constant of the left ventricular isovolemic pressure decline (Tau (τ)) (from resting values which were near normal) in contrast to healthy controls where τ had reduced as one would expect. This slowing of active relaxation resulted in an exercise-related impairment of LV filling in these patients, causing an upward displacement of the LV end-diastolic pressure-volume relation (Figure 7). Thus although passive chamber stiffness and τ were normal in some patients at rest, both became markedly abnormal during handgrip exercise, a finding that is supported by our previously performed non-invasive study during dynamic exercise (94). This small study provides a potentially attractive link between increased large artery stiffness and exercise-induced diastolic dysfunction. It may also explain how small changes in fluid balance may precipitate 'flash' pulmonary oedema in patients with HFpEF. However, the study population was highly selected – a total of 10 patients over a four year period.

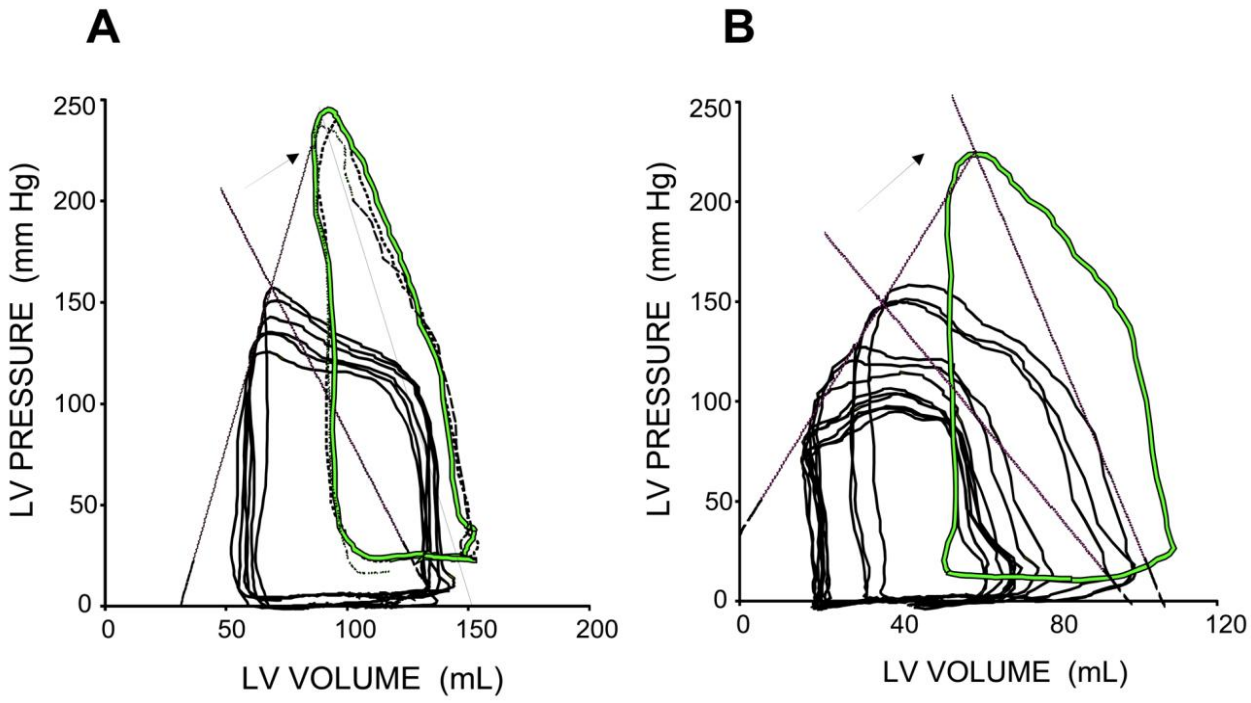

Figure 7: An example of pressure volume loops taken from patients with HfpEF at baseline and with acute increases in Ea induced by isometric handgrip (arrows). Because of elevated baseline stiffness, the "gain" is much greater with further increases, leading to severe hypertension. Note the greatly increased end-diastolic LV pressures during handgrip. (Figure from Kawaguchi et al (58))

Afterload and effects on LV active relaxation

Animal studies have demonstrated that a large acute increase in afterload in the rabbit resulted in a marked slowing of active relaxation and impaired left ventricular LV diastolic filling (93). A key coupler of this load dependent LV relaxation is Troponin I – Protein Kinase A (TnI-PKA) phosphorylation (95). This energy dependent process of phosphorylation of Troponin I by PKA decreases myofibrillar calcium sensitivity (96) and increases the rate at

which calcium dissociates from Troponin C (97) which can lead to increase rate of LV relaxation by increasing the rate of thin filament deactivation. Indeed, in a study involving transgenic mice in which PKA phosphorylation sites on Troponin I were constitutively active, acute aortic constriction led to a lengthening of τ in the wild type mice but not in the transgenic mice. (95).

Data from canine models suggests that the acute increase in afterload required to cause a slowing of active relaxation may be much less in a diseased compared with a healthy heart. Indeed in healthy hearts, modest elevations of afterload increase the rate of LV active relaxation, and a slowing of active relaxation is only observed with large acute increases in afterload. These findings can be explained by the concept of *relative load,* which represents the ratio of systolic LV pressure to isovolumetric LV pressure (98). A similar systolic LV pressure represents a higher relative load in the failing than in the normal heart. When relative load is low, afterload reserve is still available allowing the heart to face increased afterload without slowing of LV active relaxation. When relative load is high, afterload mismatch (99) occurs and a pronounced slowing of LV active relaxation is observed (98).

Myocardial energetics and LV active relaxation

The hypothesis that heart failure is due to energy-starvation has been around for decades. (100) Under aerobic condition the heart generates its energy primarily from free fatty acids (FFA) in particular long-chain fatty acids (LCFA), accounting from 60-90% of the energy generated. (101) Carbohydrate metabolism accounts for about 10-40% of energy generated by the heart, although the healthy heart is omnivorous – adapting its 'diet' as required (102). In contrast, the fetal heart utilizes predominantly glucose as its energy substrate and

the switch to free fatty acids metabolism occurs in the early postnatal period (103). During ischaemia there is a shift to relatively greater glucose metabolism. As noted below, FFA utilization requires more oxygen per unit of ATP generated than glucose utilization. Analysis of human biopsy specimens has shown a 25-30% reduction in [ATP] levels in the failing human heart. (104, 105) There is good evidence from cardiac magnetic resonance spectroscopy (MRS) studies of impaired myocardial energetic status in patients with systolic heart failure (106), and in patients with insulin resistance (107, 108). Hypertrophic cardiomyopathy (HCM), a paradigm of diastolic heart failure also displays impaired cardiac energetics as shown by a diminished resting PCr/ATP ratio. (109, 110)

In the canine rapid pacing heart failure model a reduction in high energy phosphate status precedes objective evidence of impairment of LV systolic function (111). Furthermore, normal ageing is also associated with reduced mitochondrial function (112, 113) which may further exacerbate energy impairment in heart failure patients. Interestingly our group have previously shown that there is typically a dynamic abnormality of active relaxation during exercise in HCM, and that peak exercise rather than resting time to peak filling (TTPF, a measure of rate of LV active relaxation (114)) correlates strongly with VO_2max in these patients (76). In a study by Smith CS et al (115) demonstrated in patients with left ventricular hypertrophy (LVH) and CHF (with no evidence of CAD) there was a 30% decrease in PCr/ATP ratio. Furthermore, there was a reduction in K_{for} (which is effectively the fraction of the PCr pool that exchanges with ATP each second) by 50% and reduced ATP turnover through creatine kinase (CK) reaction (the product K_{for} [Pcr]) by 65% compared with normal subjects ((P<.001). Interestingly, patients with LVH with normal EF and reduced EF heart failure had similar K_{for} and cardiac CK flux, thus demonstrating a cardiac energetic deficit in

patients with HFpEF. This is supported by animal studies which have indicated that dogs with severe LVH exhibited reduced PCr/ATP ratio and that the degree of reduction was proportional to the extent of the LVH. (116)

In the heart, the synthesis of ATP by means of oxidative phosphorylation matches the ATP demand demanded by contractile activity. However, when mitochondria become dysfunctional, FoF1 ATP synthase hydrolyses ATP rather than synthesizing it. This may provide a mechanism for reduced energy production under conditions of stress (including acute increases in LV afterload), leading to impaired LV relaxation due to reduced PKA-mediated phosphorylation of Troponin I or potentially to reduced SERCA activity.

Hypothesis

Data from these previous studies has led us to hypothesize that in HfpEF there is a dynamic patho-physiological process that occurs during exercise which results from disturbances in the ventricular-vascular coupling ratio, abnormal LV active relaxation, and an underlying reduction in myocardial energetic reserves. This leads to impaired exercise tolerance and symptoms in patients with HfpEF particular during exertion. (Figure 8)

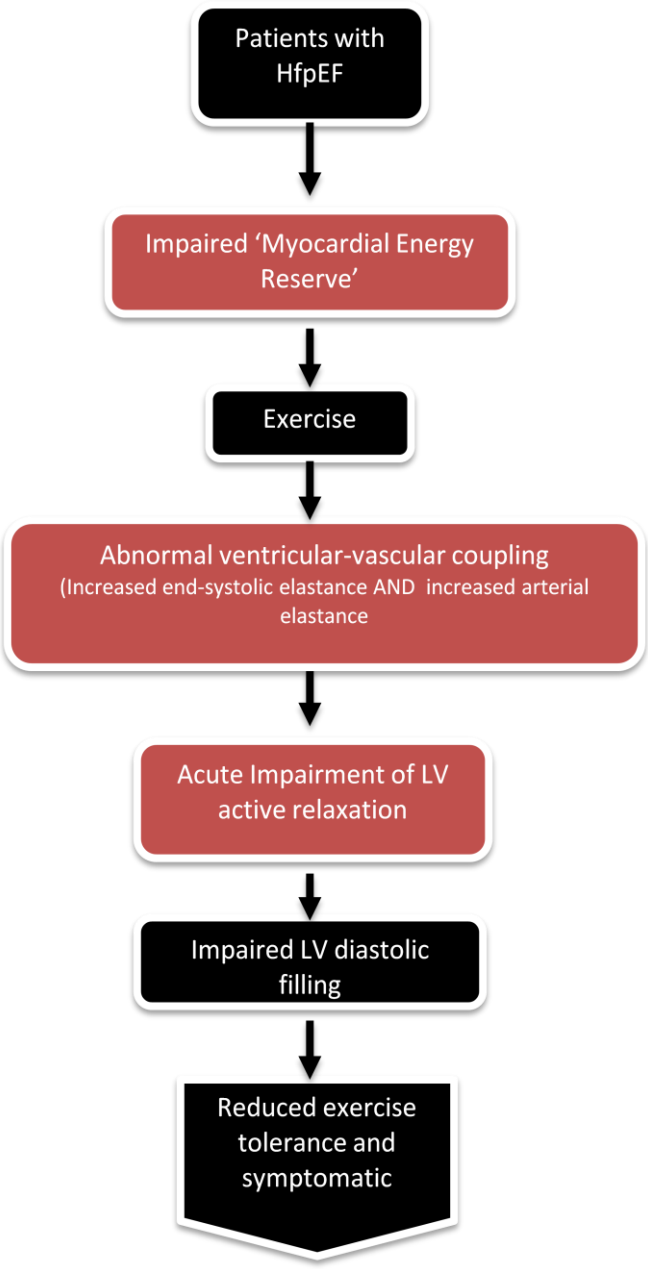

Figure 8: The paradigm

Methods

Methods

Study Participants

HfpEF patients

A total of fifty patients were recruited or referred from heart failure specialist clinics for screening. 41 patients fulfilled the criteria for HfpEF and were prospectively and consecutively recruited. They had i) signs and/or symptoms of heart failure, ii) normal LVEF and chamber size iii) objective evidence of exercise limitation on cardiopulmonary exercise testing (peak VO2 <80% of predicted) with a pattern of gas exchange indicating a cardiac cause for limitation exercise capacity, and (iv) absence of objective evidence of lung disease on formal lung function testing and/or absence of arterial desaturation during exercise and with a ventilatory reserve at peak exercise ≥ 15L. This is consistent with Yturralde and Gaasch criteria for diastolic heart failure. (19) All study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram and metabolic exercise test. Patients with severe pulmonary disease, significant valvular heart disease, atrial fibrillation, or evidence of hypertrophic cardiomyopathy were excluded similar to previous studies (77). Some patients were not involved in all the studies because of preferences or the presence of contraindications to for example magnetic resonance imaging.

Healthy controls

We studied 53 healthy controls with no cardiac history, hypertension or diabetes mellitus. Healthy controls were volunteers recruited prospectively from the community. All control subjects had a normal clinical cardiovascular examination, 12-lead electrocardiogram, echocardiogram and metabolic exercise test.

^{31}P Magnetic Resonance Spectroscopy to Measure *In Vivo* Cardiac Energetics

^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3T scanner and a linearly polarized transmit and receive ^{31}P coil with a diameter of 14 cm. Localization was achieved by ISIS (117) volume selection. The participants were positioned supine with the coil directly over the precordium. The coil was secured in place by straps wrapped around the upper body and coil. The participants were then positioned inside the magnet with the center of the coil at the isocenter of the magnet. Survey images were obtained to check the position of the coil (figure1). The subjects and/or the coil were repositioned if required to ensure that the distance between coil and septum and apex of the heart was minimized.

Localized iterative 1st order shimming was performed including the entire heart using the unsuppressed water signal acquired with the body coil as reference. A short axis cine scan was acquired to calculate the trigger delay for ECG triggering and check quality of shimming and F_0 determination. The trigger delay was calculated such that the spectra were acquired in the diastolic period. The 3-D voxel of acquisition was planned to include most of the septum and apex of the heart (Figure 1). Care was taken to minimize blood contamination from the right ventricle as much as possible. The voxel size was kept constant at 89.54ml (44x55x37mm^3) so that comparisons could be made between different subjects and scans. Initially, ^{1}H spectra were acquired from the same voxel without water suppression and repetition time of 2000 ms (total scan time of 16 sec). This helped to ensure adequate shim quality and correct F_0 determination. F_0 could be manually adjusted if necessary. Following this the ^{31} P spectrum was acquired with a repetition time of 10000 ms, 136 averages and

512 samples. A repetition time of 10000 ms was found to be optimal to adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG gated and the trigger delay was set to acquire spectra mainly in diastole. The trigger delay was measured by subtracting 250-300 from the total length of the cardiac cycle which allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes. The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program(118). Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2,3-Diphosphoglycerate (2,3-DPG) were calculated as the area under the peaks (Figure 2). PCr/ATP ratio was determined after correcting the ATP peak for blood contamination as described previously. (119)

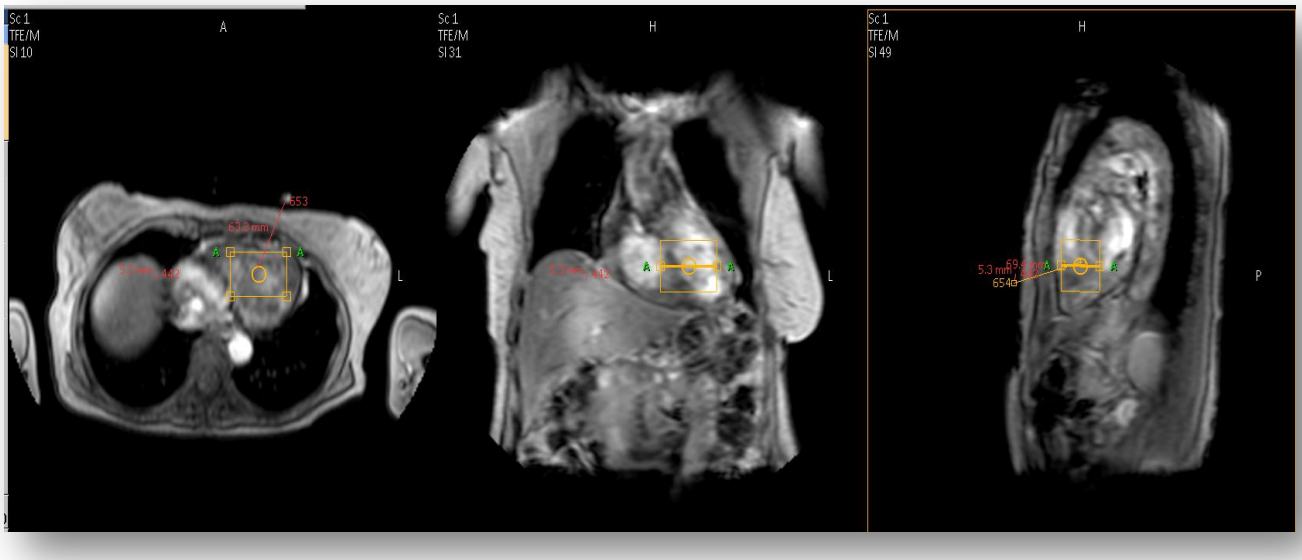

Figure 1: Survey images showing the position of voxel of acquisition and centre of the ^{31}P coil.

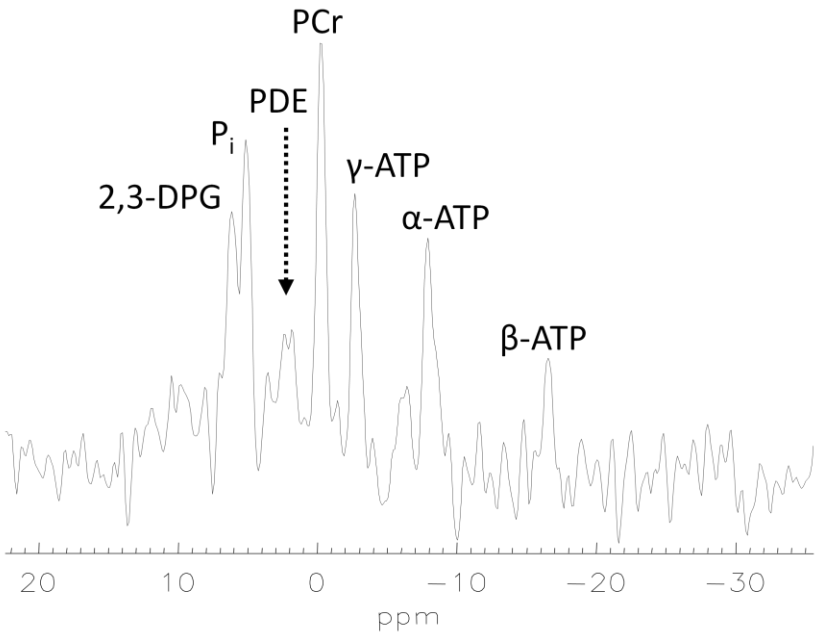

Figure 2: A typical cardiac spectra

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; PDE - Phosphodiesters; ATP - Adenosine triphosphate.

Exercise Radionuclide Ventriculography

Left ventricular ejection fraction and diastolic filling were assessed by equilibrium R-wave gated blood pool scintigraphy using a standard technique at rest and during graded semi erect exercise on a cycle ergometer as previously described in detail by our group (76). Twenty minutes after an intravenous injection of 0.03 mg/kg stannous pyrophosphate, 5 mL of blood was drawn into a heparinised syringe and incubated for 10 minutes with 750 MBq of ^{99m}Tc pertechnetate before re-injection. Studies were acquired on a small-field-of-view gamma camera fitted with a low-energy, general-purpose, parallel-hole collimator and interfaced to a dedicated minicomputer. With the patient on the cycle ergometer (Figure 3), the detector was adjusted for the left anterior oblique view with the best ventricular separation and 10° to 15° of caudal tilt. A 20% tolerance window was set about the patient's heart rate, and each RR interval was divided into 28 equal frames throughout. A constant number of frames per RR interval ensure constant temporal resolution during diastole at all heart rates. Three minutes of data were acquired at rest and at each level of exercise after a 30-second period for stabilisation of heart rate at the commencement of each stage. Exercise was performed at 50% workloads of heart rate reserve. In the study of atrial function, exercise was performed at 35% of heart rate reserve in order to adequately delineate the early and late filling component of diastolic filling because exercise at higher heart rates the early and late peaks of the first-derivative time-activity-curve are more likely to fuse. The composite cycle derived from each stage was spatially and temporally filtered. Left ventricular end-diastolic counts corrected for background gamma activity were obtained by means of a semi-automated edge detection algorithm. Data were analysed using LinkMedical MAPS software, Sun Microsystems (Figure 4). Peak left ventricular filling rate in terms of end-diastolic count per second (EDC/s) and time to

peak filling (normalised for heart rate) in milliseconds after end systole were calculated from the first derivative of the diastolic activity-time curve. Venous blood samples (≈ 5.0 mL) were obtained for weighing and for counting of blood gamma activity during each scan in order to correct for physical and physiological decay as well as for determination of relative volume changes. (120) The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously. (121, 122)

Rest and exercise systolic (SBP) and diastolic blood pressure (DBP) were determined by cuff sphygmomanometry. Pulse pressure (PP) was calculated as the difference between SBP and DBP and mean arterial pressure as (2*DBP + SBP)/3. End-systolic pressure (ESP) approximated by (2*SBP + DBP)/3. This non-invasive assessment of ESP accurately predicts LV pressure-volume loop measurements of ESP. (123) All gated blood pool scan-derived volumes were normalized to body surface area, yielding their respective indexes: end-diastolic volume index, end-systolic volume index (ESVI), stroke volume index (SVI), and cardiac index. The following indexes were calculated: a) arterial elastance index (E_aI) = ESP/SVI; b) LV systolic elastance index (E_{LV}I) = ESP/ESVI and c) arterial-ventricular coupling index = EaI/E_{LV}I = (1/EF) – 1. (88) E_aI/E_{LV}I ratio is independent of BP measurements and is therefore relatively accurate.

Figure 3: A photo of the gamma camera and the cycle ergometer used during the studies.

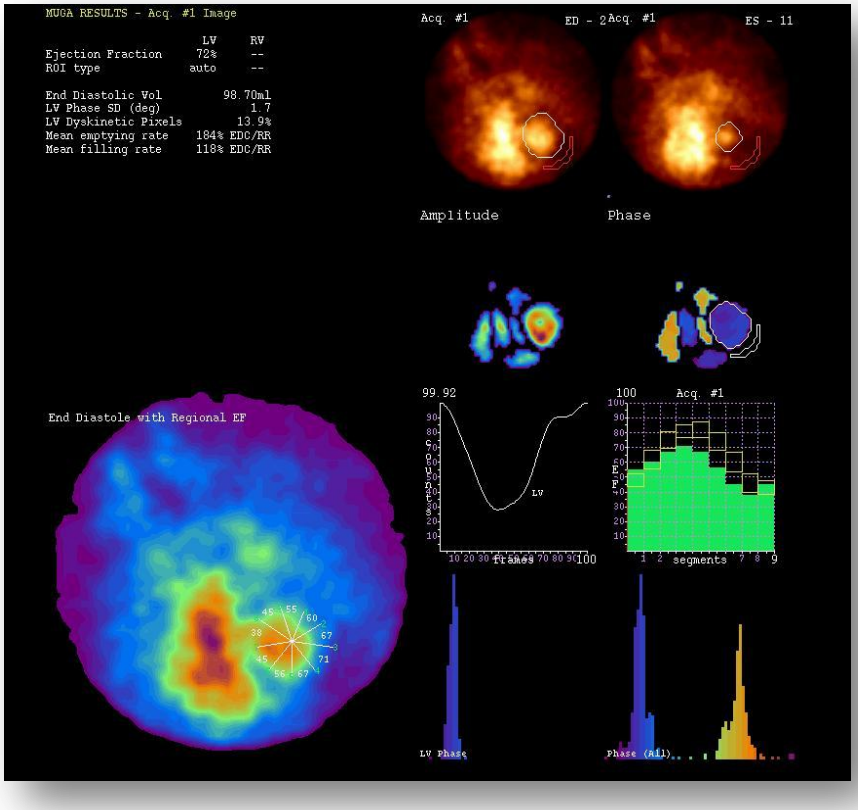

Figure 4: An example of a radionuclide ventriculography image and results

Metabolic Exercise Testing

The metabolic exercise testing was performed on a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using incremental ramp protocol (speed and inclination was increased every minute) as described previously by our group (124) with simultaneous respiratory gas analysis (125, 126). Samplings of expired gases were performed continuously, and data were expressed as 30-second means. The minute ventilation − carbon dioxide production relationship (VE/VCO_2 slope), maximal oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) was used to verify objective effort adequacy. Maximal oxygen consumption ($V0_2max$) was defined as highest value of oxygen consumption measured during the exercise period. Blood pressure and ECG were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine and a 2.5-MHz transducer. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber. LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula (127). Pulse wave Doppler sample volume was placed at the mitral valve tips to record 3 cardiac cycles. Mitral annulus velocities by pulse wave Tissue Doppler imaging (PW-TDI) were recorded from basal antero-lateral and basal Inferoseptum. LA volumes were measured by area length method from apical 2 and 4 chambers as previously described (127). Assessment of LV end-systolic elastance (E_{es}) was determined using the noninvasive single-

beat technique. (128) Arterial elastance (E_a) was calculated as the ratio of systolic pressure/stroke volume. Studies were stored digitally and analyzed off-line.

Speckle Tacking Echocardiography (STE)

STE was measured using a commercially available speckle tracking system in an ECHOPAC workstation. Myocardial deformation measurements were performed using tissue speckle tracking. In this speckle tacking system, the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image cardiac cycle and the left ventricle endocardium was traced at end-systole.(129). The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle (Figure 5). The onset of QRS complex was taken as the beginning of systole. Adequate tracking was verified in real time. A frame rate between 70-100 Hz was used. For each subject, longitudinal strain values for all LV myocardial segments in each of the apical 4 and 2 chamber views were measured and averaged to derive the global LV longitudinal strain, strain rates and velocity. Circumferential strain values were obtained in all 18 segments of the three short-axis views. The average of peak systolic circumferential strain values from the three short-axis views was calculated to derive the global LV circumferential strain and strain rates. Similarly, peak radial strain values were measured in all 18 segments at the three short-axis views and averaged to derive the global radial strain and strain rates.

In addition, cardiac rotation was computed using speckle tracking. Counter-clockwise rotation was marked as a positive value and clockwise rotation as a negative value when

viewed from the apex. In order to calculate LV torsion, LV untwist and untwist rates, the rotation traces of the basal and apical LV cross-sections were exported into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point. LV twist rates were derived from the first derivative of the LV twist curve. Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length. (130) Rotational deformation delay was also determined and defined as the magnitude of the time difference between time to peak basal rotation and time to peak apical rotation. To adjust for intra- and inter-subject differences in heart rate, all time measurements were normalized to R-R interval.

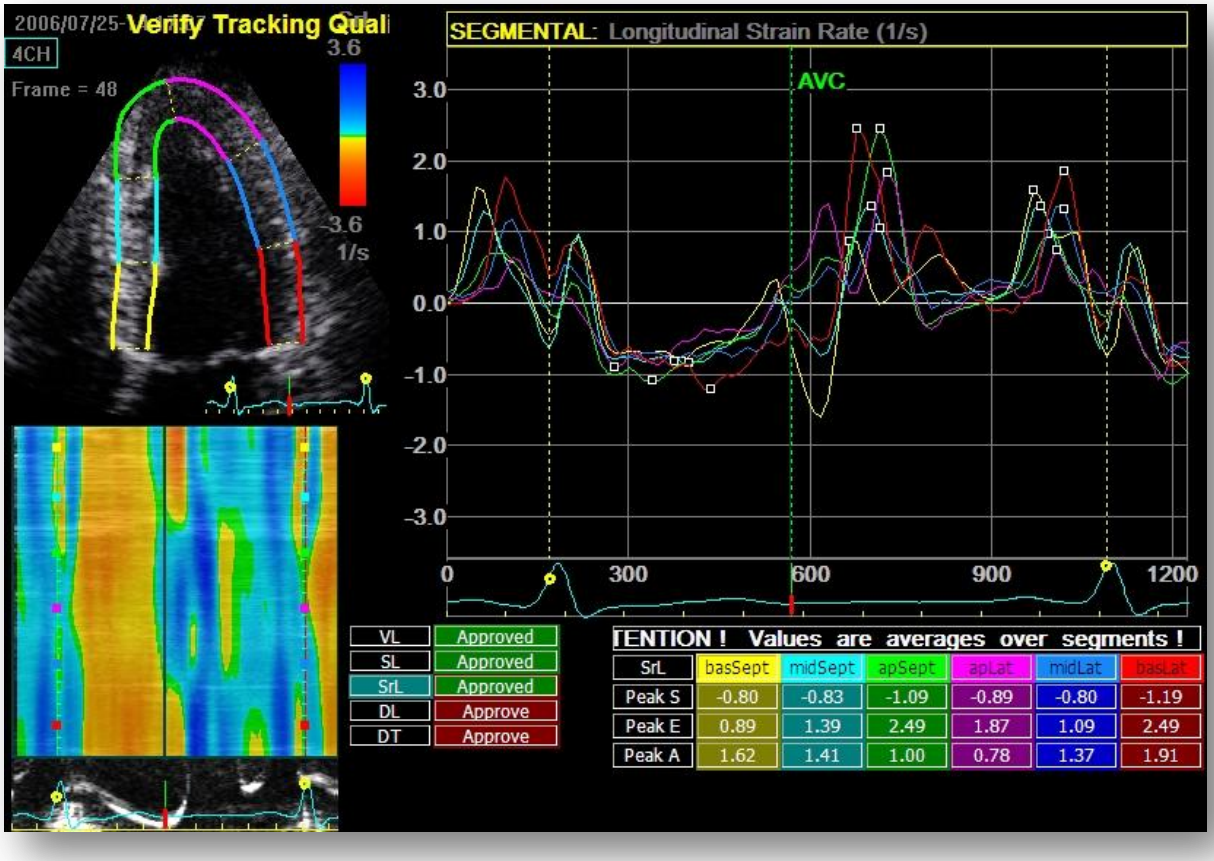

Figure 5: An example of speckle tracking derived longitudinal strain rate curves

Results

Chapter I

^{31}P Magnetic Resonance Spectroscopy to Measure *In Vivo* Cardiac Energetics in Normal Myocardium and Hypertrophic Cardiomyopathy:

Experiences at 3 Tesla

Chapter I

^{31}P Magnetic Resonance Spectroscopy to Measure *In Vivo* Cardiac Energetics in Normal Myocardium and Hypertrophic Cardiomyopathy: *Experiences at 3 Tesla*

Background

Phosphorus Spectroscopy is a non-invasive method of studying cardiac *in vivo* high energy phosphate kinetics (131). It allows for determination of PCr, ATP, Adenosine Diphosphate (ADP) and inorganic phosphate (Pi) concentrations in the myocardium. The concentrations of these substances and the ratio of PCr/ATP are measures of the cardiac energetic status. PCr is an important short-term reserve energy source that maintains a high phosphorylation potential under conditions of increased energy demand like exercise and ischaemia. The conversion of ADP to ATP by transfer of a phosphoryl group from PCr is catalysed by creatine kinase. This reaction occurs 10 times faster than ATP production via oxidative phosphorylation(132). In patients with mild to moderate heart failure, cardiac ATP flux mediated by creatine kinase is reduced by approximately 50%(133). Animal and human studies have demonstrated that a progressive reduction of the creatine pool is directly related to the severity of heart failure (134). This is largely due to a decrease in the number of creatine transporters at sites of energy production and utilisation(135). In normal myocardium two thirds of the creatine pool is phosphorylated via creatine kinase reaction to form PCr(136) and the expression and activity of this enzyme is reduced in heart failure (115, 137). Therefore in heart failure the available PCr is markedly diminished. The depletion

of PCr occurs to a greater extent compared to ATP resulting in reduced PCr/ATP ratio in heart failure as measured by MRS (138). Reduced PCr/ATP ratio is associated with increased mortality in heart failure patients (139). ^{31}P cardiac spectroscopy can also be used to monitor disease progress in heart failure patients. It has been used as an objective marker to show benefits of various treatments such as metabolic modulators (140).

Reduced PCr/ATP ratio is also seen in other conditions including ischaemic heart disease(141), left ventricular hypertrophy secondary to hypertension (142), valvular heart disease (mitral regurgitation and aortic valve disease) (119, 143), diabetes (144) and HCM (109). Interestingly, patients with genotypic HCM who do not yet have hypertrophy have a similar degree of impairment of cardiac PCr/ATP ratio as do patients with marked hypertrophy, implying that the disturbance may be an early feature of the disease and is not simply due to the hypertrophy (145).

Traditionally cardiac spectroscopy in humans has been performed using 1.5 Tesla magnets. Previous studies in animals(146) and humans demonstrated that higher field strength such as 3T (147) or 4.7T offer higher SNR. The increased sensitivity can be traded for either increased spatial resolution or improved quantification precision and hence might improve specificity of ^{31}P cardiac MRS as a diagnostic tool. Hence it is desirable to perform cardiac MRS at 3T MR scanners. However, higher field strength also result in inhomogeneities of the transmit and receive B_1 field along with restrictions of the maximum achievable B1 field strength that result in larger chemical shift displacements. Furthermore susceptibility differences between adjacent tissues have a greater effect on B_0 homogeneity, which results in line broadening. Hence in this study we test the feasibility and reliability of 31P cardiac

spectroscopy at 3 Tesla for clinical diagnostics using standard methods pre-implemented on a clinical MR scanner.

Methods:

37 Controls (22-males) and 26 HCM patients (21-males) with symptomatic non-obstructive cardiomyopathy who provided written informed consent, were included in the study. The experiment was approved by the Regional Ethics Committee at Birmingham, UK. All healthy volunteers were screened with history, echocardiography and metabolic exercise testing to rule out any structural heart diseases. All patients were recruited from cardiomyopathy clinics and had clinically proven diagnosis of non-obstructive hypertrophic cardiomyopathy. The subject characteristics are presented in table-1. The mean age of the healthy volunteers was 48 ± 16 years and that of HCM patients was 55±13 (P=ns). The mean ejection fraction on echocardiography was 64±6% in controls and 64±9% (P=ns) in HCM patients. None of the healthy volunteers had any structural heart disease or ECG abnormalities. ^{31}P cardiac spectroscopy was performed eight times in one participant both - on the same and on different days - to test the reproducibility and coefficient of variation of the test.

^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3T scanner and a linearly polarized transmit and receive ^{31}P coil with a diameter of 14 cm. Localization was achieved by ISIS (117) volume selection. The participants were positioned supine with the coil directly over the precordium. The coil was secured in place by straps wrapped around the upper body of the subject and the coil. The participants were then positioned inside the magnet with the center of the coil at the isocenter of the magnet. Survey images were obtained to check the position of the coil (figure1). The subjects and/or

the coil were repositioned if required to ensure that the distance between coil and septum and apex of the heart was minimized.

The standard phosphorus spectroscopy sequence provided by the manufacturer was used. It was based on hyperbolic secant pulses for slice selective inversion and adiabatic half passage RF pulse for non-selective excitation. In contrast to the standard procedure manual fine adjustment of F_0 was performed if the automatic F_0 determination was not correct in order to ensure the correct voxel position. In contrast to the default iterative or FASTERMAP based shimming algorithm, which was based on the selected spectroscopy VOI, an image guided shim volume was selected that included the entire myocardium. A short axis cine scan was acquired to calculate the trigger delay for ECG triggering and check quality of shimming and F_0 determination. The trigger delay was calculated such that the spectra were acquired in the diastolic period. The 3-D voxel of acquisition was planned to include most of the septum and apex of the heart. Care was taken to minimize blood contamination from the right ventricle as much as possible. The voxel size was kept constant at 89.54ml ($44x55x37mm^3$) so that comparisons could be made between different subjects and scans. Initially, ^1H spectra were acquired from the same voxel without water suppression and repetition time of 2000 ms (total scan time of 16 sec). This helped to ensure adequate shim quality and correct F_0 determination. F_0 could be manually adjusted if necessary. Following this the ^{31} P spectrum was acquired with a repetition time of 10000 ms, 136 averages and 512 samples. A repetition time of 10000 ms was found to be optimal to adequately reduce saturation effects without increasing the scan time greatly. The spectral acquisition was ECG gated and the trigger delay was set to acquire spectra mainly in diastole. The trigger delay was measured by subtracting 250-300 from the total length of the cardiac cycle which

allowed 250-300 msec of the cardiac cycle left for spectral acquisition (acquisition time is 170 msec). The total scan time was 23 minutes.

Increased chemical shift artefacts are present at 3T. In order to minimise this, slice selective inversion for ISIS encoding was based on adiabatic hyperbolic secant pulses, which achieved a pulse bandwidth between 1300 Hz (at a distance of 9 cm from the surface coil) to 2000 Hz (at a distance of 3 cm from the surface coil). This corresponds to a chemical shift displacement of 6-10% for the investigated metabolites PCr and gamma-ATP for volumes of interest that were between 3 and 9 cm away from the coil. When subjects where scanned the distance from the coil to the ROI averaged about 7.5cm and no subjects were beyond 9.0 cm. Therefore all subjects would have a chemical shift displacement less than 10% which is acceptable.

Analysis:

The spectra were analysed and quantified on jMRUI software using AMARES a time domain fitting program(118). Post-processing was performed with 15Hz Gaussian line broadening and Fourier transformation. Phase correction was performed with PCr peak as the reference peak. Quantification was performed with AMARES using a prior knowledge file to preselect the peaks. The concentrations of PCr, ATP and 2,3-Diphosphoglycerate (2,3-DPG) were calculated as the area under the peaks. Cramer Rao lower bounds (148) were then calculated. PCr/ATP ratio was determined after correcting the ATP peak for blood contamination as described previously(149).

Results:

A typical cardiac spectrum in healthy volunteer as compared to hypertrophic cardiomyopathy is shown in Figure 2. The PCR/ATP ratio was significantly lower in hypertrophic cardiomyopathy (1.42 ± 0.51) as compared to healthy controls (2.11 ± 0.57, P<0.0001) (Figure 3). The mean PCr/ATP ratio for the one participant with eight measurements was 2.11 ± 0.25 (Table-2). Bland Altman plots were used as a test of reproducibility (Figure 4). The distribution of all the data points showed a good reproducibility with a coefficient of variation of 12% in the measurement of PCr/ATP ratios which is within limits of agreement of repeated measurements. We also measured the line width of PCr peaks in the one participant who had 8 repeated scans. The mean line width was 1.363±0.204 ppm (Figure 5). The standard deviation of the line width was low which again confirmed the fact that the spectra were of good reproducible quality. As a further measure of the quality of spectra we calculated the Cramer Rao lower bounds (148), which were 6 ± 1% for the PCr peak and 10 ± 1% for the gamma ATP peak. Cramer Rao lower bounds were calculated for the whole group, which was 12 ± 6% for the PCr peak and 17 ± 9% for the gamma ATP peak (Table-3).

Due to an increase of susceptibility differences between heart muscle tissue, blood and air in the lungs at high field strength shim quality was slightly decreased at 3T in comparison to values reported for 1.5T cardiac spectroscopy. Hence multiple splitting of the ATP resonances due to J-coupling is hardly visible.

Discussion

Here we demonstrate that in-vivo cardiac ^{31}P MRS at 3T is a reliable method of measuring high energy phosphate kinetics in the myocardium. We also show reduced PCr/ATP ratio in HCM patients known to have impaired cardiac energetics as measured in 1.5T systems (145,

150). The spectra show good reproducibility indicating that ^{31}P cardiac MR spectroscopy is feasible on a clinical 3T MR system. The standard deviation for the PCr/ATP ratio for the whole group was low and comparable to previous published data on cardiac spectroscopy at 1.5 Tesla(151). This basic method of acquiring ^{31}P spectra at 3T using pre-implemented methods such as ISIS volume localisation and iterative shimming promises to be an important diagnostic and research tool. Possible applications are the comparison of PCr/ATP ratios in conditions like heart failure, ischaemic heart disease and valvular heart disease. It might also be used to monitor disease progression and study effects of medications like metabolic modulators in heart disease.

Cardiac spectroscopy at 3T continues to face some challenges which are present at 1.5T as well, but some of them are even more pronounced at high field strength. One main drawback of cardiac spectroscopy is the effect of respiratory motion and movement of the heart itself. This can result in contamination from liver and skeletal muscle of the chest wall. Careful localization of the voxel ensures minimal contamination. We particularly paid attention to this aspect while planning our voxel of acquisition. Provided the voxel is positioned carefully, no significant advantages to the quality of spectra acquired with multiple outer volume suppression bands was observed. None of our spectra show any significant contamination with skeletal muscle or liver. It is also important to ensure that the spectral acquisition is done when the heart motion is at its minimum. This is during diastole and we set the trigger delay such that all of the spectral acquisition happened during diastole. One other problem is the respiratory motion. This could be partially negated by acquiring the spectra with respiratory gating as well as cardiac gating (double triggered) and volume tracking (152, 153). Further developments in these techniques should improve quality of cardiac spectra and reduce the contamination from surrounding structures.

Higher field strength of 3T offers better spatial resolution and signal to noise ratio. These effects have been particularly noted in proton spectroscopy of the human brain (154). However, increasing susceptibility differences between muscle tissue, blood and air in the lungs cause increased B_0 field inhomogeneities and hence problems with shim convergence. Various shimming techniques like iterative and FASTMAP based (155) higher order shimming were compared. Localized iterative 1^{st} order shimming based on a volume including the entire heart offered the best and most reproducible shim quality. However, there is definitely scope for further improvement in shimming techniques. One possibility might be localized shimming based on cardiac triggered B_0-mapping as reported earlier for cardiac imaging at 3T(156). Another problem is the shortened transverse relaxation times (T2) at 3T in comparison to 1.5T. This causes an additional increase in line width of the various peaks.

No significant advantages to the quality of spectral acquisition using proton decoupling or Nuclear Overhauser Enhancement (NOE) were found. Therefore these techniques were not used. Although NOE can increase the SNR by up to 40%, this is not useful for experiments were quantification is required. NOE in particular imparts different amount of energy to phosphates in ATP and PCr which can result in altered PCr/ATP ratio. This is particularly important as the effect of NOE is different for distinct molecules and depends on conditions as the pH which might be changed in the diseased myocardium.

Due to a decrease of the maximum achievable B_1 field strength at 3T together with an increase in spectral separation compared to 1.5T, the bandwidth of the excitation pulse was

limited and hardly sufficient to excite the entire frequency range of interest. Using a simple surface coil this is especially a problem in large penetration depth. In addition, the excitation profile was not homogeneous. Hence different flip angles were applied to spins with different offset frequencies. This resulted in a frequency dependent weighting of peak intensities. Hence, the intensity of the beta ATP peak, which has a large frequency offset compared to PCr, was always significantly decreased (Figure 1) or even lost in obese subjects, where the distance between coil and VOI is large. However the determined PCr/gamma-ATP ratios were not affected, because the frequency difference between both resonances is small and the pulse frequency offset was chosen to be in-between of both.

Conclusions

^{31}P magnetic resonance spectroscopy of the myocardium at 3T is feasible and allows for reliable determination of high energy phosphate kinetics. Our reproducibility data suggests that the suggested method is robust and might be used for clinical diagnostics as well as for clinical studies. However, to take full advantage of increased SNR and spectral separation at 3T advances in shimming, coil and RF pulse design are necessary.

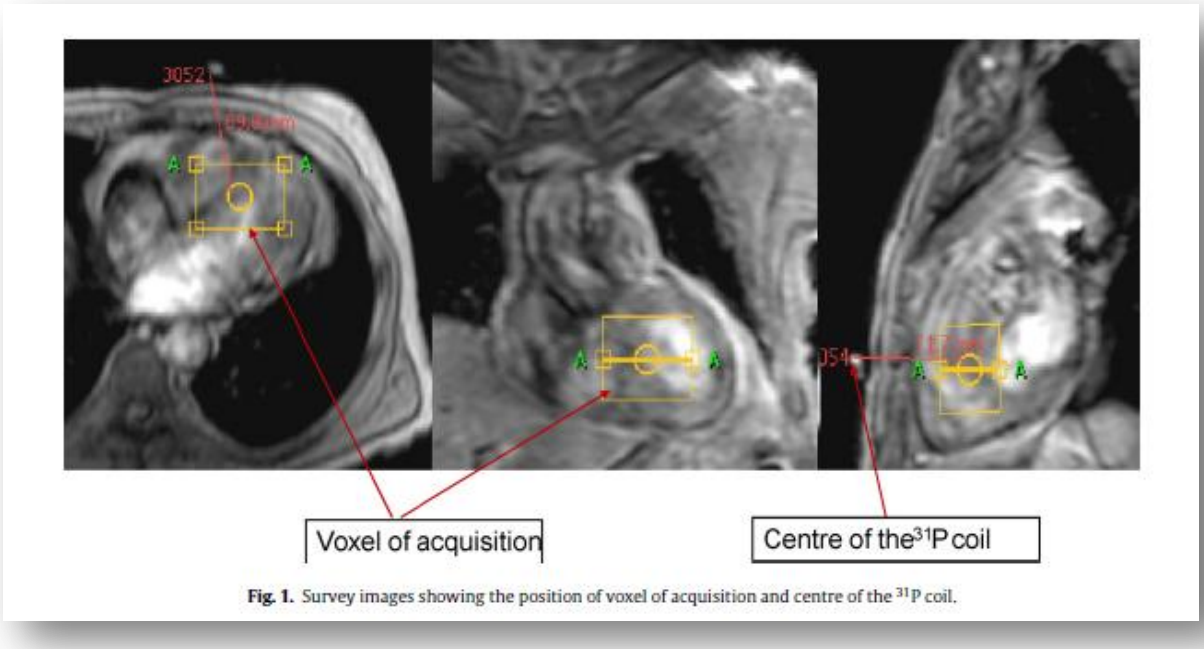

Fig. 1. Survey images showing the position of voxel of acquisition and centre of the ^{31}P coil.

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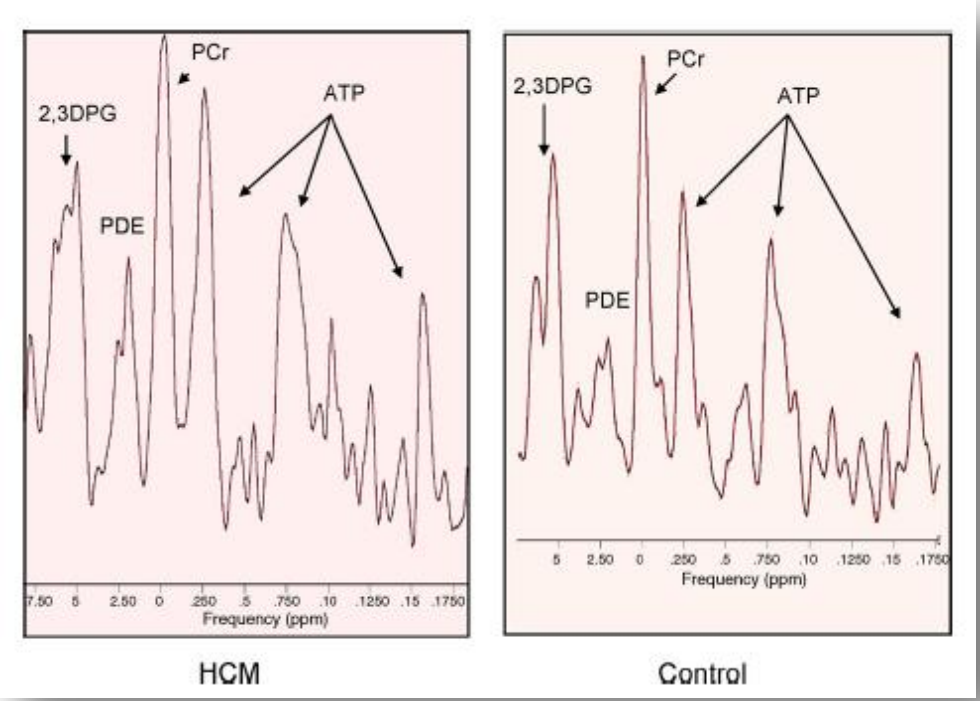

Figure 2: A typical cardiac spectra in control and HCM

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; PDE - Phosphodiesters; ATP - Adenosine triphosphate.

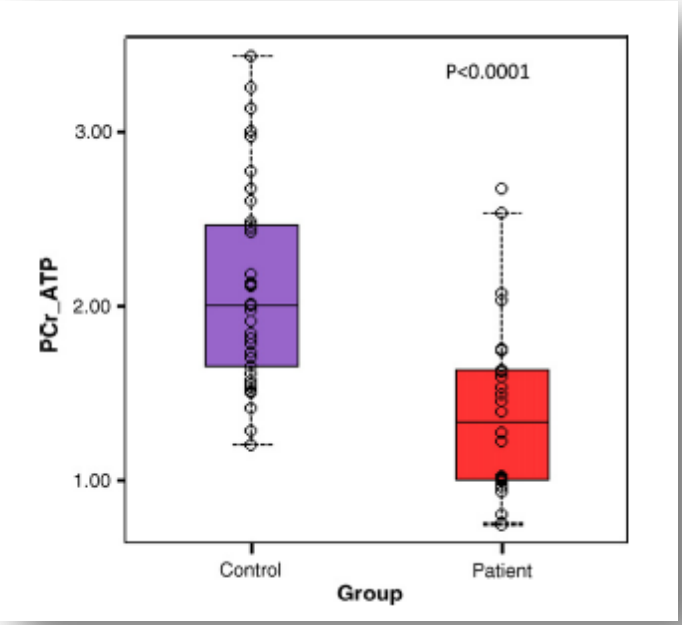

Figure 3: Box-plots of PCr/ATP ratios in controls and HCM patients

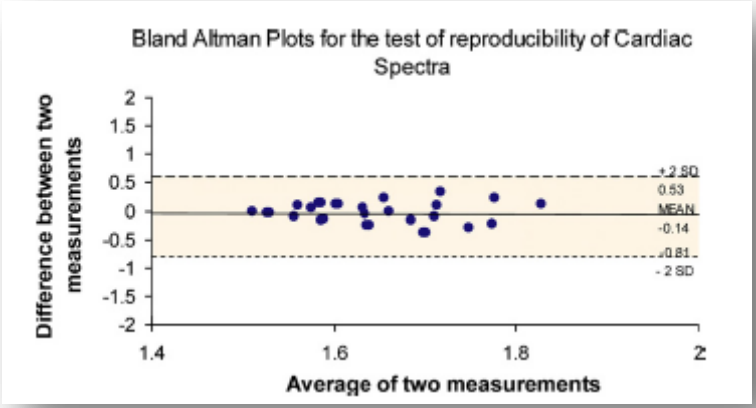

Figure 4: Bland-Altman plots of ^{31}P cardiac MRS measurements from the healthy volunteer who had 8 repeated scans. Graph plotted as difference in two measurements against mean of the same two measurements.

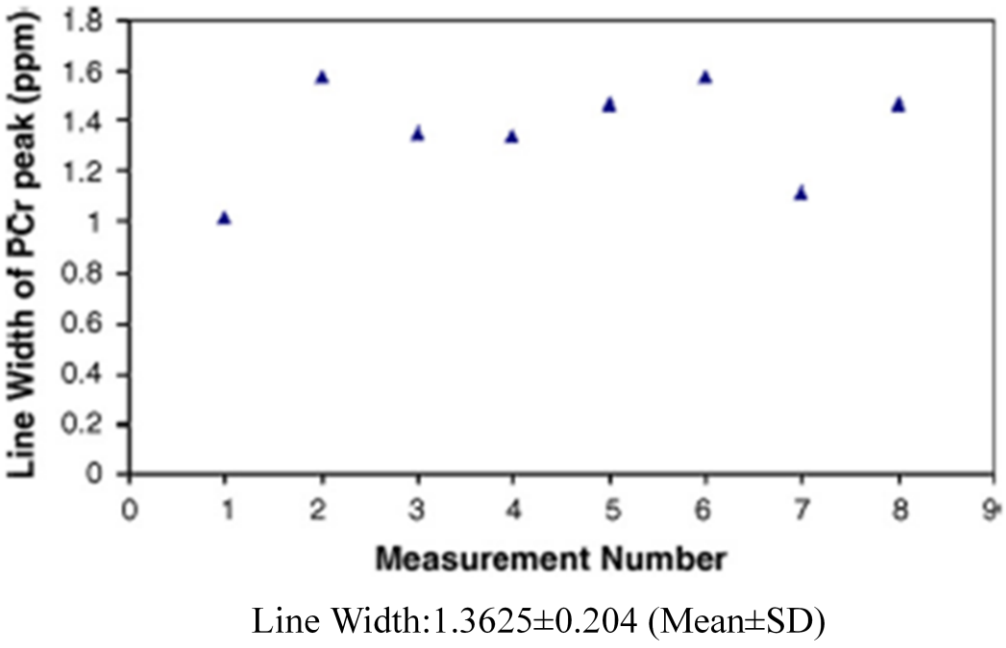

Figure 5: Line width of PCr peaks from the healthy volunteer who had 8 repeated scans expressed as parts per million (ppm)

Table 1: Baseline Characteristics			
Parameter	**Controls (N=37)**	**HCM (N=26)**	**P Value**
Age	48 ± 16	55 ± 13	ns
Male sex –no(%)	22 (59)	21 (81)	ns
EF(%)	64 ± 6	64 ± 9	ns
VO2 max	39 ± 8	24 ± 6	<0.0001
RER	1.2 ± 0.2	1.1 ± 0.1	ns
Heart rate	79.5 ± 11.7	67.5 ± 12.5	<0.01
QTc interval	421.8 ± 16.0	455.9 ± 35.0	<0.01
Systolic Blood Pressure	127.0 ± 20.4	126.1 ± 20.1	ns
Diastolic Blood Pressure	79.5 ± 9.6	75.6 ± 10.7	ns

EF- Ejection Fraction, VO2 max- Maximal oxygen consumption at peak exercise, RER – Respiratory exchange ratio, QTc- Corrected QT interval.

Table 2: Table depicting the concentrations of 2,3-DPG, ATP and PCr and the calculated PCr/ATP ratios from these measurements in the subject with eight measurements.

Subject No	2,3 DPG sum	PCr	Gamma ATP	Gamma ATP (C)	PCr/Gamma ATP Ratio	PCr/Gamma ATP Ratio (C)
Subject 1A	6.72×10^{-4}	4.90×10^{-4}	3.57×10^{-4}	2.45×10^{-4}	1.37	2.00
Subject 1B	4.27×10^{-4}	4.32×10^{-4}	3.21×10^{-4}	2.50×10^{-4}	1.34	1.72
Subject 1C	7.32×10^{-4}	6.53×10^{-4}	4.77×10^{-4}	3.55×10^{-4}	1.36	1.83
Subject 1D	1.00×10^{-3}	8.52×10^{-4}	5.12×10^{-4}	3.45×10^{-4}	1.66	2.47
Subject 1E	9.39×10^{-4}	5.53×10^{-4}	3.86×10^{-4}	2.30×10^{-4}	1.43	2.40
Subject 1F	8.29×10^{-4}	6.34×10^{-4}	4.26×10^{-4}	2.88×10^{-4}	1.48	2.20
Subject 1G	8.66×10^{-4}	5.36×10^{-4}	3.90×10^{-4}	2.46×10^{-4}	1.37	2.18
Subject 1H	8.32×10^{-4}	5.55×10^{-4}	4.05×10^{-4}	2.66×10^{-4}	1.37	2.08

PCr- Phosphocreatine; 2,3-DPG- 2,3 Diphosphoglycerate; ATP- Adenosine triphosphate; (C)- Corrected for blood contamination

Table 3: Cramer Rao Lower bounds measured to test the quality of spectra		
	PCr peak	**Gamma ATP Peak**
Healthy control (8 Measurements)	6% ± 1	10% ± 1
Whole group(Controls + HCM)	12% ± 6	17% ± 9
Controls	11% ± 5	16% ± 8
HCM	12% ± 6	17% ± 9

Chapter II

Heart Failure with Preserved Ejection Fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency

Chapter II

Heart Failure with Preserved Ejection Fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise, associated with myocardial energy deficiency

Introduction

There is increasing consensus that ≈50% of patients with the clinical features of chronic heart failure suffer from heart failure with preserved ejection fraction (HfpEF). (10) Epidemiological observations from different populations confirm that the prevalence of HfpEF is increasing as it tracks the demographic transition, especially in obese hypertensive females. HfpEF causes as many hospitalizations and incurs as severe morbidity as heart failure with reduced LVEF. Finally, HfpEF portends a significant and unimproving mortality. (12) Nonetheless, despite its unarguable clinical significance, HfpEF's pathophysiology remains controversial with some investigators proposing that diastolic abnormalities (1) and others that systolic abnormalities play a dominant role. (157) However most studies have focussed on resting parameters yet symptoms predominantly occur on exercise. One study reported a dynamic impairment of left ventricular active relaxation during isometric (handgrip) exercise (58) but the relevance of this observation to dynamic leg exercise that usually provokes breathlessness during everyday life is unclear.

The present study was designed to test the hypothesis that exercise limitation in HfpEF is due to a dynamic impairment of both LV active relaxation and of LV contractile performance

during exercise, both of which may be due to an underlying impaired myocardial energetic reserve. We used radionuclide ventriculography to measure LV active relaxation, LV contractile function and vasculo-ventricular coupling, at rest and during exercise. Furthermore, ^{31}P Cardiac Magnetic Resonance Spectroscopy (MRS) at 3-Tesla was used to measure *in vivo* myocardial energetics.

Methods

Patients

We studied 37 HfpEF patients prospectively recruited from heart failure clinics. We also studied twenty healthy controls with no cardiac history or diabetes mellitus. Study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram, metabolic exercise test, radionuclide ventriculography studies and a subgroup underwent cardiac ^{31}P MRS studies to assess cardiac energetic status. All controls had a normal cardiovascular examination, 12-lead electrocardiogram and echocardiogram. HfpEF patients were defined in accordance with ACC/AHA recommendation (158): i) symptoms and signs of heart failure, ii) EF ≥50%, iii) no valvular abnormalities. In addition we stipulated that patients should have iv) VO_2max <80% of age and gender predicted values with a pattern of gas exchange on metabolic exercise testing indicating a cardiac cause for limitation, v) absence of objective evidence of lung disease on formal lung function testing and/or absence of arterial desaturation during exercise and with a ventilatory reserve at peak exercise ≥15L. We chose to use such a definition as advocated by Coats and others (33) in order to have robust evidence that patients had exercise limitation that was cardiac rather than non cardiac in origin and so as not to prejudge the underlying pathophysiology by stipulating the presence of resting diastolic abnormalities because

diastolic abnormalities are frequently present in healthy elderly subjects (23) and it does not necessarily predict clinical heart failure (24) nor exertional dyspnoea (25). Patients with rhythm other than sinus were excluded. The investigations were performed at The University of Birmingham with approval of the Research Ethics Committee. Informed consent was obtained from all patients and controls.

Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine using a 2.5-MHz transducer. Cardiac quantifications were determined in accordance with European Association of Echocardiography. (127) LV end-systolic elastance (E_{es}), a relatively load independent measure of LV contractility, was determined using the non-invasive single-beat technique. (128) Arterial elastance (E_a), a measure of the stiffness of the entire arterial tree, was calculated as the ratio of LV end-systolic pressure/stroke volume. Studies were stored digitally and analyzed off-line.

^{31}P Cardiac Magnetic Resonance Spectroscopy (MRS)

In vivo myocardial energetics was measured by MRS at 3-Tesla as previously validated and described in detail by our group. (110) ^{31}P cardiac magnetic resonance spectroscopy was performed using a Phillips Achieva 3^T scanner and a linearly polarized transmitter and receiver ^{31}P coil with a diameter of 14 cm. The repetition time was 10000 ms with 136 averages and 512 samples. Acquisition was ECG gated and the trigger delay was set to acquire in diastole. Total scan time was 23 minutes. Java magnetic resonance user interface v3.0 (jMRUI) was used for analysis. (159) PCr and γ-ATP was used to determine the PCr/ATP ratio which is a measure of cardiac energetic state (160). Patients with ischemic heart

disease and diabetes (N=7) were excluded from the MRS studies because these conditions are known to have impaired cardiac energetics. (144, 160) Patients with contraindications were also excluded from the MRS study (N=5). One patient's spectra was excluded from the analysis due to poor quality. Three controls had contraindication to MRS study. Data were analysed separately by an investigator unaware of participants' clinical status.

Radionuclide Ventriculography

LV ejection fraction and diastolic filling were assessed by radionuclide ventriculography at rest and during graded semi erect exercise on a cycle ergometer as previously described in detail by our group. (76, 94) Three minutes of data were acquired at rest and during exercise after a 30-second period for stabilisation of heart rate at the commencement of each stage. Exercise was performed at 50% workloads of heart rate reserve. Data were analysed using LinkMedical MAPS software, Sun Microsystems (Hampshire, UK). Peak left ventricular filling rate in terms of end-diastolic count per second (EDC/s) and time to peak filling normalised for R-R interval (nTTPF) in milliseconds after end systole were calculated from the first derivative of the diastolic activity-time curve. Venous blood samples were obtained for weighing and for counting of blood gamma activity during each scan in order to correct for physical and physiological decay as well as for determination of relative volume changes. (120) The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously. (121)

All gated blood pool scan-derived volumes were normalized to body surface area, yielding their respective indexes: end-diastolic volume index (EDVI), end-systolic volume index (ESVI), stroke volume index (SVI), and cardiac index. The following indexes were calculated:

a) arterial elastance index (E_aI) = ESP/SVI; b) LV end-systolic elastance index ($E_{LV}I$) = ESP/ESVI and c) vasculo-ventricular coupling ratio (VVC) = $E_aI/E_{LV}I$ = (1/EF)–1. (88)

Metabolic Exercise Test

All participants underwent a symptom-limited erect treadmill exercise with simultaneous respiratory gas analysis. The incremental ramp protocol was such that the speed and inclination increased in a stepwise manner every minute. Exercise was terminated at the subject's request because of fatigue or breathlessness. VO_2max was obtained during peak exercise.

Statistics

Continuous variables are expressed as means ± SD. Unpaired Student's t-test (2-tail) was used to assess differences between mean values. Categorical variables were compared with Pearson Chi-Square test. All reported P values were calculated on the basis of two-sided tests and a P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using Levene's test. SPSS (v15.0) was used to perform the statistical operations.

Results

Characteristics of the Patients

HfpEF patients were generally females, overweight, aged 67±9 years old with a history of hypertension, however blood pressure was well treated (systolic BP 138±19mmHg vs. 131±23mmHg; p=0.23, in patients vs. controls) (Table 1). The tissue Doppler E/E' at the basal anterior-lateral (a measure of left ventricular end-diastolic pressure) (161), was significantly higher in patients than controls. There was also a trend (non-significant) to

higher Ees in patients than in the control group. HfpEF patients also had significantly reduced VO_2max and reduced peak HR on metabolic exercise testing. During semi-erect cycle exercise the relative stroke volume ($SVi_{EXERCISE}$ /SVi_{REST}) was lower in patients compared to controls (0.99±0.34 vs. 1.25±0.47; P=0.04), and relative cardiac output ($COi_{EXERCISE}/COi_{REST}$) was also lower (1.36±0.45 vs 2.13±0.72; p<0.001). (Table 2)

Left Ventricular Active Relaxation

nTTPF is determined by the rate of LV active relaxation (114) and by transmitral pressure gradient at the time of mitral valve opening. nTTPF was similar at rest in HfpEF patients and controls. During exercise it shortened in controls, but lengthened in patients (Table 2). Furthermore, peak filling rates during exercise were significantly reduced in patients compared to controls. (Table 2)

Left ventricular contractile function and Vasculo-Ventricular Coupling

VVC was similar at rest in HfpEF patients and controls. During exercise, LV arterial elastance, a measure of the stiffness of the entire arterial tree increased in both patients and controls but tended to increase more in patients. LV end systolic elastance, a measure of LV contractile function, markedly increased on exercise in controls but increased substantially less in patients. Accordingly the VVC ratio was essentially unchanged on exercise in patients but fell substantially on exercise in healthy controls. Furthermore whilst resting LVEF and peak emptying rate were similar in patients and controls, during exercise both were lower in patients. (Table 2)

***In vivo* Myocardial Energetic State**

At rest, cardiac PCr/ATP ratio in HfpEF patients was significantly reduced compared to healthy controls, 1.57±0.52 and 2.14±0.63, respectively, P=0.003 (Figure 1).

Discussion

The principal findings of this study are: a) HfpEF patients manifest a significant reduction in PCr/ATP ratio at rest, indicating impairment of myocardial energy 'reserves'. b) As a corollary, during exercise, the energetically demanding active relaxation stage of diastole lengthened in patients (vs. a shortening in controls) and there was also a failure of the normal increase in contractile function on exercise in patients. These combined dynamic abnormalities of both diastolic and contractile function together resulted in a lower stroke volume during exercise. c) Consistent with previous studies, HfpEF patients demonstrated chronotropic incompetence on exercise. (77) These findings underline the importance of a dynamic (rather than resting) assessment of cardiac function to comprehensively characterise patients with HfpEF.

The pathophysiology of HfpEF has been the subject of considerable controversy. These patients are typically hypertensive and exhibit impaired LV active relaxation and/or increased passive left ventricular diastolic stiffness at rest. (1) This has led many to conclude that exercise limitation is primarily a result of impaired LV diastolic filling and to the use of the term 'diastolic heart failure'. (50) However, diastolic dysfunction is also a common finding at rest in healthy elderly subjects. (23) Furthermore, 'subtle' abnormalities of systolic function, in particular long axis systolic function, are also almost universally observed in HfpEF patients despite normal LV ejection fraction. (157) This has led others to propose that HfpEF is predominantly a disorder of contractile function. (162) In order to compare both of

these possibilities, we specifically chose to define the syndrome on the basis of a limitation of exercise capacity with a cardiac basis for this limitation rather than by using resting parameter of diastolic function in order not to prejudge the role of diastolic vs. systolic mechanisms .

Little attention has been directed to changes in systolic and diastolic function during dynamic exercise, which is when the majority of patients experience their symptoms. In one study, ten patients with HfpEF were assessed with invasive pressure-volume loops and compared with age-matched controls. (58) The former had increased arterial elastance (a measure of the stiffness of the entire arterial tree), and increased LV end-systolic elastance (a measure of the stiffness of the ventricle during systole and a relatively load independent measure of the contractile state of the left ventricle (163)). Whilst diastolic abnormalities were not universally present in patients at rest, marked differences appeared during handgrip exercise. The rate of LV active relaxation increased in healthy subjects but it slowed in patients. (58) In another study from the same group, exercise-related symptoms in Afro-Caribbean hypertensive patients appeared to be strongly associated with chronotropic incompetence and an inadequate vasodilator reserve on exercise. (77)

The present study examined the patho-physiological mechanisms in HfpEF and found marked dynamic (exercise induced) abnormalities in both contractile and diastolic function of the left ventricle, and a lower peak exercise HR in patients. It is possible for an impaired HR response during exercise to cause a reduction in exercise capacity as measured by VO_2max, which is largely determined by cardiac output on exercise and the latter is simply the product of HR and SV. However, in the setting of a profound slowing of active relaxation

and increased LV passive diastolic stiffness, a larger diastolic filling period might be expected to be beneficial. In this study, despite a longer diastolic filling time, the relative change in SV was lower in patients during sub-maximal exercise. Impaired HR response may be a consequence of the heart failure as an impaired chronotropic response (associated with slow HR recovery following exercise) is typically present in systolic heart failure and is in part a manifestation of impaired vagal tone; (164) or it may be an adaptation to improve diastolic filling. The latter seems at least plausible, since increasing heart rate by atrial pacing has been shown to reduce supine resting stroke volume and cardiac output in patients with HfpEF. (2) It will be important to undertake further studies to assess whether heart rate plays a causal role in exercise limitation in HfpEF, because if so this may be amenable to rate responsive pacing.

The patients in this study had a history of hypertension but were well treated with anti-hypertensives (in most cases including vasodilators) therefore resting blood pressure and arterial elastance were not significantly higher than in the control group. Consistent with prior studies (58), at rest, LV end-systolic elastance (a measure of contractility or systolic stiffness) tended to be higher in patients although this did not reach significance. The increase in arterial elastance during exercise tended to be greater in patients vs. controls (presumably reflecting a greater increase in large artery stiffness). However, whilst left ventricular end-systolic elastance almost doubled during exercise in controls, the increase was only 35% in patients; hence VVC reduced by 33% during exercise in controls but was unchanged in patients. These findings indicate a blunting of the physiological increase in the contractile state of the left ventricle on exercise.

The physiological increase in the rate of LV active relaxation during exercise is a consequence of sympathetic activation, via cAMP-dependent protein kinase (PKA) mediated phosphorylation of key proteins including Troponin I, Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase (SERCA) and Titin. (70-72) In experimental models, large acute increases in afterload resulted in an acute impairment of LV active relaxation (93), with the threshold for this phenomenon being lower in the diseased heart, leading to the concept of 'relative load' as a determinant of afterload related impairment of LV active relaxation. (165) In the study of HfpEF patients described earlier (58), handgrip exercise was associated with a substantially greater increase in LV end-systolic pressure than in controls, potentially explaining the observed slowing of LV active relaxation. A key coupler of this load dependent LV relaxation is Troponin I – Protein Kinase A (TnI-PKA) phosphorylation (95). It is known that the energy dependent process of phosphorylation of Troponin I by PKA decreases myofibrillar calcium sensitivity (96) and increases the rate at which calcium dissociates from Troponin C (97) which can lead to increase rate of LV relaxation. Indeed, in a study involving transgenic mice in which PKA phosphorylation sites on Troponin I were constitutively active, acute aortic constriction led to a lengthening of Tau (an invasive measure of active relaxation) in the wild type mice but not in the transgenic mice. (95).

Integrating these observations, we speculate that dynamic energy impairment may account for the slowing of LV active relaxation on exercise as well as the failure of LV contractile function to increase. To increase the generalisability of this hypothesis, we avoided positively biasing our study by excluding patients with established causes of cardiac energy deficiency (ischemic heart disease and diabetes) (144, 160). Nevertheless, the PCr/ATP ratio was still substantially reduced in HfpEF patients vs. controls at rest. The lower PCr/ATP ratio

in patients indicates a reduction of high energy phosphates reserve at rest. (115, 166) Although the time required for acquisition of Cardiac MRS signals precluded the measurement of high energy phosphate status during exercise, it is likely that any basal energetic impairment will be exacerbated dynamically. This exacerbation of dynamic energetic impairment would explain the prolongation of the energy demanding active relaxation as manifest by nTTPF. Moreover, the lower hearts rates and lesser increases in LV end-systolic elastance may represent strategies to limit dynamic cardiac energy demands. The cause for this resting energy deficit may relate to insulin resistance(108), to impaired mitochondrial function as a result of ageing (113), and to neuro-endocrine activation and aberrant substrate metabolism. (167) In addition, increased myocardial fibrosis, as previously reported serologically in patients with diastolic heart failure (168), may also lead to reduced PCr/ATP ratio in HfpEF patients. This is relevant because in patients with HCM, reduced PCr/ATP has been shown to correlate with the presence of fibrotic area in the myocardium of the LV. (169)

From a clinical perspective, this study have shown that patients with HfpEF have reduced myocardial energetic status which provides the rationale to assess the therapeutic value of 'metabolic agents' (e.g. perhexiline and trimatazidine) that increase cardiac energetic status by altering cardiac substrate use (170). Indeed, trimetazidine and perhexiline have both been shown to be beneficial in systolic heart failure (124, 170) Importantly we have shown that patients with HfpEF have a dynamic pathophysiological process whereby resting parameters may be comparable to controls however during exercise HfpEF patients have impaired LV active relaxation and to a failure of LV end-systolic elastance to increase. This suggests that in detecting patients with HfpEF we cannot solely rely on resting parameters

and that we perhaps need to consider some form of exercise testing such as metabolic exercise testing as advocated by Coats and others (33) or exercise radionuclide ventriculography scans to detect abnormal LV relaxation during exercise.

Study limitations

Our radionuclide exercise protocol involved asking subjects to maintain a HR which was 50% of HR reserve above their resting HR. Since this HR reserve was calibrated to peak HR rate, the absolute workload in patients was lower. To have compared patients at the same workload would be inappropriate since this would represent a higher relative workload in patients. Moreover, most changes in SV occur in the first part of exercise with subsequent increases in cardiac output being principally due to increases in HR. (171) A small proportion of patients were on beta-blockers which may have affected their cardiovascular response to exercise, however when these patients were excluded from the analysis the findings and the level of significance remained unchanged. In addition, some patients were on calcium blockers however these were all peripherally acting (dihydropyridines for hypertension) and therefore are not expected to affect the myocardium directly. Ideally we would have liked to measure cardiac energetics during exercise however cardiac MRS studies during exercise is currently quite challenging more so if we tried to replicate the same dynamic leg exercise in the confinement of a MR scanner. A number of problems like acquisition at higher heart rates, shorter acquisition times, voxel specificity, pulse design, shorter repetition times etc are currently being addressed by our group and other groups around the world. MRS and Radionuclide studies also require a regular rhythm, thus patients with atrial fibrillation were excluded from the study. In contrast, the strength of radionuclide studies is their increasing temporal resolution at higher heart rates. This obviates the confounding E:A fusion as is

frequently experienced with exercise echocardiography. Radionuclide studies are thus not subject to systematically biasing mechanistic HfpEF towards a subgroup of patients without E:A fusion on exercise.

Conclusion

HfpEF Patients have reduced cardiac energetic reserves which when exacerbated dynamically may contribute to the abnormal LV active relaxation during exercise and to a failure of LV end-systolic elastance to increase. In addition chronotropic response was markedly impaired during exercise in these patients.

Table 1. Baseline Characteristics of the Subjects			
Variable	**Patient (N = 37)**	**Control (N = 20)**	**P Value**
Age - yr	67±9	63±7	0.51
Female sex - no. (%)	28 (76)	10 (50)	0.05
Body Mass Index	30±4	26±5	<0.01
Left Ventricular Hypertrophy - no. (%)	19 (51)	5 (25)	0.05
Diabetes mellitus - no. (%)	4 (11)	0	-
Hypertension - no. (%)	27 (73)	0	-
Ischemic Heart Disease - no. (%)	4 (11)	0	-
NYHA functional class – no.			
II	29	0	-
III	8	0	-
Drug therapy – no. (%)			
Diuretic	10 (27)	0	-
ACE inhibitor	20 (54)	0	-
ARB	6 (16)	0	-
Beta-blocker	8 (22)	0	-
Calcium blocker	10 (27)	0	-
Alpha Blocker	4 (11)	0	-
Spironolactone	2 (5)	0	-
Nitrate	3 (8)	0	-
Statins	21 (57)	0	
Metabolic exercise testing			
VO_2max (ml/kg/min)	19±4	36±8	<0.001
Respiratory Exchange Ratio (RER)	1.06±0.07	1.13±0.10	0.003
Breathing Reserve - L/min	36±15	43±18	0.16
HR - beats/min			
Rest	74±14	83±17	0.03
Peak Exercise	127±20	166±11	<0.001
ΔHR	52±16	81±14	<0.001
SBP (mmHg)			
Rest	138±19	131±23	0.23
Peak Exercise	182±26	190±30	0.30

DBP (mmHg)			
Rest	81±11	81±12	0.98
Peak Exercise	81±13	84±10	0.36
Echocardiography			
Left ventricular ejection fraction - %	64±14	63±6	0.77
Mitral E-wave velocity - m/sec	0.72±0.19	0.61±0.12	0.02
Mitral A-wave velocity - m/sec	0.80±0.20	0.59±0.17	<0.001
Ratio of E-wave: A-wave velocity	0.96±0.35	1.03±0.32	0.47
Mitral E-wave deceleration - msec	274±70	269±73	0.82
E/E' (lateral)	12±4	8±2	<0.001
E_{es}	3.07±1.07	2.60±.53	0.09
E_a	2.22±0.63	2.28±0.48	0.69

Plus-minus values are means ± SD. When patients on beta blockers were excluded from analysis, the level of significance were similar apart from resting HR (P=0.14). NYHA denotes New York Heart Association, ACE angiotensin-converting enzyme, ARB angiotensin II receptor blockers, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure, MABP mean arterial blood pressure, LA left atrium, E/E' mitral E-wave velocity-E' tissue velocity (PW-TDI) at basal anterior-lateral wall ratio, E_{es} denotes Left Ventricular End-Systolic Elastance and Ea is Arterial elastance. The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Radionuclide ventriculography at Rest and on Exercise: Diastolic Filling Characteristics, Systolic Function, Relaxation, Stiffness, and Vasculo-ventricular coupling			
Variable	**Patient (N = 37)**	**Control (N = 20)**	**P Value**
HR (beats/min)			
Exercise	97±14	114±11	<0.001
Exercise SBP (mm/Hg)	204±26	198±27	0.45
Exercise DBP (mmHg)	95±15	97±7	0.56
Exercise MABP (mmHg)	132±15	131±9	0.85
Ejection fraction (%)			
Rest	65±9	64±9	0.61
Exercise	66±9	72±8	0.05
Peak emptying rates (EDC/sec)			
Rest	382±106	400±90	0.56
Exercise	477±123	563±144	0.04
Peak filling rates (EDC/sec)			
Rest	342±120	321±111	0.54
Exercise	504±127	602±163	0.02
Time to peak filling (RR)			
Rest	0.18±0.08	0.18±0.09	0.84
Exercise	0.25±0.09	0.16±0.08	0.001
Δ nTTPF	+0.07±0.11	-0.03±0.12	<0.005
Vasculo-Ventricular Coupling ratio (VVC) (E_aI/E_{LV}I)			
Rest	0.57±0.20	0.62±0.22	0.36
Exercise	0.55±0.19	0.41±0.15	0.01
Δ VVC	-0.01±0.15	-0.25±0.19	<0.001
Relative Δ Stroke Volume Index			
Exercise	0.99±0.34	1.25±0.47	0.04
Relative Δ Cardiac Output Index			
Exercise	1.36±0.45	2.13±0.72	<0.001
Relative Δ E_{LV}I			
Exercise	1.35±0.50	1.85±0.63	0.01
Relative Δ E_aI			
Exercise	1.52±0.48	1.28±0.44	0.17

Plus-minus values are means ± SD. When patients on beta blockers were excluded from analysis, the level of significance were similar apart from peak filling rates during exercise (P=0.08). RR – values normalised for R-R interval, EDC end diastolic count. SBP systolic blood pressure, DBP diastolic blood pressure, MABP mean arterial blood pressure. Relative Δ Stroke Volume Index is $SVi_{EXERCISE}$ / SVi_{REST}, Relative Δ Cardiac Output Index is $COi_{EXERCISE}$ / COi_{REST}. Relative Δ $E_{LV}I$ is $E_{LV}I_{EXERCISE}$ / $E_{LV}I_{REST}$. Relative Δ E_aI is $E_aI_{EXERCISE}$ / E_aI_{REST}. Δ Vasculo-ventricular coupling ratio is $(E_aI/E_{LV}I)_{EXERCISE}$ - $(E_aI/E_{LV}I)_{REST}$.

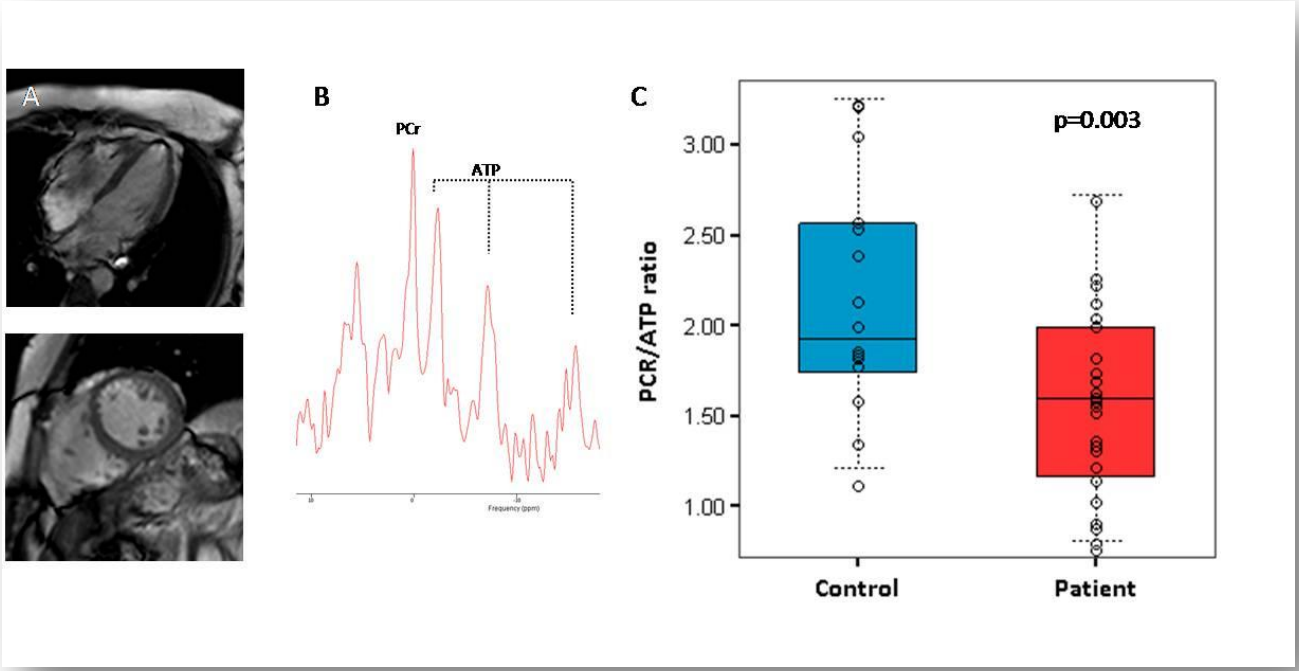

Figure 1. Panel A shows cardiac MR images of a patient with HfpEF and the corresponding localized ^{31}P MR spectra from the left ventricle is shown in panel B. The corresponding peaks of PCr and the γ-, α-, and β-phosphate (resonances of the ATP) are labeled. Panel C shows individual PCr/ γ-ATP ratio in patients with HfpEF and Controls. The PCr/ γ-ATP ratio was significantly reduced in patients with HfpEF compared to healthy controls, P= 0.003

Chapter III

Increased atrial contribution to left ventricular filling compensates for impaired early filling during exercise in Heart Failure with Preserved Ejection Fraction

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Introduction

HfpEF accounts for approximately half of patients with clinical features of chronic heart failure (10) These patients are typically elderly women who frequently have associated hypertension, diabetes, and/or coronary artery disease. (14) They have similar hospital length of stay, admission rates (11, 12) and mortality rate to that of patients with systolic heart failure (12). The prevalence of HfpEF appears to be increasing and in contrast to systolic heart failure the mortality rate of this disorder is not declining. (11)

Many patients with HfpEF have slow active relaxation and/or increased LV passive diastolic stiffness at rest. (1) In addition, there is a dynamic impairment of left ventricular active relaxation during isometric (handgrip) exercise in HfpEF. (58) However, the role of LA function during exercise remains poorly understood in HfpEF despite its key role in optimizing LV end diastolic volume, especially in the context of a possible slowing of LV active relaxation and increased LV stiffness. (1, 58) Previous studies have primarily focussed on how well geometrical aspects of the LA predicts diastolic LV dysfunction (42) and cardiovascular event (43, 44) but these observations provide little insights on how LA function plays a role in the pathophysiology of HfpEF especially during exercise when the

majority of patients experience breathlessness. In this study, using radionuclide ventriculography, we evaluated the role of LA function in the pathophysiology of HfpEF by investigating both LA and LV function at rest and during exercise.

Method

Study Participants

We studied Twenty-five HfpEF patients prospectively recruited from heart failure clinics. Fifteen healthy age-matched-controls (volunteers) with no cardiac history, hypertension or diabetes mellitus were also studied. All study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram, metabolic exercise test and radionuclide ventriculography (rest and exercise). All patients had signs and/or symptoms of heart failure with a LV ejection fraction >50% by transthoracic echocardiography and met the criteria of Yturralde and Gaasch for diastolic heart failure (19). All healthy controls had a normal clinical cardiovascular examination, 12-lead electrocardiogram, echocardiogram and a metabolic exercise test. Patients with severe pulmonary disease, significant valvular heart disease, atrial fibrillation, or evidence of hypertrophic cardiomyopathy were excluded. The investigations were performed at The University of Birmingham with approval of the Research Ethics Committee. Informed consent was obtained from all subjects.

Metabolic Exercise Testing

The metabolic exercise testing was performed on a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using incremental ramp protocol (speed and inclination was increased every minute) with simultaneous respiratory

gas analysis (125, 126). Samplings of expired gases were performed continuously, and data were expressed as 10-second means. Minute ventilation (VE), oxygen consumption (VO_2), carbon dioxide production (VCO_2) and respiratory exchange ratio (RER) were obtained. 10-second-averaged VE and VCO_2, from the initiation of exercise to anaerobic threshold, were placed in to an excel worksheet (Microsoft Excel 2003, Microsoft Corp, Bellevue, WA) to calculate the VE/VCO_2 slope via a linear regression (y= Ax + B, A = slope) as previously described. (172) Peak oxygen consumption (peak VO_2) was defined as highest value of oxygen consumption measured during the exercise period. Blood pressure and ECG were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Resting Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine (GE Healthcare) and a 2.5-MHz transducer. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber. LV volumes were obtained by biplane echocardiography, and LVEF was derived from a modified Simpson's formula (127). Pulse wave Doppler sample volume was placed at the mitral valve tips to record 3 cardiac cycles. Mitral annulus velocities by pulse wave Tissue Doppler imaging (PW-TDI) were recorded from basal antero-lateral segment. LA volumes were measured by the area length method from apical 2 and 4 chambers as previously described (127). Left ventricular hypertrophy was defined as a left ventricular mass indexed to body surface area that exceeded 88 g/m^2 for women and 102 g/m^2 for men. (127) Studies were stored digitally and analyzed off-line.

Radionuclide Ventriculography

Left ventricular ejection fraction and diastolic filling were assessed by radionuclide ventriculography using a standard technique at rest and during graded semi erect exercise on a cycle ergometer as previously described in detail by our group (173). Twenty minutes after an intravenous injection of 0.03 mg/kg stannous pyrophosphate, 5 mL of blood was drawn into a heparinised syringe and incubated for 10 minutes with 750 MBq of ^{99m}Tc pertechnetate before re-injection. Studies were acquired on a small-field-of-view gamma camera fitted with a low-energy, general-purpose, parallel-hole collimator and interfaced to a dedicated minicomputer. With the patient on the cycle ergometer, the detector was adjusted for the left anterior oblique view with the best ventricular separation and 10° to 15° of caudal tilt. A 20% tolerance window was set about the patient's heart rate, and each RR interval was divided into 28 equal frames throughout. A constant number of frames per RR interval ensure constant temporal resolution during diastole at all heart rates. Three minutes of data were acquired at rest and during exercise after a 30-second period for stabilization of heart rate. Exercise was performed at 35% workloads of heart rate reserve because at HR above >100-110/ min, the early and late LV filling curves begin to merge. The composite cycle derived from each stage was spatially and temporally filtered. Left ventricular end-diastolic counts corrected for background gamma activity were obtained by means of a semi-automated edge detection algorithm. Venous blood samples (≈ 5.0 mL) were obtained for weighing and for counting of blood gamma activity during each scan in order to correct for physical and physiological decay as well as for determination of relative volume changes.. (120) The validity of these radionuclide measures of diastolic filling at high heart rates has been established previously (121, 122). All gated blood pool scan-derived volumes were normalized to body surface area, yielding their respective indexes:

end-diastolic volume index (EDVi), end-systolic volume index (ESVi), stroke volume index (SVi), and cardiac output index (COi). In order calculate changes in these volumes during exercise, relative volume changes were determined as follows: relative change in EDVi is defined as $EDVi_{EXERCISE}$ / $EDVi_{REST}$; relative change in ESVi is defined as $ESVi_{EXERCISE}$ / $ESVi_{REST}$; relative change in SVi is defined as $SVi_{EXERCISE}$ / SVi_{REST}; relative change in cardiac output Index is $COi_{EXERCISE}$ / COi_{REST}.

The activity-time curve (ATC) (figure 1) was exported from LinkMedical MAPS software, Sun Microsystems into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The first derivative of the ATC curve was computed to represents the rate of LV volume changes. End-systole (ES) is defined as the lowest ATC point. Stroke volume (SV) is defined as the difference between end-diastolic count (EDC) and end-systolic count. Peak emptying rate (PER) was defined as the negative peak of the first derivative curve during systole and was expressed in EDC/sec. Likewise, peak early filling rate (PEFR) was defined as the early positive deflection of the first derivative curve during diastole, expressed in EDC/sec. The early LV filling was measured from the corresponding ATC segment and was expressed as percentage of stroke volume (%SV). The interval from ES to the time when PEFR occurred was defined as time to peak early filling rate (TTPEF), expressed in msec. The late positive deflection of the first derivative curve during diastole is attributed to the atrial contraction, this peak defines the peak ventricular filling rate during atrial contraction (PAFR). We used the late positive deflection of the first derivative curve as a measure of '*atrial function*' however strictly this part of the curve is also determined by ventricular properties. The atrial contribution to LV filling was measured from the corresponding ATC segment and expressed as percentage of stroke volume (%SV).

The interval from end of early filling to the time when PAFR occurred was defined as time to peak atrial filling rate (TTPAF), expressed in msec. The diastolic period was expressed as a percentage of R-R interval. The atrial component of diastolic period was defined as the percentage of the diastolic period that was spent in atrial contraction. All these parameters for diastolic function assessment have been previously described (174, 175). The early and atrial contribution of diastolic filling were fused on the activity-time curve during exercise in three patients and three healthy controls, thus these exercise data were excluded in the final analysis.

Statistics

Continuous variables are expressed as means ± SD. Unpaired Student's t-test (2-tail) was used to assess differences between mean values. ANCOVA with baseline values as covariates was performed to test for the significance of differences in between patients and controls during exercise. Categorical variables were compared with Pearson Chi-Square test. All reported P values were calculated on the basis of two-sided tests and a P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. All subjects were included in the model. SPSS (v15.0) was used to perform the statistical operations.

Results

Characteristics of Patients

HfpEF Patients were generally females, overweight, aged 66±11 years old with a history of hypertension consistent with previous large epidemiological studies (12) (Table 1). Blood pressure was well treated (systolic BP 138±17mmHg vs. 130±25mmHg; p=0.24, in patients vs. controls). The tissue Doppler E/E' at the basal anterior-lateral segment (a measure of left ventricular end-diastolic pressure) (161), was significantly higher in patients than controls (11±4 vs. 8±3, p=0.01). Left ventricular end-diastolic volume indexed for body surface area (EDVi) was significantly smaller in patients with HfpEF than controls. Patients with HfpEF also had significantly reduced peak VO_2 compared to controls (20±5 ml/Kg/min vs. 35±8 ml/Kg/min, p<0.001). The VE/VCO_2 slope was also higher in patients with HfpEF than controls (33±5 vs. 28±3, p=0.002). Resting E/E' correlated negatively with peak VO_2 (r=-0.389, P=0.019). (Table 2)

Left ventricular systolic and diastolic characteristics

At Rest

Peak emptying rate (PER), peak early filling rate (PEFR) and time to peak early filling (TTPEF) were similar in patients and controls. (Table 3)

During exercise

LVEF was significantly lower in patients compared to controls (69 ± 9 % vs. 73 ± 10 %, p<0.05). Peak early filling rate was significantly lower in patients compared to controls (387±109 EDC/sec vs. 561±156 EDC/sec, p<0.001). Peak early filling rate correlated positively with peak VO_2 (r=0.485, P=0.004) and negatively with VE/VCO_2 (r=-0.423, P=0.013) during exercise but not at rest (p=0.399 and p= 0.320, respectively).

Atrial contribution to diastolic filling

At rest

Peak atrial filling rate (PAFR) and time to peak atrial filling (TTPAF) were similar in patients and controls. The atrial contribution to LV filling was similar in patients and controls. The total diastolic period and atrial component of diastolic period were both similar in patients and controls. (Table 3)

During exercise

The atrial contribution to LV filling was significantly higher in patients than controls (46 ± 11 %SV vs. 30 ± 9 %SV, p<0.001). In addition, the atrial contribution to LV filling correlated negatively with peak early filling rate during exercise (r=-0.6, P<0.001) but not at rest (r=0.04, P<0.83). The total diastolic period and atrial component of diastolic period were both similar in patients and controls. (Table 3)

The effects of LVH on LV filling

When patients with and without LVH were analyzed separately the findings of this study remained significant for both group of patients. In patients with LVH, during exercise, patients had lower peak early filling rate (415±117 EDC/sec vs. 561±156 EDC/sec, p=0.029), the atrial contribution to LV filling was significantly higher in patients than controls (44 ± 11% vs. 30 ± 9%, p=0.004). At rest the two groups were no different. In patients without LVH, during exercise, patients had lower peak early filling rate (369±104 EDC/sec vs. 561±156 EDC/sec, p=0.001), the atrial contribution to LV filling was significantly higher in patients than controls (48 ± 11% vs. 30 ± 9%, p<0.001). At rest the two groups were no

different. When we compared patients with LVH to patients without LVH, there were no significant differences between peak early filling rate and atrial contribution to LV filling during exercise.

Discussion

Whilst there has been a considerable literature examining the role of atrial contribution to LV filling at rest in patients with HfpEF this is to the best of our knowledge, the first study to investigate LA function during exercise in patients with HfpEF and provides new insights on the role of LA function in the pathophysiology of HfpEF. The principal findings are: a) At rest, LV and atrial functions were similar in HfpEF and controls. b) During exercise, HfpEF patients showed markedly reduced early LV filling. c) HfpEF patients have increased LA contribution to LV filling during exercise. d) HfpEF patients have reduced systolic reserve.

The atrium contributes about 20-30% of total LV stroke volume. In dilated cardiomyopathy, there is a compensatory increase in the LA contribution to LV filling in patients with asymptomatic or mildly symptomatic dilated cardiomyopathy. (176) However, in patients with more advanced heart failure or highly symptomatic dilated cardiomyopathy, this compensatory response of LA contraction is reduced and attributed to elevated LV filling pressure. (177) In a study of a predominantly African American cohort of patients with hypertensive left ventricular hypertrophy (LVH), it was shown that those with and without features of HfpEF had similar systolic, diastolic and vascular function, however the cohort with features of HfpEF differed predominantly because they had evidence of left atrial dilatation and left atrial 'failure'. (45) In a separate community study, LA fractional area

change (a measure of LA emptying volume) was found to be reduced at rest in community older patients (≥65) with diastolic heart failure. (46)

Previous studies have also found that LA volume indexed to body surface area was a stronger predictor of cardiovascular event in the elderly than LV mass index or LV diastolic dysfunction, (40) and that LA volume is a marker of LV diastolic dysfunction in patients with heart failure and normal LVEF. (41) Our cohort of patients had similar LA volume to controls. This may be because patients with atrial fibrillation (AF), a common feature in HfpEF (11, 12) which is strongly associated with LA enlargement (178) were excluded from the study because radionuclide studies requires a regular rhythm and also this study's aim was to evaluate LA function. In addition, our cohort of patients were well characterized all of whom met Yturralde and Gaasch criteria for diastolic heart failure (19) and are objectively very limited in their exercise capacity as indicated by their reduced peak VO_2 and higher minute ventilation (VE/CO_2 slope) than controls, which is prognostic in diastolic heart failure. (179) The use of metabolic exercise testing as a mean to show objective exercise limitation may have potentially identified patients with HfpEF at an early stage of the condition before the development of LA dilatation.

Although, previous studies have revealed the importance of LA function and LA volume in heart failure, they provide little insight into the pathophysiology of HfpEF and even less so to what happen to LA contribution to LV filling during exercise. In this study we have demonstrated that during patients with HfpEF has an increased atrial contribution to LV filling during exercise (compared to controls). The reason for this we believe is maybe

secondary to an impaired early LV filling as previous studies have indicated that left ventricular filling impairment can modulate LA function. (180)

We found that patients with HfpEF had significantly lower peak early filling rates during exercise which suggests impairment in early LV filling and relaxation during exercise. (181) Our findings are supported by previous studies which looked at patients with asymptomatic essential hypertension, and found that hypertensive patients (with LVEF either increased by <5% or decreased with exercise) had impaired diastolic filling during exercise. (75) With respect to HfpEF, some studies have alluded to the presence of profound slowing of active relaxation and increased LV passive diastolic stiffness (1) at rest.

In this study the relative change in EDVi during exercise was non-significantly lower in HfpEF compared to controls (1% vs 10%), however this was in the context that in HfpEF, the atrial contribution to LV filling increased from 34% to 46% during exercise compared to controls where atrial contribution to LV filling remained essentially unchanged. Thus we believe that the increase in LA contribution to LV filling might represent a compensatory response to the observed abnormal early LV filling during exercise in order to maintain EDVi, and therefore we do not see a significant difference in the relative change in EDVi between the 2 groups during exercise. Furthermore, this trend towards a lesser relative increase in EDVi during exercise in patients with HfpEF coupled with the observed smaller resting EDVi might result in a substantially reduced absolute EDVi (and probably stroke volume) during exercise in these patients with HfpEF.

When patients with and without LVH were analysed separately the findings were similar.

Previous studies conducted by our group in patients with non-obstructive hypertrophic cardiomyopathy, a classic paradigm of diastolic heart failure, found that exercise left ventricular diastolic filling characteristics were a major determinant of peak exercise capacity. (76). In this study we found that during exercise and not at rest, the peak early filling rate had a significantly positive correlation with peak exercise capacity (peak VO_2) and a significantly negative correlation with VE/VCO_2 slope. Thus this impairment in early filling during exercise might in part explain why patient with HfpEF have poor exercise capacity. Furthermore, VE/VCO_2 slope has been shown to have prognostic value in patients with diastolic heart failure with respect to mortality and hospitalization. (179)

These studies were performed during sub-maximal exercise. It is possible that during more intense levels of exercise the dynamic impairment in early LV filling may worsen and that the LA compensatory response may then become insufficient or might even fail, the net result being a poor stroke volume response during exercise resulting in exercise limitation in HfpEF. Interestingly also, we observed this impairment in early LV filling during exercise in a cohort of patients with relatively normal LA volume. It is possible that this impairment in early LV filling can be further exacerbated when the LA is dilated because previous studies have shown that atrial dilatation can contribute to alterations in LA pressure and therefore reduced early diastolic filling. (47) Furthermore LA dilatation in HfpEF is associated with reduced LA function (45), reduced LA strain (during systole) and increased LA stiffness (182), the combination of which might compromise atrial kick and therefore might diminish LA compensatory response for any poor early LV filling during exercise. Furthermore when

these patients with HfpEF lose their atrial function completely (i.e. atrial fibrillation) not surprisingly the result is more severe diastolic dysfunction as well as increase hospitalization or death. (49)

We also found that at rest contractility appeared normal in HfpEF, consistent with recent data. (183) However during exercise, systolic function appear diminished as indicated by the significantly lower LVEF and a trend (non-significant) to lower peak emptying rates in patients than controls. This observation is supported by previous studies in subjects with LVH. (184) Perhaps this is not surprising because we know from previous studies that despite EF being normal, at rest, early LV longitudinal dysfunction has been detected by tissue doppler imaging (157), as well as the presence of systolic and diastolic dyssynchrony in HfpEF (56, 57), which could further exacerbate systolic reserve in HfpEF. Another factor that can limit cardiovascular reserve is chronotropic response. The metabolic exercise data reveals that HfpEF patients have significantly lower heart rate during peak exercise compared to controls indicating the presence of chronotropic incompetence as previously reported (77). This remained significant even when patients on beta-blockers were excluded from the analysis ($p<0.001$).

What we have learnt from this study which has not previously been described in HfpEF is that there is a dynamic pathophysiological process whereby early LV filling is impaired during exercise which correlates with peak exercise capacity and minute ventilation. As a result there is a compensatory increase in LA contribution to LV filling. Furthermore, there is a reduction in systolic reserve during exercise. We believe these findings play an important part in the pathophysiology of HfpEF.

Study limitations

Our radionuclide exercise protocol involved asking subjects to maintain a HR which was 35% of HR reserve above their resting HR. Since this HR reserve was calibrated to peak HR rate on metabolic exercise testing, the absolute workload and heart rate was lower in patients. Comparison of patients at the same workload would be inappropriate because this would represent a higher relative workload in patients. Furthermore, most of the changes in SV occur in the first part of exercise with subsequent increases in cardiac output being principally due to increases in HR. (171)

A minority of patients were on beta-blockers which may have some negative lusotropic effect on the myocardium however, if anything one would expect the net effect of beta-blockers would be less atrial contribution to LV filling as early LV filling is improved. To stop the beta blockers would have been unethical and could also result in rebound tachycardia. Besides, when patients on beta blocker were removed from the analysis the findings of enhanced atrial contribution to LV filling and reduced peak early filling rates during exercise remained significant ($p<0.001$ and $p<0.001$, respectively). In addition, some patients were on calcium blockers however these were all peripherally acting (dihyodropyridines for hypertension) and therefore are not expected to affect the myocardium.

Conclusion

Patients with HfpEF have increase left atrial contribution to LV filling as a compensatory response to impaired early LV filling during cycle exercise.

Table 1: Baseline characteristics

	Patient	Control	P value
N	25	15	
Females no. (%)	16 (64)	9 (60)	
Age (years)	66 ± 11	63 ± 7	0.33
BMI	30 ± 4	27 ± 5	0.03
Left ventricular hypertrophy no.	12	4	-
Hypertension no.	17	0	-
Ischaemic heart disease no.	4	0	-
Diabetes no.	3	0	-
NHYA functional class no.			
II	21	0	-
III	4	0	-
Medications no.			
Diuretic	7	0	-
ACE inhibitor	17	0	-
ARB	2	0	-
Beta-blocker	7	0	-
Calcium blocker	7	0	-
Alpha Blocker	3	0	-
Nitrate	3	0	-

Plus-minus values are means ± SD. NYHA denotes New York Heart Association, ACE angiotensin-converting enzyme, ARB angiotensin II receptor blockers.

Table 2: Metabolic exercise test and Echocardiographic parameters

	Patient	Control	P value
Metabolic exercise test			
peak VO_2 (ml/Kg/min)	20 ± 5	35 ± 8	<0.001
RER	1.07 ± 0.08	1.15 ± 0.11	0.017
% Predicted peak VO_2	58 ± 12	97 ± 17	<0.001
VE/VCO_2	33 ± 5	28 ± 3	0.002
Breathing Reserve (L/min)	40 ± 16	41 ± 16	0.92
Heart rate (beats/min)			
Rest	72 ± 15	80 ± 15	0.13
Peak	123 ± 22	164 ± 11	<0.001
Systolic blood pressure (mmHg)			
Rest	138 ± 17	130 ± 25	0.24
Peak	185 ± 29	188 ± 28	0.72
Diastolic blood pressure (mmHg)			
Rest	81 ± 11	80 ± 13	0.83
Peak	81 ± 14	83 ± 9	0.57
Echocardiography			
Ejection fraction (%)	64 ± 16	64 ± 6	0.93
EDVi (mL/m^2)	30 ± 8	40 ± 12	0.02
ESVi (mL/m^2)	10 ± 5	14 ± 4	0.02
LA Volume Index (mL/m^2)	21 ± 4	19 ± 5	0.41
MV E Vel	0.72 ± 0.18	0.63 ± 0.12	0.09
MV A Vel (m/s)	0.75 ± 0.16	0.58 ± 0.19	0.004
MV E/A Ratio	0.99 ± 0.30	1.05 ± 0.32	0.56
MV DecT (ms)	279 ± 57	265 ± 77	0.51
E/E' Antlat	11 ± 4	8 ± 3	0.01

Plus-minus values are means ± SD. LA - left atrium, E/E’ - mitral E-wave velocity-E’- tissue velocity (PW-TDI) at basal anterior-lateral ratio, EDVi –end diastolic volume indexed for body surface area, ESVi - end systolic volume indexed for body surface area. MV DecT - mitral valve deceleration time. The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 3: Radionuclide Ventriculography at Rest and during Exercise: Early and Atrial Filling Characteristics and Systolic Function

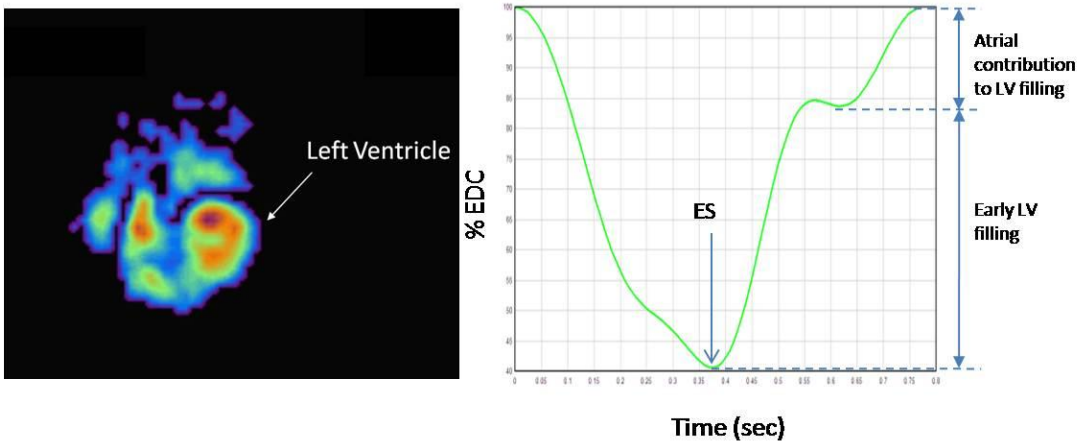
	Patient	Control	P value
Heart rate (beats/min)			
Exercise	86 ± 12	98 ± 12	<0.001
Systolic blood pressure (mmHg)			
Exercise	182 ± 27	168 ± 28	0.87
Diastolic blood pressure (mmHg)			
Exercise	92 ± 11	93 ± 8	0.40
Ejection Fraction (%)			
Rest	65 ± 9	64 ± 8	0.58
Exercise	69 ± 9	73 ± 10	0.04
Peak emptying rate (EDC/sec)			
Rest	371 ± 90	393 ± 102	0.48
Exercise	380 ± 225	520 ± 168	0.06
Time to peak emptying rate(msec)			
Rest	133 ± 66	159 ± 55	0.22
Exercise	110 ± 67	138 ± 83	0.25
Peak early filling rate (EDC/sec)			
Rest	347 ± 119	315 ± 123	0.43
Exercise	390 ± 112	561 ± 156	<0.001
Peak atrial filling rate (EDC/sec)			
Rest	213 ± 97	196 ± 94	0.59
Exercise	361 ± 104	295 ± 74	0.14
Time to peak early filling (msec)			
Rest	141 ± 59	151 ± 70	0.62
Exercise	104 ± 48	86 ± 33	0.27
Time to peak atrial filling (msec)			
Rest	99 ± 52	123 ± 112	0.29
Exercise	74 ± 16	63 ± 12	0.02
Early LV filling (%SV)			
Rest	66 ± 12	67 ± 10	0.85
Exercise	55 ± 10	70 ± 9	<0.001
Atrial contribution to LV filling (%SV)			
Rest	34 ± 12	33 ± 10	0.85
Exercise	45 ± 10	30 ± 9	<0.001
Total systolic period (%RR)			
Rest	40 ± 6	36 ± 9	0.06
Exercise	46 ± 4	49 ± 6	0.05
Total diastolic period (%RR)			
Rest	60 ± 6	64 ± 9	0.06
Exercise	54 ± 6	51 ± 6	0.05
Early component of diastolic period (%)			
Rest	66 ± 8	63 ± 12	0.33
Exercise	59 ± 11	60 ± 8	0.97

Atrial component of diastolic period (%)			
Rest	34 ± 8	37 ± 12	0.33
Exercise	40 ± 11	40 ± 8	0.97
Relative change in EDVi			
Exercise	1.01 ± 0.31	1.10 ± 0.39	0.44
Relative change in ESVi			
Exercise	0.98 ± 0.46	0.95 ± 0.59	0.91
Relative change in SVi			
Exercise	1.06 ± 0.35	1.28 ± 0.46	0.11
Relative change in COi			
Exercise	1.37 ± 0.44	1.97 ± 0.67	0.002

Plus-minus values are means ± SD. EDC end diastolic count. SBP systolic blood pressure, DBP diastolic blood pressure. %RR - % of R-R interval,

Figure 1: An example of a radionuclide ventriculography image with the activity-time curve for a healthy control.

The image show the subject's left ventricle and the corresponding activity-time curve demonstrating early and atrial contribution to LV filling. EDC – end diastolic count, ES – end systole.



Chapter IV

Myocardial contractile inefficiency and dyssynchrony in Heart Failure with Preserved Ejection Fraction and narrow QRS complex

Chapter Four

Myocardial contractile inefficiency and dyssynchrony in Heart Failure with Preserved Ejection Fraction and narrow QRS complex

Introduction

Many consider heart failure with preserved ejection fraction (HfpEF) to be a disorder of diastolic function (50), whilst others believe that it may be due to a combination of diastolic abnormalities with subtle disturbances of systolic function that are insufficient to reduce LVEF (162). Studies using tissue Doppler imaging (TDI) have demonstrated the presence of diastolic and/or systolic dyssynchrony in patients with HfpEF (56, 57). Recently a novel technique known as speckle tracking imaging (STI), has been used to quantitatively evaluate myocardial strain patterns independent of cardiac translation and insonation angle (185), which are major limitations of TDI derived myocardial strain and velocities. (186) STI is also less time-consuming than TDI and has been validated in experimental and human studies. (185, 187). In addition, STI allows for an assessment of segmental synchronicity (188) and also longitudinal strain delay index (LSDi) (189).

LSDi is derived from longitudinal strain (ε) patterns and is a strong predictor of responders to **c**ardiac resynchronization therapy (CRT) in both ischaemic and non-ischaemic patients. (189) LSDi is an index that quantifies the wasted energy in dyssynchronous ventricles, which is brought about because delayed segments do not contribute fully to end-systolic (ES) function. Furthermore, the wasted energy is greater in delayed segments with preserved

contractility compared to delayed segments with reduced contractility (i.e. scar or fibrotic myocardium).

In this study, using STI, we aim to assess LV global and segmental synchronicity, and to determine which LV segments display delayed motion, which can therefore identify potential cardiac pacing sites for correction of LV dyssynchrony. In addition, we aim to quantify the wasted energy because of systolic dyssynchrony by determining LSDi.

Method

Study Participants

HfpEF patients

We studied 38 HfpEF patients, who were prospectively and consecutively recruited from heart failure clinics. All study participants had clinical examination, 12-lead ECG, a pulmonary function test, as well as an echocardiogram and a metabolic exercise test. All patients had signs and/or symptoms of heart failure with a LV ejection fraction >50% by transthoracic echocardiography and met the criteria of Yturralde and Gaasch for diastolic heart failure (19). Patients with severe pulmonary disease, significant valvular heart disease, atrial fibrillation, or evidence of hypertrophic cardiomyopathy were excluded. Patients with rhythm other than sinus and had QRS duration> 120ms were excluded. The study was performed at The University of Birmingham with approval of the Research Ethics Committee. Informed consent was obtained from all patients and controls.

Healthy controls

We studied 33 healthy controls with no history of cardiac disease, hypertension or diabetes mellitus. Healthy volunteers from the community who responded to our advertisements were recruited on the basis that they were of similar age and gender as our patient group and had normal clinical cardiovascular examination, 12-lead electrocardiogram, echocardiogram and metabolic exercise testing.

Metabolic Exercise Testing

The metabolic exercise testing was performed on a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using incremental ramp protocol (speed and inclination was increased every minute) with simultaneous respiratory gas analysis (125, 126). Samplings of expired gases were performed continuously, and data were expressed as 30-second means. Minute ventilation, oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) were obtained. Peak oxygen consumption ($V0_2max$) was defined as the highest value of oxygen consumption measured during the exercise period. Blood pressure and ECG were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Resting Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine (GE) and a 2.5-MHz transducer. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber views. All echocardiographic measurements were averaged from 3 heart beats. LV ejection fraction (LVEF) and left ventricular hypertrophy (LVH) was determined in accordance with the guidelines. (127)

From the LV-inflow pattern (measured at the tips of the mitral valve), peak early (E) and late (A) filling velocities, E/A ratio, and E-velocity deceleration time (DcT) were measured. The isovolumic relaxation time (IVRT) was determined using pulsed-wave Doppler velocity data of the LV inflow. Tissue Doppler was applied end-expiratory in the pulsed-wave Doppler mode at the level of the lateral mitral annulus from an apical 4-chamber view. The velocities of the mitral annular systolic wave (S'), early diastolic wave (E'), and late diastolic wave (A') were noted. Lateral mitral annulus velocities were recorded to derive E/E'. Parasternal circular short-axis images were taken at the papillary (identified by the papillary muscles present) similar to previous studies. (190)

Speckle Tracking Imaging (STI)

STI was measured using a commercially available speckle tracking system in an ECHOPAC (ver. 4.2.0) workstation. Myocardial deformation measurements were performed using tissue speckle tracking. In this speckle tracking system, the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image cardiac cycle and the left ventricle endocardium was traced at end-systole.(129). The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle. The onset of QRS complex was taken as the beginning of systole. Adequate tracking was verified in real time. A high frame rate between 70-100 Hz was used as in previous studies. (191) For each subject, longitudinal strain values for all 12 LV myocardial segments in each of the apical 4 and 2 chamber views were measured to derive the LV longitudinal strain, strain rates and velocity curves. In addition, radial and

circumferential strain values for all six LV myocardial segments in the short-axis view at the papillary level were measured to derive the radial and circumferential strain curves.

Strain delay index and synchronicity assessment

As described previously by Lim et al (189), in dyssynchronous ventricles, segments which are delayed do not contribute fully to global ES function. The energy wasted per segment because of dyssynchrony is expressed by the difference between peak (ε_{peak}) and ES strain (ε_{ES}). If all the LV segments were synchronous then the strain delay index would be zero which means all the myocardial energy during the systolic period is efficiently used to push blood out of the LV. However, as LV systolic dyssynchrony worsens the strain delay index ($\varepsilon_{peak} - \varepsilon_{ES}$) also increases. When the strain delay index is high, then whilst some LV segments are contracting others are still lagging behind and thus not all potential energy is efficiently used to move blood out of the LV during systole. The strain delay index is a mathematical representation of the sum of wasted energy because of systolic dyssynchrony across LV myocardial segments:

$$\text{Strain delay index} = \sum_{1}^{n} (\varepsilon_{peak} - \varepsilon_{ES})$$

The strain delay index was determined from the longitudinal strain curves derived from 2D speckle tracking (EchoPac version 4.2.0, GE). The strain delay index proposed by Lim et al (189) is actually a magnitude of strain (%) and not of time as suggested by the word 'delay'. The onset of the QRS complex was used as the reference point for the start of systole. Strain curves derived from a single cardiac cycle were exported into DPlot graph software (Version

2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA) for analysis. The steps to determine LSDi were as follows: a) A global ε curve representing LV function was computed by averaging 12 segmental LV ε curves. b) The time to peak of the global ε curve was ascertained to determine the time of ES and the strain at ES of each of the 12 segments (ε_{ES}). c) Peak ε and time to peak ε were determined in the 12 segments as the ε curve reached its minimum value during the cardiac cycle. d) The difference (ε_{peak} - ε_{ES}) in each segment (12 segments) was summed to derive the strain delay index. (See figure 1) For segments that had positive strain or biphasic strain with the peak positive ε greater than the maximal absolute negative strain, the difference (ε_{peak} - ε_{ES}) was entered as zero. Radial and circumferential strain delay index was determined similarly but radial and circumferential ε curves from 6 segments were used (at papillary level).

Time to peak longitudinal strain (Ts-LS), circumferential strain (Ts-Circ) and radial strain (Ts-Rad) was also determined by STI. For the assessment of synchronicity, the standard deviation (SD) of Ts-LS, Ts-Circ and Ts-Rad was computed to derive Ts-LS-SD, Ts-Circ-SD and Ts-Rad-SD values respectively. Time to peak longitudinal velocity S (Ts), E (Te) and A (Ta) and peak longitudinal strain rate S (Ts-LSr), E (Te-LSr) and A (Ta-LSr) was also determined. For the assessment of synchronicity, the SD of Ts, Te and Ta were computed to derive Ts -SD, Te -SD and Ta -SD values respectively. The SD of Ts-LSr, Te-LSr and Ta-LSr was also determined to derive the Ts-LSr-SD, Te-LSr-SD and Ta-LSr-SD, respectively. For comparison, all time values were normalized for R-R interval, which was calculated by dividing the time period by the R-R interval.

Statistics

Continuous variables are expressed as mean ± SD. Unpaired Student's t-test (2-tail) was used to assess group differences. Categorical variables were compared using the Chi-Square test. A two-tailed p value of <0.05 was considered statistically significant. Variances of data sets were determined using Levene's test. Pearson correlation coefficient (r) was used to describe the relationship between two variables. All subjects were included into the model. SPSS (v15.0) was used to perform the statistical analyses.

Results

Characteristics of the Study Group

HfpEF Patients were generally females (71%), overweight (BMI>25), aged 67±9 years old and hypertensive (Table 1). Blood pressure was well treated (systolic BP 137±21mmHg vs. 134±21mmHg; p=0.49, in patients vs. controls). The tissue Doppler E/E' at the basal anterior-lateral segment (a measure of left ventricular end-diastolic pressure) (161), was significantly higher in patients than controls (11±4 vs. 7±2, p<0.001). Patients with HfpEF also had significantly reduced VO_2max compared to controls (21±5 ml/Kg/min vs. 34±7 ml/Kg/min, p<0.001). The minute ventilation-carbon dioxide production relationship (VE/VCO_2 slope) was also higher in patients with HfpEF than controls (33±6 vs. 28±3, p=0.001).

Strain Delay Index

LSDi was significantly higher in patients with HfpEF than controls (-14.36±8.24% vs. -10.73±5.62%, p< 0.05). There was a non-significant trend towards a higher radial strain delay index and circumferential strain delay index in patients with HfpEF than controls (4.80±6.34% vs. 2.27±2.39%, p= 0.05 and -7.67±5.48% vs. -5.45±4.26%, p= 0.12,

respectively). (See Table 2) There were no significant correlation between LSDi and LVEF or stroke volume.

Systolic and diastolic synchronicity

The standard deviation of time to peak systolic velocity (Ts-SD) was significantly higher in HfpEF compared to controls (0.074±0.026 RR vs. 0.060 ± 0.023 RR, $p< 0.05$). The standard deviation of time to peak early diastolic longitudinal strain rate (Te-LSr-SD) was also significantly higher in HfpEF compared to controls (0.067 ± 0.031 RR vs. 0.054 ± 0.020 RR, $p< 0.05$). There were no differences in Ts-LS-SD, Ts-Circ-SD and Ts-Rad-SD in patients with HfpEF vs. controls. (Table 3) There were no significant correlation between the parameter of diastolic dyssynchrony (Te-LSr-SD) and parameters of diastolic function such as E/E', IVRT and MV DecT.

Left ventricular segmental delay

Time to peak systolic velocity was delayed mostly in the anterior wall in patients with HfpEF compared to controls (Anterior mid: 0.208±0.094 RR vs. 0.153±0.080 RR, $p< 0.05$). (Table 4).

Subgroup analysis

There were no significant differences in LSDi, Ts-SD, Te-LSr-SD and time to peak systolic velocity in the anterior wall between patients with and without left ventricular hypertrophy. Similarly there were no significances differences in these parameters between patients with and without ischaemic heart disease.

Discussion

Whilst longitudinal dyssynchrony has been previously described in HfpEF using tissue Doppler imaging, to the best of our knowledge, this is the first study to use STI to investigate LV systolic and diastolic dyssynchrony, and to assess LV contractile efficiency in patients with HfpEF with narrow QRS complex. The principal findings are: a) patients with HfpEF exhibit a higher longitudinal strain delay index than control subjects i.e. increased myocardial energy wastage due to LV dyssynchrony b) HfpEF patients also exhibit both systolic and diastolic dyssynchrony compared to controls. c) the LV anterior wall displays the most delayed movement in patients with HfpEF.

The presence of systolic and diastolic dyssynchrony in patients with SHF has been well evaluated over the years. (192) In recent years, a number of studies using conventional echocardiography and TDI have reported subtle myocardial systolic dysfunction in HfpEF (157). TDI based studies measuring time-delay to assess synchronicity have shown that LV diastolic and/or systolic dyssynchrony was present in 60% of patients with HfpEF. (57) Interestingly, the prevalence of systolic dyssynchrony or both systolic and diastolic dyssynchrony were commoner in SHF than in HfpEF; however isolated diastolic dyssynchrony was more prevalent in HfpEF than in SHF. (57)

In this study using STI we have shown the presence of LV systolic dyssynchrony in HfpEF as evident by the higher LSDi and the higher Ts-SD (a marker of systolic dyssynchrony (57)). In addition, there was evidence of LV diastolic dyssynchrony as indicated by the higher Te-LSr (a marker of diastolic function and related to LV relaxation (τ) (193)) in HfpEF compared to controls. This is an important finding because systolic dyssynchrony in HfpEF is associated with reduced longitudinal function and lower stroke volume. (56) Indeed, in our study,

global longitudinal strain (derived by STI) and peak early diastolic velocities (derived by PW-TDI) were reduced in HfpEF patients compared to controls indicating poor long-axis function. Our findings furthermore support the view that HfpEF is not solely a disorder of diastolic function but also of systolic function (although LVEF remains 'normal) which is contrary to more traditional views that HfpEF is predominantly a disorder of diastole. (183)

The significantly increased longitudinal and the trend (although non-significant) to increased radial strain delay index and circumferential strain delay index indicate energy wastage due to LV dyssynchrony in HfpEF patients. This might lead to a reduction in cardiac energetic reserves which we had previously shown to be reduced in this group of patients.(3) However, there are other factors which may play a part in lowering myocardial energetics reserves such as insulin resistance (108) and impaired mitochondrial function as a result of ageing (113). Strain delay index can also be viewed as a marker of dyssynchrony in that if all the segments were synchronous and peaks at the same time, then strain delay index would be zero. This further reinforces the finding of systolic dyssynchrony in HfpEF. In the control group there was also evidence of systolic dyssynchrony which is reflected by the fact the LSDi was not zero. We believe that even in healthy controls there will be some degree of systolic dyssynchrony (or at least a distribution) especially in this age group with a mean age of 65 years as shown in previous studies (57).

In a study involving SHF patients, longitudinal strain delay index was shown to be a stronger predictor of CRT response than SD of time to peak myocardial velocity (TDI derived). (189) Furthermore, in the PROSPECT trial (2008), twelve echocardiographic parameters of dyssynchrony, based on both conventional and TDI, were shown to be poor predictors of

clinical or volume response to cardiac resynchronization therapy (CRT). (194) Recently, radial dyssynchrony (as measured by STI) in patients with SHF have been shown to reliably predict immediate and long-term response to CRT. (195) And more recently, longitudinal strain delay index derived from longitudinal strain (ε) patterns by STI was found to be also a potential predictor of responders to CRT in both ischaemic and non-ischaemic patients. (189) Speckle tracking appear to be superior to TDI in evaluating intra-ventricular mechanical dyssynchrony, potentially due to less angle dependency as well as better signal-to-noise of the strain signal than TDI.

From a clinical perspective, currently the management of HfpEF is primarily based on medical therapy. (196) What we have learnt from studies in SHF is that systolic dyssynchrony is a strong predictor of morbidity and mortality in these patients. (197) Indeed, CRT which aims to correct systolic dyssynchrony have been shown to improve symptoms (198) and prognosis (199) in patients with congestive heart failure. Systolic dyssynchrony is often found in patients with SHF associated with wide QRS complexes however in about 30-40% of patients with normal QRS complexes they also display evidence of systolic dyssynchrony. (192, 200) In a study on SHF, about a third of patients with QRS duration above 120 ms had the lateral wall most delayed which also means that that vast majority (two-thirds) of cases the most delayed segment is not the lateral wall. (192) This may explain why about 30% of patients with severe LVEF and wide QRS complex do not respond to CRT, as the LV lead is usually placed on the anterior-lateral wall. (199) Such patients may benefit more from multisite pacing. Interestingly, in patients with SHF and narrow QRS complex it is actually the anterior wall that displays the most delayed movement, occurring in about 25% patients. (192) In this study of patients with HfpEF with

narrow QRS complex we find that it is also the anterior wall that appears to be most delayed compared to controls.

Study limitations

Our study is limited by the relatively small sample size. Ideally we would have liked to recruit controls with similar co-morbidity as the patient group i.e. presence of coronary artery disease, hypertension etc in order to demonstrate the specificity of the findings of this study to patients with HfpEF. However, in practice we have found that even asymptomatic elderly subjects with hypertension usually have reduced peak VO_2 – hence a clear-cut distinction in this age group between hypertension with and without HFpEF is not straightforward. Furthermore, our controls are not so dissimilar to controls used in previous HfpEF studies (56, 57). The p-values quoted were unadjusted for multiple comparisons. A small proportion of patients had coronary artery disease which may have affected LV mechanics however coronary artery disease is common in HfpEF and thus is considered to be part of the syndrome (12). A proportion of patients with HfpEF were on medications which may have affected LV function however they would be expected to affect all strain parameters not selective ones. Furthermore, any variation in heart rate was corrected by the normalization time duration by the respective R-R interval.

Conclusions

Patients with HfpEF exhibits increased myocardial contractile inefficiency. They also exhibit LV systolic and diastolic dyssynchrony with the LV anterior wall displaying the most delayed motion.

Table 1: Baseline characteristics of the study population			
Variables	**Patient**	**Control**	**p value**
n	38	33	
Age, years	67±9	65±6	0.20
Female gender (%)	27 (71)	18 (55)	0.15
BMI, kg/m^2	30±4	26±4	0.003
Race, Caucasian n (%)	38 (100)	33 (100)	N/A
CAD, n (%)	4 (11)	0 (0)	N/A
Diabetes mellitus, n (%)	2 (5)	0 (0)	N/A
Hypertension, n (%)	27 (71)	0 (0)	N/A
LVH, n (%)	19 (50)	0 (0)	N/A
Medication			
Loop diuretics, n (%)	12 (32)	0 (0)	N/A
ACEi or ARB, n (%)	24 (63)	0 (0)	N/A
Beta – blockers, n (%)	7 (18)	0 (0)	N/A
Nitrates, n (%)	3 (8)	0 (0)	N/A
Calcium antagonist, n (%)	12 (32)	0 (0)	N/A
Antiplatelet agents, n (%)	13 (34)	0 (0)	N/A
Statins, n (%)	20 (59)	0 (0)	N/A
Resting heart rate, b.p.m	79±15	79±14	0.87
QRS duration, ms	90±16	94±14	0.25
Resting SBP, mmHg	137±21	134±21	0.49
Resting DBP, mmHg	82±11	81±11	0.67
VO_2max, ml/kg/min	21±5	34±7	<0.001
RER	1.07±0.09	1.09±0.09	0.31
VE/VCO_2	33±6	28±3	<0.001
Breathing reserve, L/min	36±15	40±15	0.29
Peak SBP, mmHg	181±24	191±24	0.12
Peak DBP, mmHg	82±13	88±11	0.07
Peak heart rate, b.p.m	136±18	163±9	<0.001

Data expressed as mean±SD. BMI - body mass index, CAD – coronary artery disease, DBP – diastolic blood pressure, RER – respiratory exchange

Table 2: Echocardiographic Measurements of Diastolic and Systolic function			
Variables	**Patient**	**Control**	**p value**
EF, %	64%±10%	63%±7%	0.62
IVRT, ms	140±29	151±26	0.16
MV E velocity, cm/s	0.71±0.17	0.64±0.12	<0.05
MV A velocity, cm/s	0.83±0.18	0.66±0.15	<0.001
E/A ratio	0.88±0.31	1.00±0.26	0.09
Dct, ms	256±62	246±66	0.52
TDI peak S' velocity, cm/s	0.08±0.02	0.09±0.03	0.06
TDI peak E' velocity, cm/s	0.07±0.02	0.09±0.02	<0.001
TDI peak A' velocity, cm/s	0.10±0.03	0.10±0.03	0.68
E/E'	11±4	7±2	<0.001
Global Longitudinal strain, %	-17.6±3.3	-19.9±3.5	0.006

Data expressed as mean±SD. Dct – Deceleration time of early mitral inflow EF – ejection fraction, IVRT – isovolumic relaxation time, MV A – peak Doppler late mitral inflow, MV E – peak Doppler of early mitral inflow and TDI – Tissue Doppler imaging of the lateral mitral annulus for systolic wave (S'), early diastolic wave (E') and late diastolic wave (A').

Table 3: Left ventricular strain delay index and synchronicity as measured by STI			
Variables	**Patient**	**Control**	**p Value**
Strain Delay Index			
Longitudinal (%)	-14.36± 8.24	-10.73± 5.62	<0.05
Circumferential (%)	-7.67±5.48	-5.45±4.26	0.12
Radial (%)	4.80±6.34	2.27±2.39	0.05
Longitudinal, Circumferential and Radial strain			
Time to peak longitudinal strain (Ts-LS), RR	0.403±0.116	0.400±0.040	0.86
Ts-LS-SD	0.075±0.044	0.084±0.049	0.42
Time to peak circumferential strain (Ts-Circ), RR	0.435±0.071	0.453±0.093	0.43
Ts-Circ-SD	0.064±0.026	0.053±0.027	0.14
Time to peak radial strain (Ts-Rad), RR	0.458±0.103	0.472±0.083	0.61
Ts-Rad-SD	0.042±0.037	0.047±0.049	0.70
Longitudinal velocity			
Time to peak systolic velocity (Ts), RR	0.169±0.041	0.159±0.034	0.31
Ts-SD (RR)	0.074±0.026	0.060±0.023	<0.05
Time to peak early diastolic velocity (Te), RR	0.589±0.055	0.575±0.067	0.37
Te-SD (RR)	0.067±0.035	0.056±0.021	0.13
Time to peak atrial diastolic velocity (Ta), RR	0.924±0.038	0.950±0.047	<0.05
Ta-SD (RR)	0.051±0.055	0.042±0.022	0.44
Longitudinal strain rates			
Time to peak systolic longitudinal strain rate (Ts-LSr), RR	0.182±0.040	0.176±0.034	0.56
Ts-LSr-SD (RR)	0.071±0.021	0.063±0.025	0.16
Time to peak early diastolic longitudinal strain rate (Te-LSr), RR	0.578±0.055	0.560±0.061	0.22
Te-LSr-SD (RR)	0.067±0.031	0.054±0.020	<0.05
Time to peak atrial diastolic longitudinal strain rate (Ta-LSr), RR	0.912±0.042	0.935±0.052	<0.05
Ta-LSr-SD (RR)	0.050±0.055	0.043±0.024	0.55

Data expressed as mean ± standard deviation (SD). Ts-LSr-SD - standard deviation of Ts-LSr, Te-LSr-SD - standard deviation of Te-LSr, Ta-LSr-SD - standard deviation of Ta-LSr, Ts-SD =

standard deviation of Ts, Te-SD - standard deviation of Te, Ta-SD - standard deviation of Ta, Ts-LS-SD - standard deviation of Ts-LS, Ts-Circ-SD - standard deviation of Ts-Circ and Ts-Rad-SD - standard deviation of Ts-Rad.

Table 4: Time to peak systolic velocity by segments in patients with HfpEF compared to controls

Segments	Patient	Control	P value
Anterior, apical (RR)	0.221±0.106	0.170±0.102	0.05
Anterior, mid (RR)	0.208±0.094	0.153±0.080	<0.05
Anterior, basal (RR)	0.194±0.073	0.166±0.068	0.11
Inferior, apical (RR)	0.126±0.056	0.164±0.064	<0.05
Inferior, mid (RR)	0.134±0.043	0.141±0.047	0.53
Inferior, basal (RR)	0.149±0.051	0.157±0.054	0.53
Lateral, apical (RR)	0.217±0.104	0.177±0.092	0.11
Lateral, mid (RR)	0.213±0.096	0.169±0.085	0.06
Lateral, basal (RR)	0.209±0.104	0.189±0.080	0.38
Septum, apical (RR)	0.100±0.034	0.139±0.066	<0.05
Septum, mid (RR)	0.127±0.040	0.137±0.027	0.26
Septum, basal (RR)	0.128±0.039	0.139±0.028	0.19

Data expressed as mean±SD. RR- R-R interval, Ts = time to peak systolic velocity, Te -LSr = time to peak early diastolic longitudinal strain rate

Figure 1

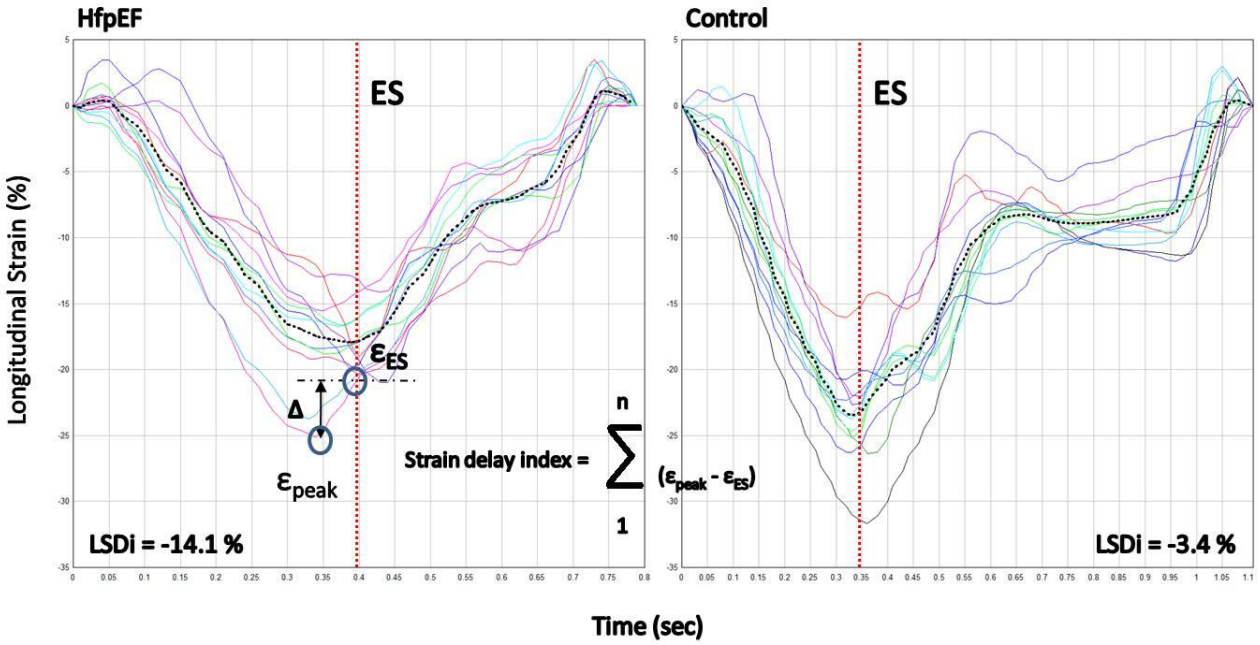


Figure 1: Longitudinal Strain delay index (LSDi) in a HfpEF patient and a healthy control: Demonstrating the sum of wasted energy (ε_{peak} - ε_{ES}) across 12 myocardial segments (colored curves) of the left ventricle. The black dotted line represents the average of all 12 curves. There is more dispersion of each strain curve peaks in the HfpEF patient compared to the control, this leads to increased LSDi in HfpEF compared to control. In addition, the average longitudinal strain is lower in the HfpEF patient compared to the control. ES – end systole, ε_{peak} – peak strain, ε_{ES} – strain at end systole.

Chapter V

Impaired Heart Rate Recovery and Chronotropic Incompetence in Patients with Heart Failure with Preserved Ejection Fraction

Chapter V

Impaired Heart Rate Recovery and Chronotropic Incompetence in Patients with Heart Failure with Preserved Ejection Fraction

Introduction

Approximately 50% of patients with the clinical features of heart failure are found to have normal left ventricular ejection fraction and normal valvular function. The term heart failure with preserved LV ejection fraction (HfpEF) is applied to these patients. (77) They are typically elderly women who frequently have associated hypertension, diabetes, and/or coronary artery disease. (14) They have similar hospital length of stay, admission rates (11, 12) and mortality rate to that of patients with systolic heart failure (12). The prevalence of HfpEF appears to be increasing and in contrast to systolic heart failure the mortality rate of this disorder is not declining. (11)

The pathophysiology of HfpEF has been a matter of considerable controversy. Impaired left ventricular relaxation, increased passive left ventricular stiffness and contractile dysfunction (despite the presence of a normal left ventricular ejection fraction) each appear to contribute to exercise limitation. However one recent study in HfpEF reported an associated between an impaired heart rate (HR) response to exercise and exercise limitation. (77) However there are a number of important caveats. Firstly, the patients were mainly African American hypertensives and the relevance to HfpEF in a Caucasian population is unknown. Secondly, the patient numbers were relatively small. Thirdly, many

of the patients (and the hypertensive controls without breathlessness) were taking beta blockers. Although these were discontinued 24 hours prior to the study the potential of either ongoing beta blockade (chronotropic incompetence (201)) or of rebound effects (202) to have influenced the findings cannot be excluded. Thus, in this study we aimed to assess HR response to exercise and during recovery in a larger group of patients with HfpEF who were not taking beta-blockers using maximal symptom limited erect treadmill metabolic exercise testing.

Methods

Study Participants

HfpEF patients

We studied 41 HfpEF patients prospectively and consecutively recruited from heart failure clinics. All study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram and metabolic exercise test. All patients met the criteria of Yturralde and Gaasch for the diagnosis of diastolic heart failure. (19) They had (i) signs and/or symptoms of heart failure, (ii) objective evidence of exercise limitation on cardiopulmonary exercise testing (peak VO2 <80% of predicted) with a pattern of gas exchange indicating a cardiac cause for limitation exercise capacity, (iii) normal LVEF and chamber size, and (iv) LV hypertrophy and/or evidence of diastolic dysfunction on Doppler echocardiography. Patients with severe pulmonary disease, significant valvular heart disease, atrial fibrillation, or evidence of hypertrophic cardiomyopathy were excluded similar to previous studies (77). HfpEF patients on beta-blockers or non-dihydropyridines calcium blockers (e.g. verapamil and diltiazem) were also excluded in order accurately assess chronotropic response and HR recovery. The investigations were performed at The

University of Birmingham with approval of the Research Ethics Committee. Informed consent was obtained from all subjects.

Healthy controls

We studied 41 healthy controls with no cardiac history, hypertension or diabetes mellitus. In addition 16 newly diagnosed hypertensive controls from the community were studied to explore the possibility of hypertension *per se* as a cause of cardiac autonomic dysfunction. The vast majority of these hypertensive controls were studied prior to the commencement of any antihypertensive therapy. None was taking heart rate lowering medication. All control subjects had a normal clinical cardiovascular examination, 12-lead electrocardiogram, echocardiogram and metabolic exercise test. Healthy controls were volunteers recruited prospectively from the community.

Metabolic Exercise Testing

The metabolic exercise testing was performed on a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using incremental ramp protocol (speed and inclination was increased every minute) as described previously by our group (124) with simultaneous respiratory gas analysis (125, 126). Samplings of expired gases were performed continuously, and data were expressed as 30-second means. The minute ventilation − carbon dioxide production relationship (VE/VCO_2 slope), maximal oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) was used to verify objective effort adequacy. Peak oxygen consumption (peak VO_2) was defined as the average values of VO_2 measured during the last 30 seconds. Blood pressure and ECG

were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Chronotropic incompetence is defined as an inadequate HR response to exercise. Two methods were used to assess chronotropic response. The first, was percentage of the HR reserve used during maximal exercise (%HHR) which was determined as the change in HR from rest to peak exercise as a percentage of HR reserve (the difference between the predicted maximal heart rate and the resting heart rate). A failure to use 80 percent of the HR reserve was considered to be evidence of chronotropic incompetence, (203) which is an independent predictor of mortality. (204) The second method was to calculate the peak exercise HR as a percentage of predicted maximal HR (%Max-PPHR). In this case, chronotropic incompetence was defined as a peak exercise HR less than 80% of the maximum age predicted peak HR. (205) HR recovery post exercise was defined as the reduction in the HR from the HR at peak exercise to the HR at one minute after the cessation of exercise. Abnormal HR recovery was defined as a reduction of ≤12 beats per minute in the first minute of exercise. (206) Predicted maximal HR for an individual was calculated using the more recently determined formula of Tanaka et al (208 - 0.7 x age in years). (207)

Resting Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine and a 2.5-MHz transducer. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber views. All echocardiographic measurements were averaged from 3 heart beats. LV ejection fraction was calculated from

LV volumes (LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV)) by the modified biplane Simpson rule in accordance with the guidelines. (127) From the LV-inflow pattern (measured at the tips of the mitral valve), peak early (E) and late (A) filling velocities, E/A ratio, and E-velocity deceleration time (DcT) were measured.

Statistics

Continuous variables are expressed as mean ± SD. Variances of data sets were determined using Levene's test. Comparisons were performed with one-way ANOVA if the data were normally distributed. Categorical variables were compared using the Chi-Square test. Pearson correlation coefficient (r) was used to describe the relationship between variables. A two-tailed p value of <0.05 was considered statistically significant. SPSS (v15.0) was used to perform the statistical analyses.

Results

Characteristics of Patients

HfpEF Patients were generally females (70%), overweight, aged 69±8 years old with a history of hypertension. Healthy controls were of similar gender (63% females) and age (67±6 years old). (Table 1) Patients with HfpEF had significantly reduced peak VO_2 compared to healthy controls (20±4 ml/Kg/min vs. 31±6 ml/Kg/min, $p<0.001$). The minute ventilation--carbon dioxide production relationship (VE/VCO_2 slope) was also higher in patients with HfpEF than healthy controls (33±6 vs. 29 ± 4, $p<0.001$). (Table 2)

Chronotropic response to maximal exercise testing

HfpEF patients vs. matched healthy controls had similar resting heart rate and predicted maximal HR, 78±14 vs. 79±13 (p=0.99) and 160±6 vs. 161±4 (p=0.53), respectively. HfpEF patients had lower peak HR response and lower change in HR (the difference between peak HR and resting HR) during peak exercise compared to matched healthy controls, 139±22 vs. 171±18, p<0.001 and 60±22 vs. 93±21, p<0.001, respectively. (Figure 1). Chronotropic incompetence was significantly more common in patients with HfpEF compared to matched healthy controls as measured by %HHR and %Max-PPHR. In addition, abnormal HR recovery 1-minute post exercise was also significantly more common in patients with HfpEF compared to matched healthy controls. (See table 3 and figure 2). Peak oxygen consumption (peak VO_2) correlated directly with peak exercise heart rate (r=0.57, p<0.001). (Figure 3) Chronotropic incompetence and impaired heart recovery remained highly significant in patients with HfpEF when compared with hypertensive controls. There were no significant differences between hypertensive controls and healthy controls with respect to chronotropic response during peak exercise and heart rate recovery following exercise.

Discussion

The principal findings of this study are: a) HfpEF patients had similar resting and predicted maximal HR compared to matched-healthy controls, but during peak dynamic exercise, HfpEF patients displayed significant chronotropic incompetence. b) Abnormal HR recovery 1-minute post exercise was more common in HfpEF patients compared to matched healthy controls. c) Hypertensive controls showed similar chronotropic response to peak exercise and heart rate recovery following exercise as healthy controls.

Chronic heart failure is characterised by impaired exercise tolerance often due to breathlessness and fatigue. Metabolic exercise testing is an objective tool to measure exercise limitation in patients with CHF as indicated by reduced maximal oxygen consumption (peak VO_2) and an increase in the ventilatory response to exercise (the relation of ventilation (VE) to carbon dioxide production (VCO_2) or VE/VCO_2 slope). (208) In this study we found that patients with HfpEF have reduced peak VO_2 and higher VE/VCO_2 slope compared to older controls, which are supported by previous reports. (209) Indeed, VE/VCO_2 slope has been shown to have prognostic value in patients with diastolic heart failure with respect to both mortality and hospitalization. (179)

In healthy subjects, the initial increase in HR during exercise results from a withdrawal of the physiological vagal tone present at rest and sympathetic tone is responsible for further increases in HR as exercise continues. (210) Post exercise sympathetic withdrawal contributes to early HR recovery and at a later stage parasympathetic reactivation plays a larger role in HR recovery. (211)

In chronic heart failure (CHF) associated with LV systolic dysfunction there is impaired autonomic function (212) as a result of an impaired vagal tone (164) as well as an over-activity of sympathetic function which results in reduced responsiveness to beta adrenergic stimulation due to both reduced adrenoreceptor number and reduced downstream signalling (213). The clinical sequalae of these autonomic changes include an impaired HR response to exercise (chronotropic incompetence) which may contribute to exercise limitation. (214, 215) Indeed studies have shown that as the severity of systolic heart failure worsens the more common chronotropic incompetence during exercise becomes in patients

with CHF. (216) Nevertheless, even in asymptomatic patients with reduced LVEF and LV dilatation poor HR response has been reported. (217) An impaired heart rate recovery following exercise is also common in patients with systolic heart failure (218) and appears to be primarily due to low vagal tone (164). Impaired HR recovery following exercise also appears to be a powerful predictor of mortality not only in patients with systolic heart failure but also predicts cardiovascular mortality in apparently healthy subjects. (206)

In this study, we found HfpEF patients had lower maximal HR response during maximal exercise than controls, which is important because maximal HR response is associated with coronary disease and cardiovascular mortality. Furthermore, we found that 34% of patients with HfpEF have chronotropic incompetence during maximal exercise when defined by, %Max-PPHR and 63% when defined by %HHR. These proportions are quite similar to findings in patients with CHF due to systolic dysfunction. (219) HR recovery was also found to be impaired in a significant proportion of patients with HfpEF, which suggest the presence of parasympathetic imbalance.

Borlaug BA *et al* (77) showed in hypertensives (mainly African Americans) that chronotropic incompetence was a powerful predictor of the presence of symptoms of heart failure. However, this relationship between chronotropic incompetence and HfpEF may or may not be causal. Chronotropic incompetence in HfpEF may be an adaptation to improve diastolic filling, since increasing HR by atrial pacing has been shown to reduce supine resting stroke volume and cardiac output in patients with HfpEF. (2) It will be important to undertake further studies to assess whether HR plays a causal role in exercise limitation in HfpEF, because if so, this may be amenable to rate responsive pacing.

The precise mechanism of impaired autonomic function in HfpEF is unclear, some have proposed a peripheral factor responsible rather than central. (77) Studies in CHF have revealed a blunted baroreflex control could play an important role (220) secondary to reduced arterial compliance (221), impaired central reflex integration, and a decrease in end-organ responsiveness (220). Increased sensitivity of muscle ergoreceptors and peripheral chemoreceptors has also been linked to autonomic impairment in CHF. (214, 222) A review on this topic has been discussed by our group elsewhere. (212)

Conclusions

Patients with HfpEF have chronotropic incompetence during maximal exercise and abnormal heart rate recovery post exercise.

Table 1: Baseline characteristics of the study population				
	Healthy Controls	**Hypertensive Controls**	**HfpEF Patients**	**P Value**
	n=41	**n=16**	**n=41**	
Females no. (%)	26 (63)	7 (43)	29 (70)	0.17
Age (years)	67±6	68±6	69±8	0.02
BMI no. (%)	26±4	27±3	31±4*†	0.21
Obesity no. (%)	21 (51)	13 (81)	35 (85%)	0.003
Hypertension no. (%)	0	16 (100)	27(68)	N/A
Ischaemic heart disease no. (%)	0	0	1(2)	N/A
Diabetes no. (%)	0	0	3(7)	N/A
NHYA functional class no. (%)				
II			34(83)	N/A
III			7(17)	N/A
Medications				
Diuretic	0	2 (13)	9(22)	N/A
ACE inhibitor	0	1 (6)	18(44)	N/A
ARB	0	0	7(17)	N/A
Calcium blocker	0	1 (6)	14(34)	N/A
Alpha Blocker	0	0	4(10)	N/A
Spironolactone	0	0	1(2)	N/A
Nitrate	0	0	3(7)	N/A

Plus-minus values are means ± SD. NYHA denotes New York Heart Association, ACE angiotensin-converting enzyme, ARB angiotensin II receptor blockers. BMI body mass index. Obesity defined as BMI>25.

*P<0.05 vs. Hypertensive controls

†P<0.05 vs. Healthy controls

Table 2: Metabolic exercise test and Echocardiographic parameters				
	Healthy Controls	**Hypertensive Controls**	**HfpEF Patient**	**p value**
	n=44	**n=16**	**n=41**	
Metabolic exercise test				
VO_2 max (ml/Kg/min)	31 ± 6	29 ± 5	20 ± 4*†	<0.001
Respiratory Exchange Ratio (RER)	1.11 ± 0.10	1.12 ± 0.09	1.07 ± 0.09	0.04
% Predicted VO_2 max	93 ± 21	84 ± 13	60 ± 10*†	<0.001
VE/VCO_2	29 ± 4	30 ± 3	33 ± 6†	<0.001
Breathing Reserve (L/min)	38 ± 14	34 ± 17	35 ± 14	0.58
Heart rate (beats/min)				
Rest	79 ± 13	87 ± 13	78 ± 14	0.07
Peak	171 ± 18	163 ± 11	139 ± 22*†	<0.001
Systolic blood pressure (mmHg)				
Rest	135 ± 20	153 ± 16†	139 ± 21	<0.01
Peak	190 ± 20	193 ± 21	183 ± 26	0.29
Diastolic blood pressure (mmHg)				
Rest	82 ± 9	87 ± 9	82 ± 11	0.16
Peak	88 ± 10	93 ± 9	82 ± 11*†	<0.01
Echocardiography				
Left ventricular ejection fraction - %	64 ± 5	63 ± 7	64 ± 10	0.88
Mitral E-wave velocity - m/sec	0.62 ± 0.14	0.68 ± 0.16	0.66 ± 0.14	0.30
Mitral A-wave velocity - m/sec	0.71 ± 0.15	0.80 ± 0.17	0.85 ± 0.19†	<0.001
Ratio of E-wave: A-wave velocity	0.93 ± 0.25	0.88 ± 0.23	0.80 ± 0.18†	0.03
Mitral E-wave deceleration - msec	237 ± 62	250 ± 44	265 ± 64	0.13

Plus-minus values are means ± SD. The minute ventilation − carbon dioxide production relationship (VE/VCO_2 slope)

*P<0.05 vs. Hypertensive controls

†P<0.05 vs. Healthy controls

Table 3: Chronotropic incompetence in patients with HfpEF compared to matched controls

	Healthy Controls (N=41)	Hypertensive Controls (N=16)	HfpEF Patients (N=41)	p Value
Chronotropic incompetence using %Max-PPHR method, No. (%)	1 (2)	0 (0)	14 (34)	**<0.001**
Chronotropic incompetence using %HHR method, No. (%)	1 (2)	0 (0)	26 (63)	**<0.001**
Abnormal heart rate recovery, No. (%)	1 (2)	1 (2)	9 (23)	**0.01**

Figure 1

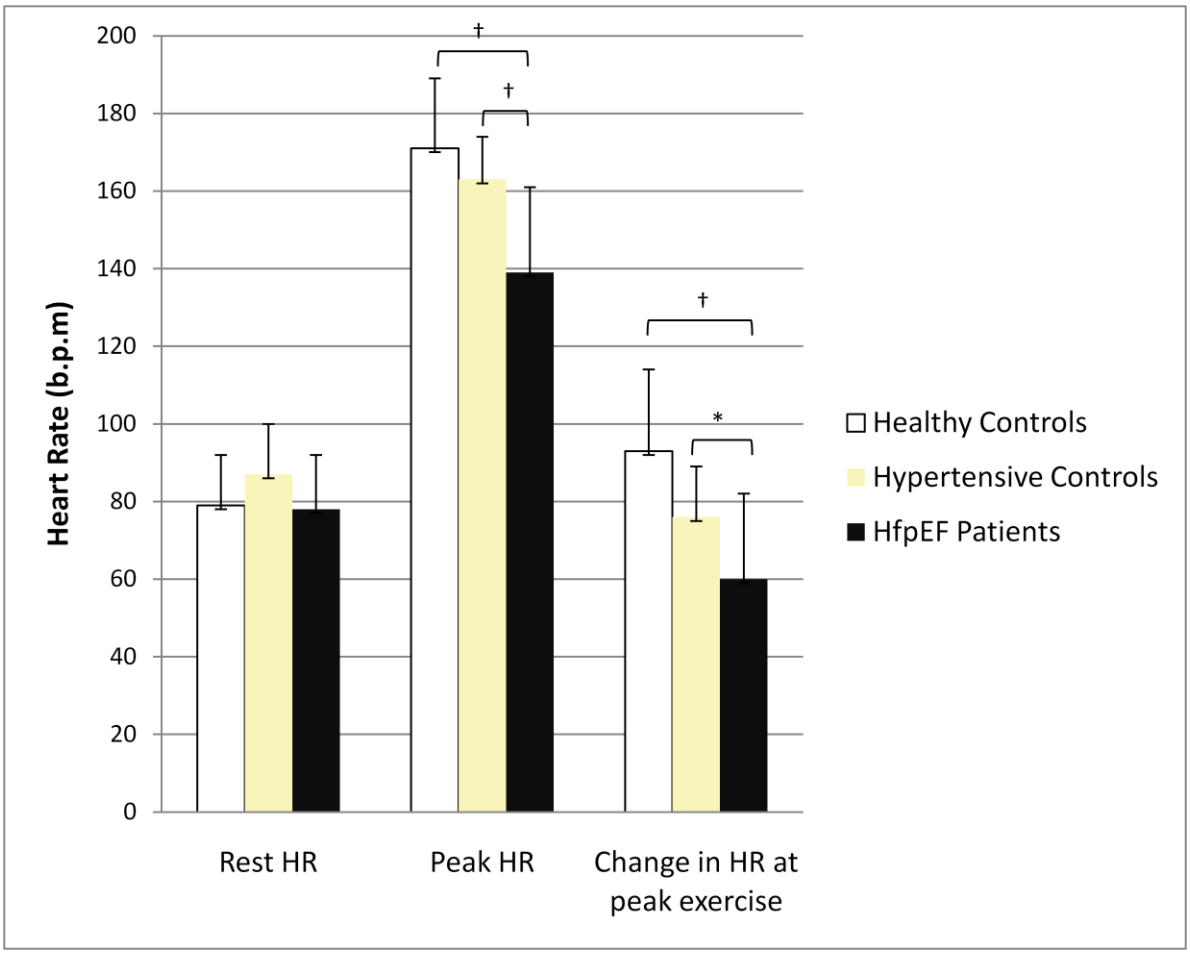


Figure 1: Differences in heart rate response during exercise and post exercise in patients with HfpEF, hypertensive controls and healthy controls. (Only significant differences are shown)
*p<0.05
†p<0.001

Figure 2

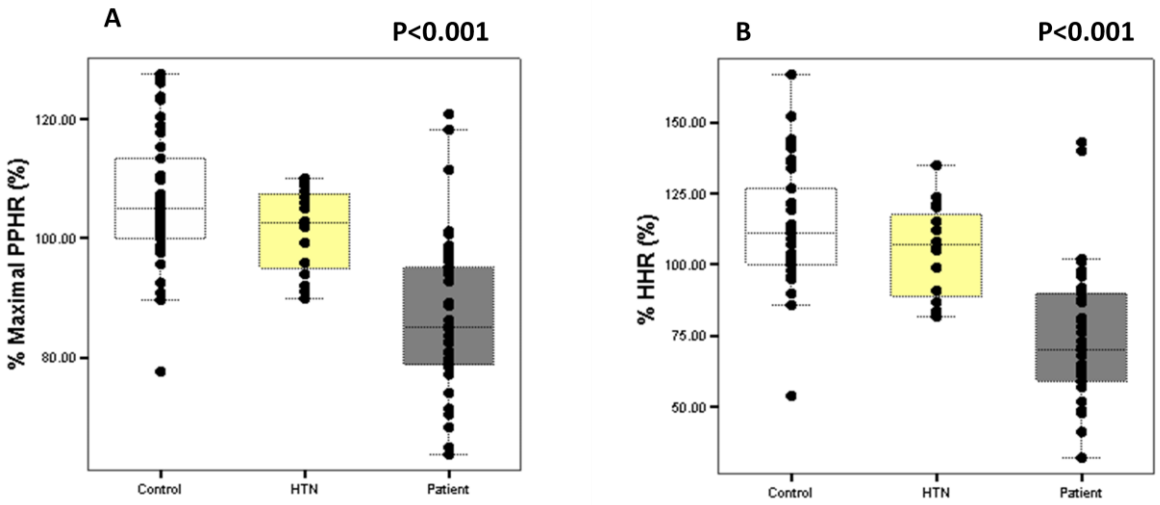


Figure 2: Abnormal chronotropic response in patients with HfpEF compared to hypertensive controls and healthy controls during exercise. Panel A: chronotropic incompetence in HfpEF patients as measured by % of heart rate reserved used at peak exercise (%HHR). Panel B: chronotropic incompetence in HfpEF patients as measured by peak exercise HR as a percentage of predicted maximal HR (%Max-PPHR).

Figure 3

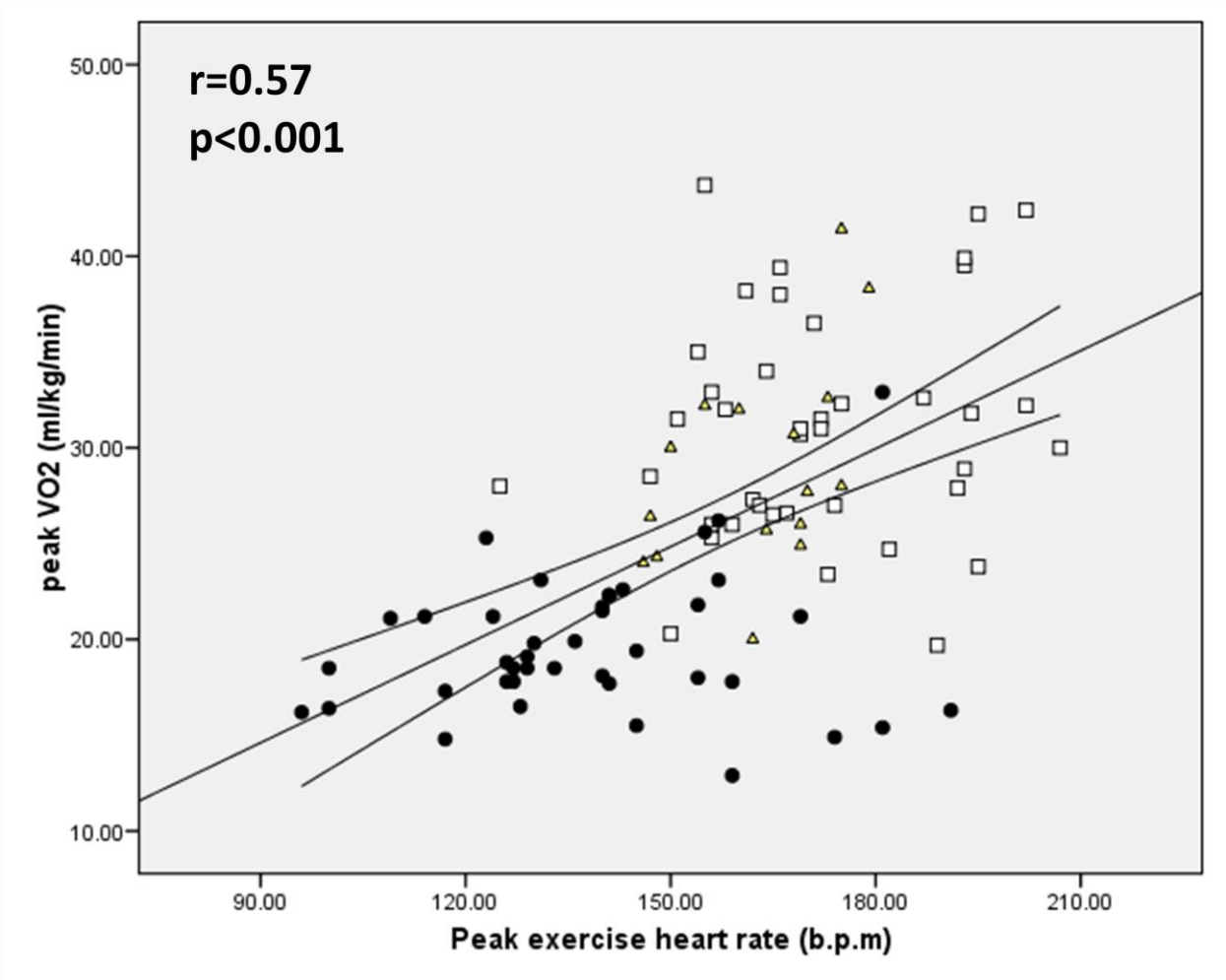


Figure 3: Peak oxygen consumption correlating directly with peak exercise heart rate. Black dots – HfpEF patients, triangles – hypertensive controls and squares – healthy controls.

Chapter VI

Left Ventricular Torsion and Strain patterns in Heart Failure with Normal Ejection Fraction are similar to Age-related changes

Chapter VI

Left Ventricular Torsion and Strain patterns in Heart Failure with Normal Ejection Fraction are similar to Age-related changes

Introduction

Heart failure with normal ejection fraction (HfnEF) comprises approximately half of patients with clinical features of chronic heart failure (11) with hospital admission/readmission rates and length of stay similar to that of patients with systolic heart 'failure'. (12) These patients are often overweight or obese elderly women who frequently have associated hypertension, diabetes, and/or coronary artery disease. (12) The prevalence of HfnEF appears to be increasing and mortality rate is only a little less than that of systolic heart failure. (11) Many patients with HfnEF have diastolic dysfunction, a feature that is also commonly found in normal aging. (223) No study has attempted to directly compare the physiological changes in LV torsion and untwist associated with aging and those found in patients with HfnEF. This is important because of the potentially significant overlap in the two processes especially when it comes to diagnosis and understanding the pathophysiology of HfnEF. In this study, we aim to investigate what features of LV biomechanics e.g. LV torsion and untwist as well as LV strains, are related to HfnEF and which are age-related changes. We used 2D ultrasound speckle-tracking echocardiography (STE) to noninvasively evaluate LV torsion and untwist in young and older healthy volunteers as well as in patients with HfnEF. STE estimation of LV torsion has been shown to be concordant with those analyzed by tagged

magnetic resonance imaging (MRI). (224) Metabolic exercise testing was used to objectively measure exercise capacity in patients with HfnEF and healthy controls.

Method

Study Participants

HfnEF patients

We studied 40 HfnEF patients prospectively and consecutively recruited from heart failure clinics. All study participants had clinical examination, 12-lead electrocardiogram, pulmonary function test, echocardiogram and metabolic exercise test. All patients had signs and/or symptoms of heart failure with a LV ejection fraction >50% by transthoracic echocardiography and met the criteria of Yturralde and Gaasch (19) for diastolic heart failure. Patients with severe pulmonary disease, significant valvular heart disease, atrial fibrillation, or evidence of hypertrophic cardiomyopathy were excluded similar to previous studies (77). Pulmonary function test was performed to identify patients with severe pulmonary disease. The investigations were performed at The University of Birmingham with approval of the Research Ethics Committee. Informed consent was obtained from all subjects.

Healthy controls

We studied 53 healthy controls with no cardiac history, no hypertension or diabetes mellitus. 27 healthy controls were under the age of 50 yrs and they were classified as young controls, the remainder were classified as older controls (n= 26). All healthy controls had a normal clinical cardiovascular examination, 12-lead electrocardiogram and metabolic exercise test. They were healthy volunteers from the community.

Metabolic Exercise Testing

The metabolic exercise testing was performed on a Schiller CS-200 Ergo-Spiro exercise machine which was calibrated before every study. Subjects underwent spirometry and this was followed by symptom-limited erect treadmill exercise testing using incremental ramp protocol (speed and inclination was increased every minute) with simultaneous respiratory gas analysis (125, 126). Samplings of expired gases were performed continuously, and data were expressed as 30-second means. Minute ventilation (VE/VCO_2 slope), oxygen consumption, carbon dioxide production, and respiratory exchange ratio (RER) were obtained. Peak oxygen consumption ($V0_2max$) was defined as highest value of oxygen consumption measured during the exercise period. Blood pressure and ECG were monitored throughout. Subjects were encouraged to exercise to exhaustion with a minimal requirement of RER > 1.

Resting Echocardiography

Echocardiography was performed with participants in the left lateral decubitus position with a Vivid 7 echocardiographic machine and a 2.5-MHz transducer. Resting scans were acquired in standard apical 4-chamber and apical 2-chamber views. All echocardiographic measurements were averaged from 3 heart beats. LV ejection fraction was calculated from LV volumes (LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV)) by the modified biplane Simpson rule in accordance with the guidelines. (127) LVEDV and LVESV were indexed to body surface area (BSA). From the LV-inflow pattern (measured at the tips of the mitral valve), peak early (E) and late (A) filling velocities, E/A ratio, and E-velocity deceleration time (DcT) were measured. The isovolumic relaxation time (IVRT) was determined using pulsed-wave Doppler velocity data of the LV inflow. Tissue Doppler was

applied end-expiratory in the pulsed-wave Doppler mode at the level of the lateral mitral annulus from an apical 4-chamber view. The velocities of early diastolic wave (E') was noted. Lateral mitral annulus velocities were recorded to derive E/E'. Parasternal circular short-axis images were taken at three distinct levels: LV basal level with the cross section as circular as possible (identified by the mitral valve), papillary and apical (no papillary muscles present) similar to previous studies. (190) Area-length method was used to determine LV mass indexed to BSA. (127) Left ventricular hypertrophy was defined as a left ventricular mass indexed to BSA that exceeded 88g/m^2 for women and 102g/m^2 for men.(127)

Speckle Tracking Echocardiography (STE)

STE was measured using a commercially available speckle tracking system in an ECHOPAC (ver. 4.2.0, GE, USA) workstation. Myocardial deformation measurements were performed using tissue speckle tracking. In this speckle tracking system, the displacement of speckles of myocardium in each spot were analyzed and tracked from frame to frame. We selected the best-quality digital two-dimensional image cardiac cycle and the left ventricle endocardium was traced at end-systole.(129). The region of interest width was adjusted as required to fit the wall thickness. The software package then automatically tracked the motion through the rest of the cardiac cycle. The onset of QRS complex was taken as the beginning of systole. To adjust for intra- and inter-subject differences in heart rate, all time intervals were normalised to R-R interval by expressing the time interval as a percentage of the R-R interval (% RR). Adequate tracking was verified in real time. Regarding adequate tracking quality, the system (ECHOPAC (ver. 4.2.0, GE, USA)) automatically generates an acceptable or unacceptable tracking quality. We systematically accepted only segments that received an acceptable tracking quality for analysis with visual control of tracking quality to

ensure adequate automatic tracking. This was done by verifying adequate tracking quality of endocardial and epicardial borders by the system. To optimize speckle tracking, two-dimensional gray-scale harmonic images were obtained at a frame rate of 70 – 100 frames/s. For each subject, longitudinal strain values for all LV myocardial segments in each of the apical 4 and 2 chamber views were measured and averaged to derive the global LV longitudinal strain, strain rates and velocity. Circumferential strain values were obtained in all 18 segments of the three short-axis views. The average of peak systolic circumferential strain values from the three short-axis views was calculated to derive the global LV circumferential strain and strain rates. Similarly, peak radial strain values were measured in all 18 segments at the three short-axis views and averaged to derive the global radial strain and strain rates.

In addition, cardiac rotation was computed using speckle tracking. Counter-clockwise rotation was marked as a positive value and clockwise rotation as a negative value when viewed from the apex. In order to calculate LV torsion, LV untwist and untwist rates, the rotation traces of the basal and apical LV cross-sections were exported into DPlot graph software (Version 2.2.1.4, HydeSoft Computing, LLC, Vicksburg, USA). The LV twist curve was generated by calculating the difference between apical and basal rotations at each corresponding time point. LV twist rates were derived from the first derivative of the LV twist curve. Peak LV torsion was derived from LV twist divided by LV diastolic longitudinal length as previously described. (130) Rotational deformation delay was also determined and defined as the magnitude of the time difference between time to peak basal rotation and time to peak apical rotation. (130) Peak untwist rate at the E wave was used to determine peak untwist rate as described in previous studies. (190) Of the 93 subjects in the study, 63

(68%) subjects had both adequate LV basal and apical images for speckle tracking to complete analysis of all LV rotational parameters, which is comparable to previous studies. (130)

Reproducibility of STE

Inter-observer measurement variability was determined by two independent observers who measured LV torsion in 10 randomly selected controls. To obtain the intra-observer variability, the first observer performed the analysis at two separate occasions at 1 month apart. We performed Bland-Altman plots to assess reproducibility of measurement. Our results showed that for LV torsion, intra-observer reproducibility was 0.24 ± 0.58 (bias ± 1.96 standard deviation of the difference (STD)) with a mean of 3.06°/cm and 2.82°/cm. Inter-observer reproducibility was 0.15 ± 0.69 (bias ± 1.96 STD) with a mean of 2.82°/cm and 2.67°/cm.

Statistics

Continuous variables are expressed as means ± SD. Comparisons were performed with one-way ANOVA if the data were normally distributed. Categorical variables were compared with Pearson Chi-Square test. A P value of <0.05 was considered to indicate statistical significance. Variances of data sets were determined using F-test. Pearson correlation coefficient (r) was used to describe the relationship between variables. All subjects were included into the model. Bland Altman plot was used to assess data reproducibility using MedCalc (v9.2.1.0). Variables of interest that were found to correlate with the dependent variable on univariate analysis were included in a stepwise linear regression analysis to identify independent variables. SPSS (v15.0) was used to perform most of the statistical

operations.

Results

Patient characteristics

Patients with HfnEF were generally females, overweight, mean aged of 67 ± 10 with a high prevalence of hypertension, which has been similarly described in previous large epidemiological studies (12). HfnEF patients also had significantly reduced VO_2max and reduced peak HR on metabolic exercise testing compared to older controls (see table 1). The E/E' at the lateral mitral annulus was significantly higher in patients than in older controls (see table 2).

Longitudinal, radial and circumferential strains

Aging

Global longitudinal, radial and circumferential strains were preserved with advancing age. However, longitudinal strain rate E was lower and longitudinal strain rate A was higher with aging. Longitudinal velocity (peak S and E) was also significantly reduced with aging (see table 3).

HfnEF

Compared to older controls, global longitudinal and radial strains were preserved in patients with HfnEF. Longitudinal strain rates and velocity were also comparable to older controls. However, global circumferential strain was significantly increased in patients with HfnEF compared to older controls (-24.7 ± 4.7 and -20.0 ± 4.9, respectively, P= 0.003). Global circumferential strain rate peak S and E were significantly higher in HfnEF patients compared to older controls.

LV torsion and Untwist

Aging

LV torsion is significantly increased with aging (table 4). This is in part due to the time to peak apical rotation occurring later during systole with aging with the resulting trend of peak rotational deformation delay decreasing with advancing age (p= 0.07), as well as a trend for increased LV basal rotation with aging (p=0.07). Peak LV untwist rate was preserved with aging and thus the times to 15%, 25%, 50% and 75% untwist were not significantly delayed with aging.

HfnEF

Compared to older controls, LV torsion and peak untwist rate were preserved in patients with HfnEF. Peak rotational deformation delay was similar in HfnEF and older controls. Furthermore, the times to 15%, 25%, 50% and 75% untwist were not delayed in HfnEF patients when compared to older controls. (See figure 1).

Associations with LV torsion

On univariate analysis, LV torsion was significantly correlated with age, BMI, Dct, E/A ratio, E/E', LVEDV Index, LVESV Index, LV mass index, VO_2max, global radial strain, global circumferential strain and rotational deformation delay. In the multivariate analysis, a linear-regression model was used to examine LV torsion as the dependent variable and found that rotational deformation delay, global circumferential strain, Dct and LVEDV Index were independent predictors of LV torsion (r^2= 0.65, P= 0.010, P=0.004, P=0.004 and P=0.045, respectively).

Associations with LV Untwist rate

On univariate analysis, LV untwist rate was significantly correlated with age, BMI, Dct, E/A ratio, E/E', LVEDV Index, LVESV Index, LV mass index, VO_2max, global radial strain, global circumferential strain, rotational deformation delay, peak LV torsion. In the multivariate analysis, a linear-regression model was used to examine LV untwist as the dependent variable and found that LV torsion and BMI were independent predictors of LV untwist rate (r^2= 0.65, P<0.001 and P=0.013, respectively).

Discussion

The principal findings of the present study are: a) LV torsion increases with advancing age due to in part reduced rotational deformation delay and increased LV basal rotation. However, LV torsion is unchanged in HfnEF compared to older controls. b) LV untwist, peak untwist rate, LV longitudinal and radial strain are preserved with age and in patients with HfnEF. c) Circumferential strain and strain rate are enhanced in patients with HfnEF. d) LV torsion is independently predicted by rotational deformation delay, circumferential strain and MV deceleration time. e) LV untwisting rate is independently determined by peak LV torsion and by BMI.

LV torsion is the net result of counter-clockwise rotation of the base with respect to clockwise rotation of the apex along the LV long axis. Normal LV torsion is a component of systolic function (225) and contributes to an energy-efficient ejection (226). The subsequent LV untwisting is a key determinant of diastolic function (227, 228) because it helps to generate the intra-ventricular pressure gradient (IVPG) during isovolumic relaxation (229) thus creating a suction effect to allow early diastolic filling to occur once the mitral valve opens. (230) In this study, we found LV torsion increased with aging consistent with

previous STE studies (130, 231) and tagged MRI studies (232). The reason for this increased LV torsion is unclear but in patients with aortic stenosis (233) and hypertrophic cardiomyopathy (234) where LV torsion is also enhanced, the explanation appear to be related to under-perfusion of the sub-endocardium leading to reduce sub-endocardial myofibres function which normally counteracts the LV twisting generated by the sub-epicardial myofibres. (235) In a tagged MRI study, aging was associated with a decrease of contractile function in the sub-endocardium relative to that in the sub-epicardium without changes in ejection fraction. (236) This impairment of sub-endocardial contractile function may be secondary to sub-endocardial fibrosis, asymptomatic sub-endocardial infarction (236) or reduced sub-endocardial perfusion (237). The net effect is increased LV torsion and the preservation of EF in the elderly and to reduce myocardial oxygen demand (238, 239).

In addition, since LV torsion is determined by instantaneous basal and apical rotation, any changes in the magnitude of rotational deformation delay between apex and the base of the LV will affect LV torsion. Indeed we found that LV torsion is independently predicted by rotational deformation delay as well as circumferential strain. We found in young controls the peak apical rotation occurs earlier than peak basal apical rotation, which may be explained by the start of electrical activation sub-endocardially in the right-handed helix near the apical septum with subsequent spread of the electrical activity towards the base. (240) The reduction of rotational deformation delay with aging resulted in greater LV torsion. This appear to be primarily because time to peak apical rotation occurs later in systole and closer to the timing of peak basal rotation with advancing age. The reason why peak apical rotation occurs later in systole and close to the timing of peak basal rotation still remains to be investigated but some have postulated it may be due to an increase in elastic

and collagenous tissue in the conduction system with aging (241) and also due to prolonged contraction duration (prolonged active state) (242). Other studies have indicated that the increased in LV torsion with aging is related to significantly increased peak apical rotation. (232)

In this study, we found patients with HfnEF had preserved LV torsion and untwisting rate compared to older controls, at least at rest, which is consistent with previous studies. (190, 243) In a study involving a heterogeneous group of patients with diastolic dysfunction (e.g. hypertrophic cardiomyopathy, hypertension and amyloidosis), LV twist and untwist rate were found to be significantly increased in patients with mild diastolic dysfunction. However, in patients with advanced diastolic dysfunction with increased filling pressure, LV torsion was normalized or reduced. (244) In addition, we found LV torsion to be an independent predictor of LV untwisting rate. This is perhaps not surprising, considering ventricular torsion during systole provides the potential energy for the later subsequent rapid untwisting recoil and so therefore the greater the LV torsion the more potential energy is stored for subsequent higher LV untwisting rate. Interestingly, peak LV untwisting rate has been found to be an independent predictor of the time constant of isovolumic relaxation (τ) and IVPG. (245) It is possible therefore for the observed increased LV torsion (therefore increase potential energy for subsequent LV untwisting recoil) to be a compensatory mechanism for reduced ventricular relaxation associated with aging. (246) In patients with HfnEF, ventricular relaxation is also impaired compared to matched controls. (1) In this study, we find untwisting rate to be preserved with HfnEF compared to older controls, which would suggest that LV untwisting becomes dissociated from LV relaxation rate in this population. Indeed, in patients with HfnEF, τ does not correlate with untwisting

rate. (190) Furthermore, we found that untwisting is not delayed with aging or in patients with HfnEF (compared to older controls), which is reflected by the lack of significant differences in LV untwisting rate. We also found LV longitudinal and radial strain to be preserved with aging and in patients with HfnEF (compared to older controls).

In this study we found that patients with HfnEF have reduced VO_2max and higher VE/VCO_2 slope compared to older controls, which are supported by previous reports. (209) Indeed, VE/VCO_2 slope has been shown to have prognostic value in patients with diastolic heart failure with respect to mortality and hospitalization. (179) Furthermore, we demonstrated that patients with HfnEF had increased circumferential strain and strain rate compared to older controls and that this was an independent predictor of LV torsion. It may be that circumferential strain is a marker of compensation to sustain LV torsion in order to preserve ejection fraction in these patients with HfnEF.

What we learn from this study is that many of the changes in LV biomechanics (e.g. LV torsion and untwist) in HfnEF are also present in older controls at rest. The parameters that do separate the two groups (i.e. patients and older controls) are the enhanced circumferential strain and strain rate as well as marker of increased LV end-diastolic pressure such as E/E'. During exercise patients with HfnEF and similar age controls can be clearly differentiated by VO_2max and VE/VCO_2 slopes. It is possible that the pathophysiology of HfnEF is a dynamic process with marked changes occurring on exercise and that studying these patients at rest might not be informative. Thus, to fully appreciate the role of LV torsion and untwist in the pathophysiology of HfpEF we believe that patients with HfpEF need to be investigated under exercise conditions.

Study limitations

Our study is limited by the relatively small sample size. There were a slightly greater proportion of females in the HfnEF group vs. the older control group, but the differences were relatively small and would not be expected to influence the results significantly. Statistically there were no differences in age between the HfnEF group and the older control group. A small proportion of patients had coronary artery disease which may have affected LV mechanics however coronary artery disease is common in HfnEF and thus is part of the syndrome. (12) A proportion of patients with HfnEF were on medications which may affect LV function however they would be expected to affect all strain parameters not selective ones.

Conclusions

Aging is associated with increased LV torsion secondary to reduced rotational deformation delay and increased peak basal rotation. LV untwist rate, longitudinal and radial strain are preserved with aging. LV torsion and strain patterns in patients with HfnEF are similar to age-related changes apart from circumferential strain, which is enhanced in patients with HfnEF. Independent determinant of LV torsion are rotational deformation delay, circumferential strain, LVEDV Index and MV deceleration time. And, LV untwisting rate is independently predicted by peak LV torsion and BMI.

Table 1: Baseline clinical characteristics and metabolic exercise parameters of the study population				
Variables	**Young**	**Older**	**HfnEF**	**P-value**
n	27	26	40	
Age, years	30 ± 8	64 ± 7†	67 ± 10	<0.001
Female gender (%)	7 (26)	14 (54)†	29 (73)*	<0.001
BMI, kg/m^2	25 ± 3	26 ± 5	30 ± 4*	<0.001
LVH, n (%)	0	5 (19)	15 (38)	<0.001
CAD, n (%)	0	0	4 (10)	na
Diabetes mellitus, n (%)	0	0	2 (5)	na
Hypertension, n (%)	0	0	29 (73)	na
Medication, n (%)				
Loop diuretics, n (%)	0	0	13 (33)	na
ACEi or ARB, n (%)	0	0	26 (65)	na
Beta – blockers, n (%)	0	0	7 (18)	na
Nitrates, n (%)	0	0	3 (8)	na
Calcium antagonist, n (%)	0	0	12 (30)	na
Antiplatelet agents, n (%)	0	0	14 (35)	na
Statins, n (%)	0	0	21 (53)	na
Resting heart rate, b.p.m	78 ± 10	82 ± 16	79 ± 15	0.498
Resting SBP, mmHg	114 ± 10	132 ± 22†	137 ± 21	<0.001
Resting DBP, mmHg	72 ± 8	82 ± 11†	82 ± 11	0.002
VO_2max, ml/kg/min	44 ± 7	35 ± 8†	21 ± 5*	<0.001
RER	1.28 ± 0.11	1.12 ± 0.10†	1.07 ± 0.89	<0.001

VE/VCO_2	28 ± 4	28 ± 7	33 ± 6*	<0.001
Breathing reserve, L/min	52 ± 6	44 ± 3	36 ± 15	0.001
peak SBP, mmHg	169 ± 19	191 ± 27†	183 ± 26	0.012
peak DBP, mmHg	74 ± 10	84 ± 10†	83 ± 13	0.004
peak heart rate, b.p.m	178 ± 11	164 ± 11†	136 ± 19*	<0.001

Data expressed as mean ± SD. BMI - body mass index, CAD – coronary artery disease, DBP – diastolic blood pressure, LVH - Left Ventricular Hypertrophy, RER – respiratory exchange ratio, SBP – systolic blood pressure.

*P<0.05 vs. Older group

†P<0.05 vs. Young group

Table 2: Echocardiographic Measurements on Diastolic and Systolic function				
Variables	**Young**	**Older**	**HfnEF**	**P-value**
EF, %	62 ± 5	63 ± 6	63 ± 14	0.744
IVRT, ms	72 ± 13	149 ± 26†	139 ± 29	<0.001
MV E velocity, cm/s	78 ± 15	62 ± 11†	71 ± 17	0.001
MV A velocity, cm/s	50 ± 10	65 ± 14†	82 ± 20*	<0.001
E/A ratio	1.6 ± 0.3	1.0 ± 0.3†	0.9 ± 0.4	<0.001
Dct, ms	243 ± 65	263 ± 67	253 ± 64	0.533
LVEDV indexed , ml/ m^2	49 ± 12	37 ± 12†	30 ± 8	<0.001
LVSDV indexed , ml/ m^2	19 ± 6	13 ± 4†	11 ± 5	<0.001
LV mass Index, g/m^2	67 ± 10	85 ± 12†	97 ± 18*	<0.001
E/E'	5 ± 1	8 ± 2†	11 ±4*	<0.001

Data expressed as mean ± SD. Dct – Deceleration time of early mitral inflow EF – ejection fraction, IVRT – isovolumic relaxation time, MV A – peak Doppler late mitral inflow, MV E – peak Doppler of early mitral inflow, LVEDV indexed – left ventricular end diastolic volume indexed to body surface area (BSA), and LVSDV indexed – left ventricular end systolic volume indexed to body surface area (BSA)

*P<0.05 vs. Older group

†P<0.05 vs. Young group

Table 3: Global longitudinal, radial and circumferential strains				
Variables	**Young**	**Older**	**HfnEF**	**P-value**
Global Longitudinal Strain, %	-18.4 ± 2.8	-18.2 ± 2.9	-17.8 ± 3.3	0.763
Global Longitudinal Strain Rate peak S, 1/sec	-1.16 ± 0.21	-1.11 ± 0.16	-1.16 ± 0.21	0.541
Global Longitudinal Strain Rate peak E, 1/sec	1.53 ± 0.29	1.24 ± 0.33†	1.32 ± 0.28	0.002
Global Longitudinal Strain Rate peak A, 1/sec	0.83 ± 0.25	1.23 ± 0.21†	1.30 ± 0.31	<0.001
Global Longitudinal Velocity peak S, cm/sec	4.54 ± 0.72	3.73 ± 0.90†	3.72 ± 0.85	<0.001
Global Longitudinal Velocity peak E, cm/sec	-5.26 ± 1.49	-3.02 ± 1.09†	-3.05 ± 0.77	<0.001
Global Longitudinal Velocity peak A, cm/sec	-3.16 ± 0.96	-4.35 ± 0.94†	-4.24 ± 1.09	<0.001
Global Radial Strain, %	31.6 ± 9.5	28.3 ± 10.1	21.8 ± 10.5	0.006
Global Radial Strain Rate Peak S, 1/sec	1.36 ± 0.30	1.4 ± 0.34	1.47 ± 0.28	0.518
Global Radial Strain Rate Peak E, 1/sec	-1.41 ± 0.42	-1.39 ± 0.43	-1.71 ± 0.43	0.033
Global Radial Strain Rate Peak A, 1/sec	-0.62 ± 0.23	-1.41 ± 0.50†	-1.21 ± 0.39	<0.001
Global Circumferential Strain, %	-17.6 ± 3.6	-20.0 ± 4.9	-24.7 ± 4.7*	<0.001
Global Circumferential Strain Rate Peak S, 1/sec	-1.46 ± 0.27	-1.53 ± 0.27	-1.80 ± 0.31*	0.001

Global Circumferential Strain Rate Peak E, 1/sec	1.66 ± 0.39	1.77 ± 0.64	2.29 ± 0.40*	<0.001
Global Circumferential Strain Rate Peak A, 1/sec	0.67 ± 0.20	1.39 ± 0.41†	1.52 ± 0.40	<0.001

Data expressed as mean ± SD.

*P<0.05 vs. Older group
†P<0.05 vs. Young group

Table 4: LV torsion and Untwist				
Variables	**Young**	**Older**	**HfnEF**	**P-value**
peak Apical Rotation, °	8.5 ± 4.6	8.8 ± 5.1	10.4 ± 5.4	0.380
Time to peak Apical rotation %RR	33 ± 12	42 ± 6†	41 ± 8	0.002
peak Basal Rotation, °	-4.7 ± 2.6	-7.5 ± 2.7	-7.4 ± 5.0	0.025
Time to peak Basal rotation, %RR	43 ± 14	43 ± 11	34 ± 10	0.034
Peak rotational deformation delay %RR	19 ± 14	11 ± 9	10 ± 8	0.010
peak Torsion, °/cm	1.4 ± 0.8	2.2 ± 0.9†	2.5 ± 1.2	0.001
Time to peak Torsion, %RR	38 ± 8	39 ± 4	40 ± 7	0.870
peak Twist rate S, °/sec	83 ± 41	111 ± 51	111 ± 46	0.066
Time to peak Twist rate S, %RR	19 ± 12	17 ± 7	22 ± 9	0.222
peak Untwist rate E, °/sec	-79.5 ± 40	-110 ± 35	-129 ± 55	0.002
Time to peak Untwist rate E, %RR	49 ± 7	54 ± 8	53 ± 9	0.154
Time to 15% Untwist, %RR	45 ± 7	48 ± 7	45 ± 6	0.361
Time to 25% Untwist, %RR	47 ± 7	51 ± 7	48 ± 7	0.184
Time to 50% Untwist, %RR	53 ± 11	61 ± 14	56 ± 10	0.148
Time to 75% Untwist, %RR	62 ± 16	75 ± 18	73 ± 18	0.032

Data expressed as mean ± SD. %RR – % of R-R interval.

*P<0.05 vs. Older group
†P<0.05 vs. Young group

Figure 1

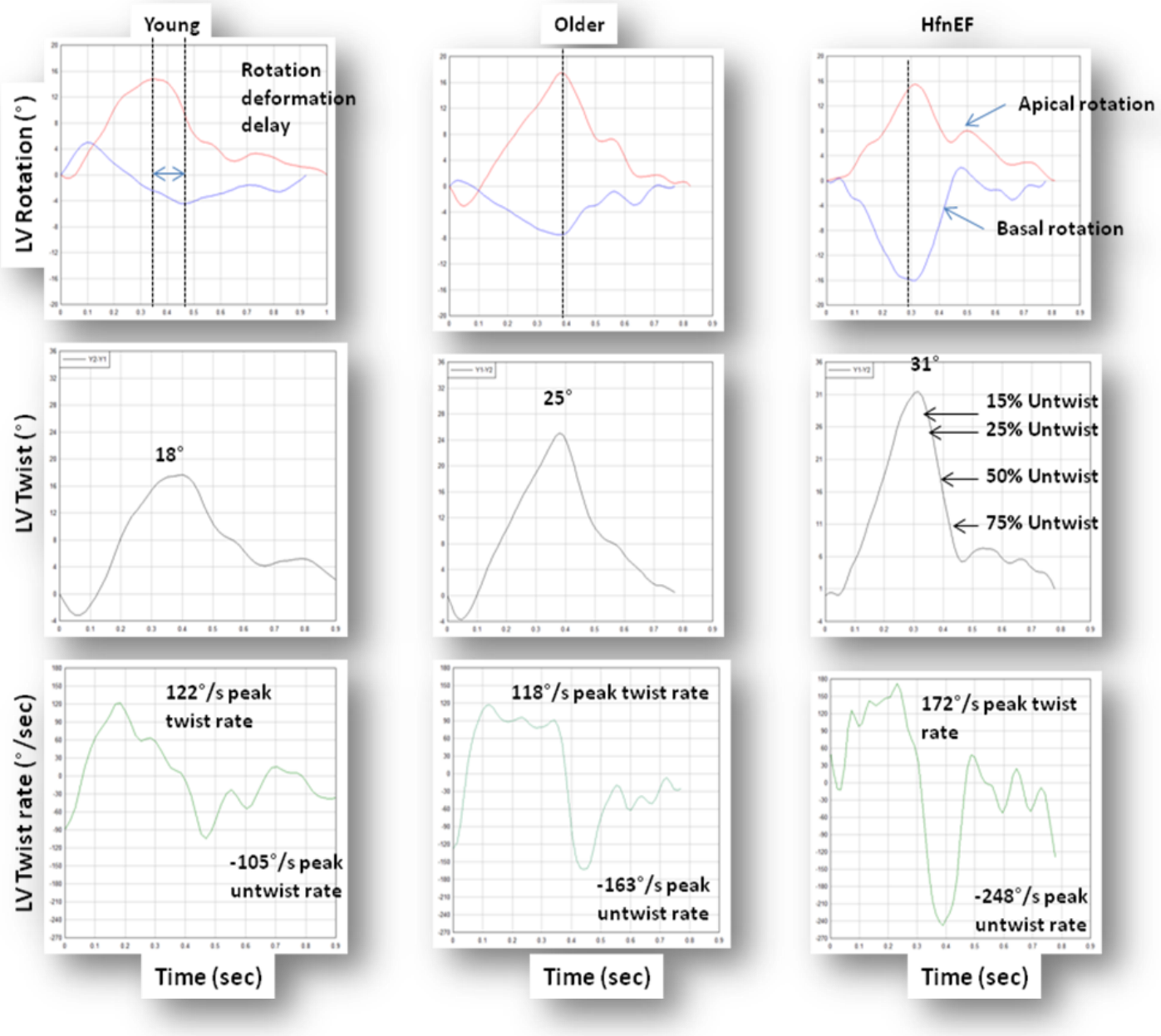


Figure 1: Profiles of apical rotation, basal rotation, LV twist and LV untwist rate.

Profiles of apical rotation, basal rotation, LV twist rate and LV untwist rate in a young control, older control and a patient with HfnEF, over one cardiac cycle. Notice the longer rotational transformation delay in the young control resulting in a lower peal LV twist compared to the older control. Rotational transformation delay, LV twist and untwisting rate in the patient with HfnEF are comparable to the older control.

Conclusions

Conclusions

About 50% of patients with the clinical features of chronic heart failure suffer from heart failure with preserved ejection fraction (HFpEF); (10, 11) Its prevalence is increasing and HFpEF causes as many hospitalizations and incurs similar morbidity and almost as high mortality as heart failure with reduced LVEF. (11, 12) Many consider HFpEF to be a disorder of diastolic function (50), whilst others believe that it may be due to a combination of diastolic abnormalities with subtle disturbances of systolic function that are insufficient to reduce LVEF. (52, 162) In addition, studies using tissue Doppler imaging (TDI) have demonstrated the presence of diastolic and/or systolic dyssynchrony in patients with HFpEF (56, 57). The typical patient with HFpEF is an elderly female with systolic hypertension due to increased large artery stiffness.

Diastolic function is influenced by the passive elastic properties of the LV and by energy dependent process of active relaxation. Increased myocardial mass or changes in extra-myocardial collagen network (50) can cause increased LV passive diastolic stiffness at rest (1). Recently, Zile *et al* reported that patients with HFpEF had abnormal LV relaxation and increased LV stiffness, and that the diastolic pressure-volume relationship was also shifted up and to the left. (1) These data suggest that left ventricular stiffness can modulate cardiac function in HFpEF patients. A study by van Heerebeek et al examined shifts in titin isoform expression between patients with systolic and diastolic heart failure. (67) Titin is a large cytoskeletal protein which contributes to resting stiffness of the myocardium. (59) In this small, highly selected group (n=4 with DHF, n=5 with SHF) they demonstrated a shift towards the stiffer N2B isoform in the DHF group and a shift towards the longer N2BA

isoform in the SHF group when compared with previously published results from healthy controls. (67) This may contribute to the observed high diastolic stiffness in HfpEF. A shift to expression of the shorter N2B isoform in response to increased arterial stiffness would increase 'contractility' (to compensate for increased aortic impedance) at the price of increased LV systolic stiffness

The interaction between the heart and the systemic vasculature, termed ventricular-vascular coupling (VVC) is essential for the heart to achieve maximal cardiac work, power and chamber efficiency while maintaining physiological blood pressures and cardiac outputs. (80, 81) VVC is indexed by the ratio arterial elastance/end-systolic elastance. LV end-systolic stiffness (Ees) is calculated from the slope of the end-systolic pressure-volume relation. Arterial stiffness (Ea) is a measure of impedance and is determined by the ratio of systolic pressure/stroke volume. Ventricular-vascular interaction is important in the context of HfpEF because of its important effects on diastolic filling. (247) In patients with HFpEF, the resting VVC is lower than in younger individuals (85) but similar to asymptomatic hypertensive elderly patients (45, 91) and falls within a range where cardiac work and efficiency are not compromised. (84)

Recently, Borlaug *et al* found that although Ea and Ees were increased in hypertensive patients with or without compared to control patients, in hypertensive patients increased Ees was associated with increased myocardial contractility; this was not the case in HfpEF patients where myocardial contractility was depressed, (248) suggesting that Ees in HfpEF patients does not necessarily reflect LV contractility (i.e. muscle mass) but rather changes in extra-myocardial collagen (e.g. titin (67)) and fibrosis. Indeed, Martos et al recently

demonstrated in that there was marked serological evidence of active fibrotic processes HfpEF patients. (249)

However, although the resting VVC ratio is within the physiological range in patients with HFpEF, the absolute values of Ees and Ea are considerably elevated indicating increased arterial and ventricular systolic stiffness and this becomes important during exercise. In young healthy subjects exercise is associated with an increase in contractility and in the rate of LV active relaxation, although the latter is attenuated with increasing age. (73) In HfpEF these physiological changes on exercise are profoundly deranged and this appears central to the pathophysiology of the disorder. Indeed, one of our study demonstrated that changes in LV torsion, untwist and LV strain and strain rate in patients with HfpEF at rest were actually quite similar to changes found as part of normal aging process. (6) However, during exercise, HfpEF patients had reduced systolic and diastolic function as well as evidence of delayed LV untwisting and LV suction. (250)

In a small study by Kitzman *et al,* HFpEF patients underwent invasive cardiopulmonary exercise testing. They found HFpEF patients exhibited a shift of the LV end-diastolic pressure volume relation upward and to the left at rest, and during exercise increases in LV filling pressure during exercise were not accompanied by increases in end-diastolic volume index (EDVi), indicating limitation to LV filling during exercise and a failure of the Frank-Starling mechanism. (74) More recently a study conducted by Kawaguchi et al, reported a dynamic impairment of left ventricular active relaxation during isometric (handgrip) exercise in a group of HFpEF patients. (58) Ennezat *et al* (78) found that HFpEF patients had greater arterial elastance response to exercise which was accompanied by reduced systolic function

as measured by LVEF, stroke volume and cardiac output. Similar findings were reported by Wachter et al, (using pressure-volume loop analysis with atrial pacing) in a small population of HfpEF patients (n=17) a blunted frequency-dependent increase in cardiac output secondary to reduced LV relaxation reserve with increased LV passive stiffness. (251)

In our own study, we found using radionuclide ventriculography that during cycle exercise HFpEF patients demonstrated marked disturbances of ventricular-vascular coupling and of both systolic and diastolic function that appeared to be responsible for exercise limitation. LV active relaxation was paradoxically slowed (measured by the time to peak filling from the time activity curve). Arterial elastance fell less and ventricular end systolic elastance increased much less during exercise than in age matched controls, the latter indicating a failure of contractile reserve. (3) The impaired LV diastolic filling during exercise may be partly compensated by increased left atrial contribution during the final stages of diastolic filling, (4) until atrial failure and eventually atrial fibrillation supervene later in the natural history of the disease resulting in more severe diastolic dysfunction as well as increase hospitalization or death. (49)

These findings suggest a potentially attractive link between increased large artery stiffness and exercise-induced diastolic dysfunction. Animal studies have demonstrated that a large acute increase in afterload in the rabbit resulted in a marked slowing of active relaxation and impaired left ventricular LV diastolic filling (252). However, acute increase in afterload required to cause a slowing of active relaxation may be much less in a diseased compared with a healthy heart. However, the concept of *relative load,* which represents the ratio of systolic LV pressure to isovolumetric LV pressure (165) allows for the possibility that an

increase in afterload required to cause a slowing of active relaxation may be much less in a diseased heart compared with a healthy heart. A similar systolic LV pressure represents a higher relative load in the failing than in the normal heart. When relative load is low, afterload reserve is still available allowing the heart to face increased afterload without slowing of LV active relaxation. When relative load is high, afterload mismatch (99) occurs and a pronounced slowing of LV active relaxation is observed (98).

A key coupler of this load dependent LV relaxation is Troponin I – Protein Kinase A (TnI-PKA) phosphorylation (95). This energy dependent process of phosphorylation of Troponin I by PKA decreases myofibrillar calcium sensitivity (96) and increases the rate at which calcium dissociates from Troponin C (97) which can lead to increase rate of LV relaxation by increasing the rate of thin filament deactivation. We demonstrated using magnetic resonance spectroscopy (MRS) that HfpEF patient had substantially reduced myocardial energetic reserve at rest compared to controls (decreased PCr/ATP ratio).The lower PCr/ATP ratio in patients indicates a reduction of high energy phosphates reserve at rest. (115, 166) This might explain why HfpEF patients are particularly prone to impaired LV active relaxation during exercise and impaired contractile reserve. (3) This is consistent with a previous smaller study which found a deficit in myofibrillar energy delivery could contribute to heart failure in patients with LVH. (253) The cause for this resting energy deficit may relate to insulin resistance (108), to impaired mitochondrial function as a result of ageing (113), and to neuro-endocrine activation and aberrant substrate metabolism. (167) In addition, increased myocardial fibrosis, as previously reported serologically in patients with diastolic heart failure (249), may also lead to reduced PCr/ATP ratio in HfpEF patients. This is relevant

because in patients with HCM, reduced PCr/ATP has been shown to correlate with the presence of fibrotic area in the myocardium of the LV. (169)

In addition, myocardial contractile inefficiency and dyssynchrony may also be a contributory factor. We showed that in HfpEF there was contractile inefficiency as a result of systolic dyssynchrony as measured by speckle tracking imaging. Which is similar to previous studies that shown using TDI that LV diastolic and/or systolic dyssynchrony was present in 60% of HfpEF patients. (56, 57) Studies in CHF have shown that systolic dyssynchrony is a strong predictor of morbidity and mortality in these patients. (197) Indeed, CRT which aims to correct systolic dyssynchrony have been shown to improve symptoms (198) and prognosis (199) in patients with CHF. In these cases, where patients have CHF and broad QRS complex, it is often the lateral wall that displays the most delayed movement. (192) In those patients with CHF and narrow QRS complex it is actually the anterior wall that displays the most delayed movement, occurring in about 25% patients. (192) In our own study we found that the LV anterior wall appears to be the most delayed segment.

Another issue that we had addressed, a point often overlooked as a cause of exercise intolerance is autonomic dysfunction. An earlier study had demonstrated chronotropic incompetence in HfpEF, however the subjects were on rate-limiting drugs such as beta-blockers which causes chronotropic incompetence. (77) We studied a relatively large number of patients with HfpEF who were not on heart rate (HR) limiting medication and found that patients with HfpEF demonstrated chronotropic incompetence during peak metabolic exercise testing and abnormal HR recovery following exercise compared to age-

gender-matched healthy controls and hypertensive patients. There is no doubt that chronotropic incompetence plays a significant role in exercise limitation in HfpEF as peak VO_2 is essentially determined by cardiac output (CO) which is in turn is defined by HR and stroke volume (SV). However, it is unclear whether chronotropic incompetence is adaptive (increasing diastolic filling time) or maladaptive. Further studies are required to resolve this matter.

Ultimately the aim of understanding the pathophysiology of HfpEF is to propose a new therapy for condition that unlike systolic heart failure has no proven therapy that improves morbidity and mortality. Our group has previously demonstrated the beneficial short-term effects of perhexiline in patients with chronic heart failure (of both ischaemic and non-ischaemic aetiology) in a phase 2 double-blind, randomized, placebo-controlled trial. (124) Perhexiline works by modifying myocardial substrate utilization from free fatty acids (FFAs) to carbohydrates. (254) Perhexiline inhibits both carnitine palmitoyl transferase-1 (CPT-1) and CPT-2, which are involved in mitochondrial uptake of long chain fatty acids. This results in a reduction in myocardial fatty acid β-oxidation, and an increase in glucose utilization at a reduced oxygen cost for energy production. (255) High levels of FFA can induce mitochondrial uncoupling that wastes energy. (256) We found in a multi-centre study that perhexiline therapy provides symptomatic relief in the majority of patients (chronic heart failure and/or refractory angina) with minimal side effects or toxicity. (257) Our studies indicate a myocardial energetic impairment in HfpEF and impaired LV relaxation during exercise. This has formed the scientific rationale for the potential use of perhexiline in HfpEF. Thus, we have successfully secured a project grant from the British Heart Foundation

for a randomized placebo-controlled trial looking at the effectiveness of perhexiline in HfpEF. This trial is currently underway and is recruiting.

In addition, recently there has been much interest in the drug 'Ranolazine' which is a late sodium channel inhibitor (I_{Na}). (258, 259) This agent has very interesting diastolic properties and has been shown to have therapeutic benefit in conditions of diastolic dysfunction due to elevated cellular Na^+ and Ca^2 concentrations by reducing these cellular Na^+ and Ca^2 concentrations. (260) What we understand is that HfpEF is a syndrome characterized by increased LV passive stiffness and impaired relaxation particularly during exercise. (1, 3, 58) In addition, Coronary artery disease and diabetes are highly prevalent in this population and there is evidence indicating abnormal calcium handling which may contribute to the impaired diastolic function such as relaxation and stiffness. In patients with ischaemic heart disease, Ranolazine has been reported to cause a downward shift of the LV pressure–volume relationship, to increase peak filling rate and to increase wall lengthening during isovolumic relaxation of ischaemic regions of the LV. (261, 262) It is possible that Ranolazine can improve LV compliance and improve LV diastolic relaxation at rest and during exercise (when ischaemia induced diastolic dysfunction is most likely to occur).

Future studies should focus on teasing out what the primary causes of HfpEF, what are primary and secondary effects of the HfpEF syndrome. Impaired chronotropic response may be a primary event contributing to HfpEF or it could be a secondary as a result of HfpEF. Impaired chronotropic response is typically present in systolic heart failure and is in part a manifestation of impaired vagal tone; (164) or it may be an adaptation to improve diastolic filling. Increasing HR by atrial pacing has been shown to reduce supine resting stroke volume

and cardiac output in patients with HfpEF. (2) But this is not be the normal physiological response since these patients were supine and at rest, the response might be different if they were undergoing physical dynamic exercise. Possible ways to address the issue whether chronotropic response is a primary or secondary event are: a) design a study involving the drug Ivabradine (263) (suppresses the sino-atrial node) then exercise HfpEF patients (metabolic exercise testing) to assess changes in peak VO_2. If peak VO_2 does not change with Ivabradine compared to without, this would suggest that chronotropic incompetence is not a primary event. However, peak VO_2 is largely determined by cardiac output on exercise and the latter is determined by HR and SV. It is highly likely that that VO_2 will diminish with the use of Ivabradine. b) A failure to increase HR and peak VO_2 in response to isoprenterenol (β_1 and β_2 agonist) might suggest chronotropic incompetence to be a primary event in HfpEF. However, chronotropic incompetence is not simply a function of beta receptor down regulation but also impaired resting vagal tone. The initial increase in heart rate during exercise is a result of vagal withdrawal and when resting vagal tone is low this effect is diminished.

The specific limitations of each study of this thesis have been discussed in detail in the appropriate chapters. In general, we would have liked to study a larger group of patients and to have better matched cohort of controls with similar co-morbidities such as hypertension, diabetes and/or ishaemic heart disease undergoing MUGA and MRS scans. However such cohort of carefully matched controls without heart failure would be in practice quite difficult to attain. In addition, it would have been interesting if we had more invasive cardiovascular measurements (to measure actual intra-cavity pressures and volumes) such as pressure-volume loop analysis or Swan-Ganz catheter measurements at

rest and during exercise. However, of course this would be difficult to gain consent from sufficient number of patients and has major ethical implications especially with respect to the control cohort. What also potentially could have been very interesting was to investigate cardiac energetic using MRS during exercise in order to assess whether cardiac energetics in terms of PCr/ATP ratio reduces further during exercise. This currently is not possible because of the complexity of acquiring cardiac MRS spectra during exercise at higher HR and problems with localization due to the movements of the heart when respiration rate increases upon exercise with added interference from the liver and lungs. A small proportion of patients in the study had coronary artery disease which may have affected LV mechanics however coronary artery disease is common in HfpEF, making up a third of the population (12) and thus could be considered as part of the clinical syndrome. It is possible that myocardial ischaemia can lead to impaired LV filling during exercise in HfpEF patients with ischaemic heart disease. This is supported by some evidence suggesting that statins improves survivial in HfpEF patients (264), although this may be more related to statins' beneficial properties on LVH, fibrosis and arterial compliance. (265) In addition we cannot completely exclude the effects of microvascular disease and how it may affect LV systolic and diastolic function during exercise. This is an important research question which we have plans to study further by using adenosine stress MRI scans to measure myocardial perfusion reserve index, which is an marker of coronary microvascular function as previously used by our group. (266)

In summary, work from this thesis indicates that HfpEF is defined by a) reduced myocardial energetics reserves, b) impaired LV relaxation during exercise, c) failure of LV to increase contractile function during exertion, d) impaired chronotropic response during peak

exercise with impaired heart rate recovery, e) enhanced left atrial contribution to LV filling during exercise and f) LV contractile inefficiency with systolic and diastolic dyssynchrony at rest. (Figure 1)

Our studies suggest that the pathophysiology of the HfpEF is one of a dynamic process with complex interaction between various processes such as increased LV stiffness, abnormal myocardial energetic, increased central arterial tree stiffness, abnormal autonomic functions and other factors such as cardiac dyssynchrony and contractility inefficiency. (3, 4, 6)

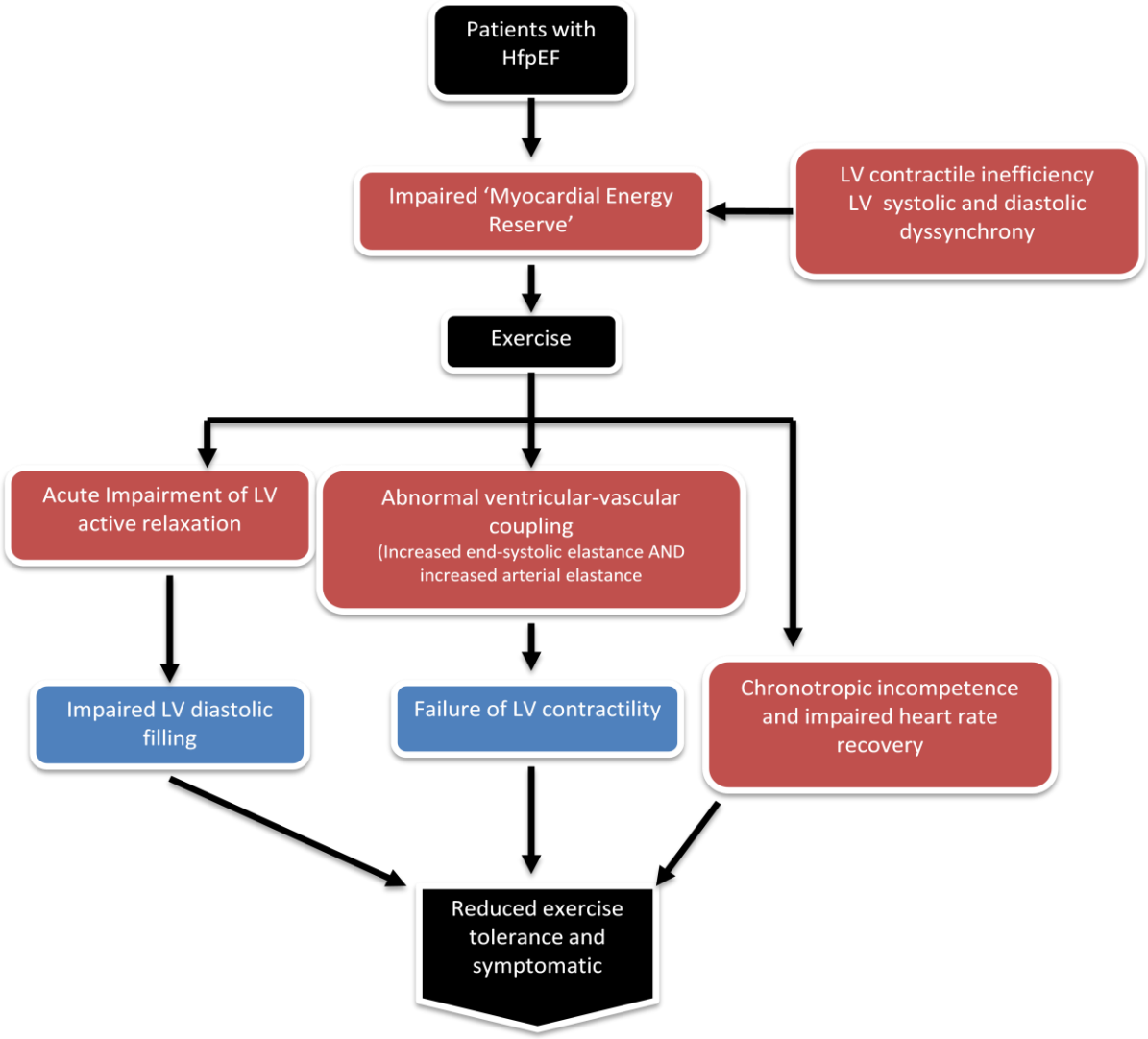


Figure 1: Pathophysiology of heart failure with preserved ejection fraction.

Published original research articles

The following original research articles were published using results and work carried out in this PhD thesis.

1. **Phan TT**, Abozguia K, Nallur SG, Mahadevan G, Ahmed I, Williams L, Dwivedi G, Patel K, Steendijk P, Ashrafian H, Henning A, Frenneaux M. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. ***J Am Coll Cardiol*** 2009;**54**(5):402-409 (3)

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Abbreviations

%HHR - % of heart rate reserve used at peak exercise

%Max-PPHR - peak exercise HR as a percentage of predicted maximal HR

A’ - the velocities of the mitral annular late diastolic wave

ACE - angiotensin-converting enzyme

ARB - angiotensin II receptor blockers

ATC - activity-time curve

BMI - body mass index

BSA - body surface area

CAD – coronary artery disease

CRT - cardiac resynchronization therapy

DBP - diastolic blood pressure

DcT - E-velocity deceleration time

E/E’ - mitral E-wave velocity-E’ tissue velocity (PW-TDI)

E’ - the velocities of the mitral annular early diastolic wave

Ea - arterial elastance

EDC - End diastolic count

EDC - end-diastolic count

EDVI - end-diastolic volume index

E_{es} - left ventricular end-systolic elastance

ES - end-systole

ESVI - end-systolic volume index

HfpEF - Heart failure with preserved ejection fraction

HR – heart rate

IVRT - isovolumic relaxation time

LA - left atrial

LSDi - longitudinal strain delay index

LV - Left ventricular

LVEDV - left ventricular end-diastolic volume

LVEF – left ventricular ejection fraction

LVESV - left ventricular end-systolic volume

LVH - left ventricular hypertrophy

MABP - mean arterial blood pressure

MRS - Magnetic Resonance Spectroscopy

MV A – peak Doppler late mitral inflow

MV E – peak Doppler of early mitral inflow

nTTPF - Time to Peak left ventricular Filling

NYHA- New York Heart Association

PCr – phosphor-creatine

peakVO_2 - peak oxygen consumption

PER - peak emptying rate

RER - respiratory exchange ratio

RR – values normalised for R-R interval

S’ - the velocities of the mitral annular systolic wave

SBP - systolic blood pressure

SD - standard deviation

STE - Speckle Tracking Echocardiography

SV - stroke volume

SVI - stroke volume index

Ta - peak longitudinal velocity A

Ta –SD – standard deviation of peak longitudinal velocity A

Ta-LSr - longitudinal strain rate A

Ta-LSr-SD – standard deviation of longitudinal strain rate A

TDI - Tissue Doppler imaging

Te - peak longitudinal velocity E

Te –SD – standard deviation of peak longitudinal velocity E

Te-LSr - longitudinal strain rate E

Te-LSr-SD – standard deviation of longitudinal strain rate E

Ts - peak longitudinal velocity S

Ts –SD – standard deviation of peak longitudinal velocity S

Ts-Circ - time to peak circumferential strain

Ts-Circ-SD - standard deviation of Ts-Circ

Ts-LS - time to peak longitudinal strain

Ts-LSr - longitudinal strain rate S

Ts-LSr-SD – standard deviation of longitudinal strain rate S

Ts-LS-SD - standard deviation of Ts-LS

Ts-Rad - time to peak radial strain

Ts-Rad-SD - standard deviation of Ts-Rad

VCO_2 - carbon dioxide production

VE - minute ventilation

VE/VCO_2 slope - the minute ventilation – carbon dioxide production relationship

VO_2 - oxygen consumption

VVC - Vasculo-ventricular coupling

γ-ATP - gamma peak adenosine triphosphate

ε - longitudinal strain

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